University of Medicine, Faculty of Medicine, Health and Biological Sciences

University of Southampton

Regulation of lung Th1 and Th2 $CD4^+$ T cell responses

Kong-Sang Wan, MD

Thesis submitted for Doctor of Philosophy

March 2001

ACKNOWLEDGEMENT

First, I have to thank my late parents who taught me the real meaning of the word "courage". Also, I have to express my appreciation to my partner, Tiffany and my daughter, Kerry for staying by my side during my hard time in England.

I deeply thank Professor Anne M Hooke for setting an example for me that age should not be an obstacle in learning and it gave me the drive to go forward since 1985. I would like to acknowledge Professor Stephen T Holgate, my supervisor, who offered this research opportunity to such a mature clinician and fully supported this study programme.

In these past three years, I was indebted to my direct supervisor, Dr. Kevan Roberts who designed this exciting and interesting project for me and with the help of Dr. Zeina Jaffar, they have taught me all the excellent scientific techniques in basic immunology while I worked in the project. They were also giving me instructions and helping me to complete this thesis without any complaint; even though they had to face a lot of headaches that I caused.

Finally, I would like to thank Mrs. Jasmine B Barley, Mr. Kerry W Taylor, and Mrs. Sue Martin who have provided me a perfect working environment to finish this research project. I have to thank all the colleagues in the Duthie Laboratory; they have given me all their friendly support, especially during the period that I suffered from the terrible traffic accident.

ABSTRACT

A large body of evidence suggests that mucosal T cells orchestrate the inflammatory processes in asthma. However, little is known of how T cell responses in the airways regulated. To address this issue the DO11.10 ovalbumin (OVA)– specific T cell receptor transgenic mouse was used to model Th1- and Th2-mediated pulmonary inflammation. Following the adoptive transfer into BALB/c mice, DO11.10 T cells bearing a Th1 or Th2 phenotype entered the lungs of recipient mice. Moreover, the inhalation of an aerosolized solution of OVA resulted in the onset of a pulmonary neutrophila and eosinophilia, respectively. Stimulation of lung mononuclear cells (LMC) with OVA 323-339 peptide induced the production of interferon-γ in Th1-recipients, and IL-4 and IL-5 in Th2-recipient mice. In addition, higher levels of IL-4 and IL-5 were found in the bronchoalveolar fluid (BALF) obtained from Th2 recipient mice that inhaled OVA. Invariably, inhalation of OVA by either Th1 or Th2 recipients resulted in the LMC entering a state of growth arrest, since proliferative responses by LMC to antigen were minimal. These data demonstrate that the injected Th1 and Th2 DO11.10 T cells retained their phenotype *in vivo* following inhalation of OVA aerosols.

Prostanoids produced at tissue sites exert multiple actions and are thought to be important in maintaining homeostatsis in the mucosal environment. The extent to which prostaglandins produced at the site of inflammation influence the immune response remains unclear. Using the DO11.10 model of pulmonary inflammation the role of cyclooxygenase-2 (COX-2) and COX-2 dependent prostanoids in regulating pulmonary inflammation was examined. High levels of prostaglandins were produced in the airways of both Th1 and Th2 recipient mice. Treatment of mice with either indomethacin or lysine-aspirin (inhibitors of both COX-1 and COX-2) dramatically reduced the levels of all the prostaglandins in the BALF. In contrast, treatment with the COX-2 specific inhibitor NS-398 markedly reduced the levels of PGI_2 present in the BALF. However, the levels of PGE_2, $PGF_{2\alpha}$, PGD_2 and thromboxane B_2 were unaffected. Interestingly, treatment of the mice with inhibitors of COX activity also had a marked effect on the magnitude of pulmonary inflammatory that developed. It was observed the treatment mice with either the COX-2 selective inhibitor NS-398 or with the non-specific inhibitors indomethacin or lysine aspirin augmented the level of pulmonary eosinophilia in the Th2 recipients. This was associated with the increased levels of IL-4 and IL-5 in the BALF of these mice. Collectively, these data demonstrate that PGI_2 influences the Th1/Th2 balance in favour of Th1 responses.

Table of Contents

Table of Figures

Abbreviations

Ab	antibody
AEC	airway epithelial cell
Ag	antigen
AIAR	aspirin-induced asthma and rhinitis
AM	alveolar macrophage
APC	antigen presenting cell
ARDS	adult respiratory distress syndrome
ASA	aspirin
BAL	bronchoalveolar lavage
BALF	bronchoalveolar lavage fluid
BSA	bovine serum albumin
BTL	B leukotreine receptor
cAMP	cyclic AMP
CCR	CC chemokine receptor
cDNA	complementary DNA
CMI	cell-mediated immunity
cpm	counts per minute
CTLA	cytotoxic T lymphocyte antigen
CXCR	CXC chemokine receptor
COX	cyclooxygenase
CysLT1, CysLT2	cysteinyl leukotreine receptors

DC	dentritic cell
DTH	delayed-type hypersensitivity
ECP	eosinophil cationic protein
EDTA	ethylene diamine tetaacetic acid
ELISA	enzyme linked immunosorbant assay
EP1 (2,3,4)	prostaglandin E receptor 1 (2,3,4)
EPO	eosinophil peroxidase
FACS	fluorescence activated cell scanner
FCS	foetal calf serum
FcγR	functional Fcγ receptor
FcεRI	high affinity IgE Fc receptor
FcεRII	low affinity IgE Fc receptor
FITC	fluorescence isothiocyanate
GM-CSF	granulocyte macrophage-colony stimulating factor
HBSS	Hank Us balanced salt sd uti o
HEPES	N-2-Hydroxyethyl piperazine-N'-2ethanesulfonic acid
Ia	MHC class II histocompatibility molecule of mice
ICAM	intracellular adhesion molecule
IgE	immunoglobulin E
IgG	immunoglobulin G
IL	interleukin
IFN-γ	interferon-γ
IM	interstitial macrophage

INDO	indomethacin
ip	intraperitoneal
IP receptor	prostcyclin receptor
Iv	intravenous
LFA	lymphocyte function associated antigen
LMC	lung mononuclear cell
LO	lipoxygenase
LPS	lipopolysaccharide
LTB4	leukotriene B4
MBP	major basic protein
M-CSF	macrophage-colony stimulating factor
2-ME	2 mercapto-ethanol
MHC	major histocompatibility complex
MIP	macrophage inflammatory protein
mRNA	messenger RNA
NEC	nasal epithelial cell
NK	natural killer cell
NO	nitric oxide
NSAID	non-steroid anti-inflammatory drugs
OD	optic density
OVA	chicken egg ovalbumin
PAF	platelet activating factor
PBS	phosphate buffered saline

PCR	polymerase chain reaction
PE	phycoerythrin
PG (D2, E2, F2α, I2, J2)	prostaglandin
PGHS	prostaglandin H synthase
PMN	polmorphonuclear leukocyte
RT	reverse transcription
TAE	Tris-acetate electrophoresis buffer
TCR	T cell receptor
Tg	transgenic
TGF	transforming growth factor
TNF	tumor necrosis factor
TXA2 (B2)	thromboxane A2 (B2)
VLA-4	very late antigen-4

Chapter One

Introduction

1.1 Cells involved in lung mucosal immune responses

There are two fundamentally different types of responses to infection, innate (natural) responses and adaptive (acquired) responses. The innate responses use phagocytic cells (neutrophils, monocytes, and macrophages), cells that release inflammatory mediators (basophils, mast cells, and eosinophils), and natural killer cells. The molecular components of innate responses include complement, acute phase proteins, and cytokines. Adaptive responses involve the proliferation of antigen-specific B and T cells, which occurs when the surface receptors of these cells bind to antigen. Antigen presenting cells (dendritic cells, macrophages, B cells, and epithelial cells) display the antigen to T lymphocytes, which elicits a response to the antigen. B cells secrete immunoglobulins, the antigen-specific antibodies responsible for eliminating extracellular microorganisms. T cells help B cells to make antibody and can also eradicate intracellular pathogens by activating macrophages and by killing virally infected cells. Innate and adaptive responses usually work together to eliminate pathogens.

1.2 Innate immunity

The innate immune system consists of all the immune defenses that lack immunologic memory. Thus, a characteristic of innate responses is that they remain unchanged however often the antigen is encountered. The effector mechanisms of innate immunity, which include antimicrobial peptides, phagocytes, and the alternative complement

pathway, are activated immediately after infection and rapidly control the replication of the infecting pathogen. The strategy of the innate immune response may not be to recognize every possible antigen, but rather to focus on a few, highly conserved structures present in large groups of microorganisms (pathogen-associated molecular patterns). It includes bacterial lipopolysaccharide, peptidoglycan, lipoteichoic acids, mannans, bacterial DNA, double-stranded RNA, and glucans, which have common features. First, pathogen-associated molecular patterns are produced only by microbial pathogens, and not by the host. Second, the structures recognized by the innate immune system are usually essential for the survival or pathogenicity of microorganisms. Third, they are usually invariant structures shared by entire classes of pathogens. Mutations that inactivate the receptors or signaling molecules involved in innate immune recognition and mutations that render them constitutively active could lead to immunologic abnormalities. The first type of mutation would be expected to result in various types of immunodeficiencies. The second type of mutation would trigger inflammatory reactions and could thus contribute to a wide variety of conditions with inflammatory component, including asthma, allergy, arthritis, and autoimmunity.

1.3 Adaptive immunity

The adaptive component is organized around two classes of specialized cells, T cells and B cells. The size and diversity of this repertoire increase the probability that an individual lymphocyte will encounter an antigen that binds to its receptor, thereby triggering

activation and proliferation of the cell. This process termed clonal selection, accounts for most of the basic properties of the adaptive immune system. The development of lymphocytes and the myeloid lineage from primordial stem cells in the fetal liver and in bone marrow is influenced by interactions with stromal cells and by cytokines. The initial stages of lymphocyte development do not require the presence of an antigen, but once these cells express a mature antigen receptor, their survival and further differentiation become antigen-dependent.

Adaptive immune responses are generated in secondary lymphoid tissue, which include lymph nodes, spleen, and mucosa-associated lymphoid tissue. The mucosa-associated lymphoid tissues, including the tonsils, adenoids, and Peyer's patches, defend mucosal surfaces. Diffuse collections of lymphoid cells are present throughout the lung and the lamina propria of the intestinal wall. The adaptive immune system has a tremendous capacity to recognize almost any antigenic structure, but because antigen receptors are generated at random, they bind to antigens regardless of their origin, bacterial, environmental, or self. The role of the innate immune system function is to signal the presence of infection. The signals induced on recognition by the innate immune system, in turn, control the activation of adaptive immune responses; the adaptive immune system responds to a pathogen only after it has been recognized by the innate immune system. Self-antigens are not recognized by receptors of the innate immune system and, do not induce the expression of costimulatory signal. After activation, helper T cells control other components of adaptive immunity, such as the activation of cytotoxic T cells, B cells and macrophages. Innate immune recognition therefore appears to control all the

major aspects of the adaptive immune responses through the recognition of infectious microbes and the induction of signals required for the triggering of adaptive immunity.

1.3.1 The distribution of leukocytes in the lung

Several compartments of the lung contain large numbers of lymphocytes, which includes the intravascular pool, the interstitial pool and the bronchoalveolar space pool. Although the migratory route and mechanisms promoting movement from the blood to the alveolar space is poorly understood, the lung is more than a passive filter for damaged lymphocytes and the site of accumulated lymphocytes in the bronchial wall. An integrated understanding of the lung immune system will have implications towards the areas of tolerance and allergy in the respiratory tract and immunization against pulmonary infections.

1. The intravascular lymphocyte pool

   When labeled lymphocytes injected *iv* in different species, an early high concentration of lymphocytes in the lung was found. This lung intravascular pool of lymphocytes is large, on a per gram basis nearly ten times larger than for the liver and kidney. However, nothing is known about the interaction of lymphocytes with pulmonary endothelial cells characterization of such processes will be very important in defining how lymphocytes are reduced to the lung and factors which regulate the mobilization of lymphocytes in the pulmonary vasculature.

2. The lung interstitium

Considerable numbers (15-48 x 10^6 g^{-1}) of interstitial lymphocytes are recovered when lung tissue has been digested with enzyme after vascular perfusion to remove the intravascular lymphocytes. Interstitial lymphocytes have a characteristic subset composition, size, distribution and cytokine production profile. However, whether the lymphocytes in the interstitial space are in transit from the blood to the alveolar space, or whether they represent a separate pool with specific functions is not clear.

3. The bronchoalveolar space

Using bronchoscopes, subsegmental parts of the lung can easily be lavaged in humans. BAL has also been performed in many other species. The majority of BAL cells are alveolar macrophages, but about 10% of the nucleated cells are lymphocytes, mostly T cells that express markers typical for memory lymphocytes. Experimentally, inhalation of lipopolysaccharide by healthy humans induces lymphocytes in BAL within 3 hours. Clinical data lead to a similar conclusion: after exposure to allergens, the number of CD4+ T cells in the peripheral blood decreases, leading to the suggestion that these lymphocytes migrate to the lung. There is some evidence in experimental animals that lymphocytes can leave the bronchoalveolar space by entering the lung parenchyma and reaching the draining lymph nodes via afferent lypmphatics. In conclusion, the principle issues that remain unresolved are: (1) Where do the lymphocytes in the bronchoalveolar space come from? (2) Are they in equilibrium with lymphocytes in the blood?

Multiple lines of evidence suggested that several cytokines produced by alveolar macrophages, mast cells, lymphocytes, neutrophils, eosinophils, and epithelial and endothelial cells control the inflammatory response in various lung diseases. For instance, asthma is a well-characterized disease with respect to pulmonary Th2-like T cell activation and cytokine production. The presence of both activated T cells and lipid mediators has been demonstrated in blood, bronchial biopsies, and bronchoalveolar lavage (BAL) that correlates with the level of eosinophilia and disease severity. Moreover, a recent study has demonstrated different T cell-derived cytokine profiles present in BAL fluid of allergic and nonallergic asthmatic subjects. Allergic asthmatic patients were characterized by increased levels of IL-4 and IL-5, and elevated IgE levels were found in these allergic subjects. In contrast, non-allergic asthmatic subjects had elevated levels of IL-2 and IL-5, with IL-2 contributing to T cell activation. In both types of asthma, the close correlation of IL-5 levels with the number of eosinophils suggests that IL-5 is responsible for the characteristic eosinophilia of asthma.

1.3.2 Antigen presentation in the lung

Molecules recognized by receptors on lymphocytes are generically referred to as antigens and can range from small chemical structures to highly complex molecules. Both the T cell receptor and the B cell receptor recognize only a small part of a complex antigen, the antigenic epitope. However, not all antigens are naturally immunogenic, for instance, haptens must be coupled to larger immunogenic molecules (carriers) to stimulate a response. In contrast, carbohydrates must be coupled to proteins in order to be

immunogenic, as is the case for the polysaccharide antigens used in Haemophilus influenzae type b vaccine. Therefore, antigen presentation to T cells entails the display of antigen as peptide fragments bound to MHC molecules. The main antigen-presenting cells (APC) for T cells are dendritic cells, macrophages, and B cells.

1.3.2.1 Dendritic cells

Dendritic cells (DC) are phenotypically heterogeneous, possibly reflecting different stages of maturation (Steinman RM 1991; Peter JH et al 1996). Lung DCs constitutively express MHC class II and I antigen as well as the costimulatory molecules CD80, CD86 and the adhesion molecules ICAM-1, ICAM-3 and LFA-1 (Vremec D et al 1997, Banchereau J et al 1996). They are low-density, loosely adherent, nonphagocytic and have long dendritic processes evident both in tissue sections and in cell suspensions. The density of DCs varies from 100-800 per mm^2 epithelial surface in the large airways, to 75 per mm^2 in the epithelium of the small airways of the peripheral lung (Schon-Hegard MA et al 1991). The density of intraepithelial DCs in the lung rapidly increases in rats in response to stimuli such as chemical irritants and bacterial LPS (McWilliam AS et al 1996; Crowley M et al 1989). In man, increased numbers of DCs are found in the lung mucosa of subjects with allergic respiratory diseases or the submucosa of the bronchial biopsies taken from atopic asthmatics when compared to normal control individuals (Moller GM et al 1996). Similarly, increased numbers of DCs have been reported in both the epithelium and Lamina propria of the conducting airways of atopic asthmatics and in the nasal mucosa of grass-pollen sensitive rhinitis (Fokken WJ et al 1989). A reduction in

DC numbers in the airway epithelium has been reported to occur in atopic asthmatics treated with inhaled corticosteroids (Moller GM et al 1996).

DCs are highly efficient antigen presenting cells (APCs) and unique in their ability to elicit responses from naïve T cells. They acquire foreign antigens in peripheral sites, process them, and subsequently migrate to the T cell areas of lymph nodes. In lungs, aeroantigens entering the bronchoalveolar spaces are processed by DCs, which possess elongated processes entering into the lumen of the airway. Freshly isolated DCs from the tracheal epithelium and lung parenchyma are poor APCs, but their stimulatory activity is increased after overnight culture (Capuis F et al 1997). This suggests that DCs are stimulated and become mature during the migration from tissues to regional lymph nodes. Holt's study showed that in the lung and airway mucosa, the functional phenotype of the DC is actively controlled by the microenvironment, for instance, alveolar macrophages and cytokines (Holt PG et al 1993). Elimination of alveolar macrophages results in the rapid enhancement of the APC activity of resident lung DCs (Thepen T et al 1989; 1991). Also the APC activity of the functionally mature DCs is inhibited when co-cultured with alveolar macrophages. The neutralizing antibody of GM-CSF (Bilyk N et al 1993) can ablate this inhibitory activity.

1.3.2.2 Pulmonary macrophages

Macrophages derived from blood-borne monocytes possess receptors for carbohydrates that are normally exposed on the cells of infectious agent, such as mannose, and therefore can discriminate between 'foreign' and 'self' molecule. In addition, it has receptors for

antibodies and complement, so that the coating of microorganisms with antibodies, complement or both enhances phagocytosis. The engulfed microorganisms are subjected to a wide range of toxic intracellular molecules or persist and contribute to the adaptive immune response by acting as professional antigen-presenting cells. Phagocytes also remove the body own dead or dying cells. Dying cells in necrotic tissue release substances that trigger an inflammatory response, whereas cells that are dying as a result of apoptosis (programmed cell death resulting in the digestion of DNA by endonucleases). Alveolar macrophages (AMs) and interstitial macrophages (IMs) originate from the bone marrow (Van Oud Alblas AB et al 1983) and are related to each other, yet are phenotypically distinct. AMs have a high potential for microbicidal activity, while IMs are responsible for immunoregulatory and accessory functions (Franke-Ullmann G et al 1996). AMs reside within the fluid layer overlaying the alveolar and airway epithelia and can be collected by endobronchial lavage, while IMs are located in the lung parenchyma (Brain JD et al 1988) and can be extracted from lung tissue by enzymatic digestion. IMs and AMs exhibit size, functional and phenotypic differences, which may reflect the stage of differentiation and differing physiological roles of these cells in their distinct locations. The major role of AMs is to phagocytose and remove pathogenic organisms and soluble antigens from the alveoli and to inhibit the local immune response. AMs are poor APCs for priming T cells even when they express high levels of MHC class II (Lipscomb MF et al 1986; Kradin RL et al 1991). A number of laboratories have implicated AMs in the suppression of T cell proliferative responses (Holt PG et al 1979; McCoombs CC et al 1982). This is effected by the production of mediators which include prostaglandin E2 (Monick M et al 1987), TGF-β (Roth MD et al 1993), and NO (Fu YH et al 1992).

However, the suppressive activity of AMs is lost by treatment with LPS or selected cytokines such as GM-CSF (Bilyk N et al 1993).

1.3.2.3 Airway epithelial cells

Various epithelial cells including respiratory epithelial cells can express MHC class II molecules and have been reported to capable of antigen presentation (Mayer et al 1987, Kalb TH 1991). Both class I and class II antigens were uniformly and strongly expressed throughout the major, lobar, and segmental bronchi, the bronchiolar epithelium and the alveolar epithelium (Allan R et al 1989). In both the rat and human systems, colonic epithelial cells have been found capable of inducing T cell proliferation in response to antigenic stimulation (Bland PW 1989). Since those respiratory epithelial cells vastly outnumber all other constituents of the mucosal surface exposed to airborne infectious agents and allergens, its ability to process and present antigen to T cells may have physiologic importance. Moreover, the site of these cells juxtaposed to the external environment may make them important in the regulation of local immune responses.
Nasal epithelial cells (NEC) and airway epithelial cells (AEC) are similar to conventional APC in that they express CD80 and CD86 and functional Fcγ receptor (FcγR) and contain acidic compartments in which class II MHC molecules and processed peptide associate. Moreover, treating NEC and AEC with anti-CD80 and anti-CD86 antibodies could block T cell proliferation (Salik E et al 1999). In conclusion, upregulation of respiratory epithelial cells accessory function may aid in protective responses against specific pathogens or in inducing inflammatory responses. An improved understanding of antigen

presentation by respiratory epithelial cells may lead to better treatment of chronic inflammatory disease of the upper and lower respiratory tract and the development of vaccine against respiratory infections.

1.3.3 The humoral response

Protective immunity can be divided into cell-mediated immunity and humoral immunity. Specific immunity is mediated by antibodies made in a humoral immune response that can be transferred to naïve recipients with immune serum containing specific antibody. Selective production of Th2 cells that activate the B cells to produce different types of antibody, help drive driving the humoral immune response. Antibodies consist of two identical heavy chains and two identical light chains that are held together by disulfide bonds. The amino acid sequence of the constant region of the heavy chains specifies five classes of immunoglobulins (IgG, IgA, IgM, IgD, and IgE), four subclasses of IgG, and two subclasses of IgA. These classes and subclasses have different functions. Each type of antibody can be produced as circulating molecule or as a membrane bound form. The latter type has hydrophobic transmembrane sequence that anchors the molecule in the B cell membrane, where it functions as the B cell receptor. The B cells that develop earliest during ontogeny are referred to as B1 cells. Most of B1 cells express CD5, an adhesion and signaling cell-surface molecule. They are the source of natural antibodies, which are IgM antibodies and are frequently polyreactive. Most B cells lack the CD5 molecule, and because they develop slightly later in ontogeny, they are referred as B2 cells. Before they encounter antigen, mature B2 cells coexpress IgM and IgD antibodies on their cell surface, but by the time they become memory cells, they have usually switched to the use

of IgG, IgA, or IgE as their antigen receptors. The B cell responses occur in the germinal centres discrete within the spleen and lymph nodes, where they encounter the antigen undergo immunoglobulin class switching and begin to produce IgG, IgA, or IgE, and somatic hypermutation of their antigen-receptor genes occurs. The final stages of differentiation of B2 cells into antibody-secreting plasma cells occur within the secondary lymphoid tissues but outside the germinal centres. Although generally short-lived (a few days) some plasma cells survive for weeks, especially within the bone marrow.

1.3.4 The T cell response

Antigen specific T cells play the main role in the cell-mediated immune response that encompasses all adaptive immunity and cannot be transferred to a naïve recipient with serum antibody. Stem cells continuously migrate from the bone marrow to the thymus, where they develop T cells. Recent evidence suggests that, despite the partial degeneration of the thymus that occurs at puberty, T cells continue to develop in thymus throughout life (Jamieson BD et al 1999). T lymphocytes are a subset of lymphocytes defined by heterodimeric receptors associated with the proteins of the CD3 complex. Most T cells have α:β heterodimeric receptors but γ:δ T cells have a γ:δ heterodimeric receptor. The α:β T cell receptor recognizes short peptides that result from the intracellular processing of protein antigens, which are presented to the T cell receptor by MHC molecules on the cell surface of APC. However, T cells require at least two signals to become activated; one is the complex of a peptide and MHC molecules, and the other is a costimulatory signal mediated by, for example, the CD80 and CD86 molecules on the

surface of the APC. Recognition of an antigen in the absence of costimulatory signal leads to permanent inactivation or apoptosis of the T cell. Essentially, the principle molecules constitute receptor-ligand pairs important driving T cell activation are CD28-CD80/CD86; CD40-CD154; and CD2-CD58 (Lenschow DJ et al 1996, Grewal IS et al 1998, Holter W et al 1996). Moreover, activated dendritic cells are particularly potent stimulators of naïve T cells, because they express large amounts of the costimlatory B7 and CD40 (van Kooten C et al 1997).

1.3.4.1 T lymphocytes in the lung

Lymphocytes are the predominant effector cell in specific immune responses. They comprise approximately 8% to 10% of lung cells recovered by BAL (Pabst R 1992). The vast majority of these lymphocytes are T lymphocytes (60% to 75%) and the ratio of CD4+ to CD8+ lymphocytes is approximately 1.5:1, the same as in blood. B cells comprise 1% to 10% of the lung lymphocyte population. Approximately 10% of the lymphocyte are null or plasma cells. The location and proportion of lymphocytes in the lung vary between species. In rats, rabbits, and chickens, large numbers of lymphocytes are located in the BALF, while in hamsters, mice and humans, the majority of lymphocytes are present in the interstitium, and are diffuse in the mucosa, alveolar septa and pleura. The majority of T cells present in the lung tissue in normal and asthmatic individuals are CD45RO+ memory cells (Saltini C et al 1990), which react promptly to recall antigens (Akbar AN et al 1988). Analysis of allergic inflammation in the lungs of mice has greatly contributed to our knowledge of the behaviour of lung T cells to inhaled antigens. Exposure of mice to aerosolized antigens has been used to induce a pulmonary

allergic response dependent on CD4+ T cell (Guvett SH et al 1994). This approach emphasizes the important role played by CD4+ T cells in the allergic inflammation in the lungs. However, the minority of T cells are γδ T cells, estimated to be 8-20% in mice, which have been implicated in down-regulating primary IgE responses in rats to inhaled soluble protein antigens (McMenamin C et al), but they are not required for maximal inhibition of the IgE response (Brain W et al 1998).

(1) CD4+ T Lymphocyte

It has become clear that the nature of the immune response initiated by CD4+ T cells is at least partly dependent on the selection or preferential activation of particular subsets of CD4+ T cells, knows as Th1 and Th2 cell, which secrete defined patterns of Cytokine. Th1 cells were characterized by the predominant secretion of IL-2, IFN-γ, and TNF-β, whereas Th2 cells characteristically predominantly secreted IL-4, IL-5, IL-6, and IL-10. Other Cytokine, such as TNF-α, IL-3, and GM-CSF, were produced by both Th1 and Th2 cell subsets (Mormon TR et al 1986; 1991). Broadly speaking, Th1 cells participate in delayed-type hypersensitivity reactions (Cher DJ et al 1987), but also provide help for B lymphocyte immunoglobulin synthesis under certain circumstances. It can cause inflammation and also may downregulate Th2-driven actions. For example, IFN-γ inhibits Th2 proliferation (Mosmann TR et al 1986) but expands Th1 cells (Takagi M et al 1990). In addition, IFN-γ inhibits IL-4 actions, including IgE synthesis (Finkleman FD et al 1988; Diaz P et al 1989), mast cell proliferation (Corrigan CJ et al 1988), and induction of MHC class II molecules on B cells (Corrigan CJ et al 1990). Th2 cells, by their pattern of secretion of B lymphocyte co-stimulatory cytokines, enhance the

synthesis of all immunoglobulins, including IgE, in immune responses. IL5 has specific eosinophil proinflammatory actions which include promoting their differentiation, activation, and maturation (Rothenberg ME et al 1989; Secor WE et al 1990). Similarly, Th2 products can modulate Th1 activity. For instance, IL-10 suppresses Th1 cell cytokine secretion (Fiorentino DF et al 1989); IL-4 antagonizes IFN-γ actions (Wierenga EA et al 1990;Kay AB et al 1991). Thus not only may T cell products promote airway inflammation, but it is speculated that the tendency toward inflammation in asthma may represent a CD4+ cell imbalance with the equivalent of Th2 cell products being dominant. Some T-lymphocytes clones secreting cytokines characteristic of both Th1 and Th2 cells are termed Th0 cells (Firestein GS et al 1989).

(2) CD8+ Lymphocyte

Most cytolytic T lymphocytes express the CD8+ cell surface co-receptor. Most of these T cells have antigen receptors that recognize antigenic peptide complexes to class I MHC molecules (Jorgensen JL et al 1992). Cell-mediated cytolysis is an effector mechanism evolved to combat intracellular parasitism by viruses, certain bacteria, and some protozoan parasites. Cytolytic cells also play a critical effector role in allograft and tumor rejection. It should be noted that a subset of CD4+ T cells with antigen receptors for peptide-class II MHC complexes can also express cytolytic activity (Kaplan DR et al 1984; Keshav S et al 1990).

1.3.5 Inflammatory cells infiltrating the lung

1.3.5.1 Pulmonary neutrophils

Polymorphonuclear (PMN) leukocytes such as neutrophils or eosinophils are never found in the lower respiratory tract under normal conditions. They are potent inflammatory cells recruited by alveolar macrophages via specific chemoattactants, and participate in the destruction and clearance of invading organisms that have reached the alveolar space (Hunninghake GW. et al 1980; Fantone JC et al 1982). These cells produce toxic O2-derived species and the myeloperoxidase-derived hypohalite ion, all of which can kill microorganisms (Martin WJ et al 1984; Janoff A 1985). Futhermore, neutrophils possess specific enzymes, eg. lysozyme that can destroy bacterial cell walls and other barriers to eliminate potentially infectious agents (Gadek JE et al 1984). For examples, leukocyte elastase can digest elastin, collagen, laminin, proteoglycans, and fibronectin. The recruitment of inflammatory cells to the alveolar spaces, however, represents a "two-edged sword." Inflammatory cells are clearly necessary for the inflammatory or immune response when an infection is developing in the alveolar spaces. However, the release of potent oxidants and toxic proteins by these inflammatory cells can injure the alveolar structures and interstitium of the lung. Evidence has accumulated for IL-8, β2 integrins and their ligand ICAM-1 in neutrophil adhesion to the transmigration across endothelial cells (Ferro TJ et al 1993). However, the different mechanisms of migration across endothelial and airway epithelial monolayers remain obscure.

1.3.5.2 Pulmonary eosinophils

Eosinophils are derived from haemopoietic stem cells in the bone marrow. They have the capacity to secrete a number of mediators including basic proteins (MBP, ECP, EPO), membrane-derived mediators (LTC4, PAF, PGE1, PGE2), proteases (phospholipase D, histaminase), chemokines (MIP-1α, RANTES) and cytokines (IL-1α, IL-2, -3, -4, -5, -6, -8, GM-CSF, TNF-α, TNF-β) (Wardlaw AJ et al 1995). These proteins are toxic and are likely to result in epithelium damage. Additional damage may cause the interaction of eosinophils with the bronchial epithelium, resulting in the induction of epithelial proteases (Herbert CA et al 1991). Eosinophils play an important role in the host defense against parasites and the allergic diseases such as asthma, rhinitis and atopic dermatitis. Eosinophils are a important source of cytokines such as IL-3 (Kita H et al 1991), GM-CSF (Moqbel R et al 1991), IL-5 (Desreumaux P et al 1992), IL-6 (Hamid Q et al 1991), TGF-α (Wong DTW et al 1990), and TGF-β (Ohno I et al 1992), which prolong the inflammatory response and contribute to airway fibrosis. Eosinophils express the low affinity IgE receptor CD23, the carbohydrate binding protein MAC-2 (Cherayil BJ et al 1989), FcγRIII receptors for IgG and IgA receptors, and receptors for certain components of the complement cascade. The high affinity IgE receptor has been found on eosinophils in some instances, although it is unclear whether it is expressed in asthma (Gounni AS et al 1994). It appears that different receptors are able to mediate the release of different mediators (Tomassini M et al 1991). IL-3 (Rothenberg ME et al 1988), IL-5 (Lopez AF et al 1988) and GM-CSF (Owen Jr WF et al1987) can all increase the activation status of eosinophils, and eosinophils present in the airways of asthmatics have been shown to express the activation maker CD69 (Hartnell A et al 1993). Eosinophils also express

several adhesion receptors such as β1 and β2 integrins, E-selectin and P-selectin ligands and L-selectin, which enable them to interact with the vascular endothelium. The recruitment of eosinophils to the lungs of OVA-sensitized guinea pigs following aerosol challenge is blocked by a monoclonal antibody against VLA-4 (Pretolani M et al 1994). Whether this is a consequence of VLA-4 binding to VCAM-1 or other ligands is not resolved. However, both VLA-4 and VCAM-1 are involved in the recruitment of eosinophils and T cells to the trachea of aerosol-challenged mice (Nakajima H et al 1994).

1.3.5.3 Mast cells and Basophils

All mast cells are derived from progenitors present in the bone marrow and can be found throughout the connective tissue, particularly near blood and lymphatic vessels. Mast cells and basophils express the high affinity IgE receptor (FcεRI) and can be activated by allergen. Following activation, basophils are capable of releasing a range of inflammatory mediators, which are divided into primary mediators and secondary mediators. The primary mediators are produced before degranulation and stored in the granules, including histamine, proteases, eosinphil chemotactic factor, neutrophil chemotactic factor and heparin. The secondary mediators are either synthesized after target-cell activation or are released by the breakdown of membrane phospholipids during the degranulation process such as PAF, LT, PG, bradykinin and cytokines (Gordon JR et al 1990). Mast cells recovered by BAL from the airways of asthmatics exhibit an increased spontaneous release of histamine and PGD2 and responsiveness to allergen and other secretory stimuli (Flink KC et al 1985). There is also evidence to implicate the basophil

in the response (Bascom RM et al 1988). The degranulation of mast cells is initiated by a number of stimuli, including allergen cross-linkage of bound IgE, C3a, C4a, C5a, ACTH and calcium ionophore.

1.4 The development of Th1 and Th2 effector CD4+ T cells

On encountering antigen naïve CD4+ T cells change into either an effector functional phenotype or memory phenotype effector CD4+ T cells can be categorized as being either Th1 or Th2 (type1 or type 2) depending on the cytokines they produce (Table 1.1).

	T helper cell type 1 (Th1)	T helper cell type 2 (Th2)
Cytokines Profile	IFN-γ, TNF-β, IL-2	IL-4, IL-5, IL-6, IL-9 IL-13, IL-10 (in mice)
Chemokines Receptors	CXCR-3, CCR-5	CXCR-4, CCR-3 (Eotaxin R) CCR-4, CCR-7, CCR-8
Cytokine Receptors		
IFN-γ:	IFN-γRα	IFN-γRα, IFN-γRβ
IL-12:	IL-12Rβ$_1$, IL-12Rβ$_2$	IL-12Rβ$_1$
Aproptosis	+++	--
Other Markers		
CD30:	--	++
ST2L/T1:	--	++
GATA-3:	--	++
T-bet	++	--

Table 1.1
Properties of Th1 and Th2 effector cells

Th1 cells produce IFN-γ, IL-2, IL-3 and TNF-β and play a critical role in directing cell-mediated immune responses, important for the clearance of intracellular pathogens (Mosmann TR et al 1986, Steven TR et al 1988, Brewer JM et al 1994). Th2 cells produce IL-3, IL-4, IL-5, IL-6, IL-10 and IL-13 and have been associated with allergy and are important for humoral responses (Coffman R et al 1986, Finkelman FD et al 1988, Mosmann TR et al 1989). Naïve T cells reside in the recirculating lymphocyte pool and move continuously from lymphoid organs to blood and back (Ford WL et al 1975). When naïve T cells recognize MHC-peptide complexes displayed on antigen presenting cells (APCs) they are triggered to proliferate. It is generally thought that naïve $CD4^+$ T cells (Th0) have the potential of differentiating into either Th1 or Th2 subset. The pattern of differentiation is influenced by a number of factors that include amount and route of antigen exposure, genetic factors (Constant SL et al 1997, Romagnani S et al 1999). The type of APCs and cytokine present during lymphocyte stimulation has a major contribution of differentiation of $CD4^+$ lymphocytes. For instance, IL-12 produced by macrophages and dendritic cells induces IFN-γ expression, which inhibits proliferation of Th2 cells resulting in preferential differentiation toward the Th1 subset. In contrast, IL-4 itself is a potent stimulus for the development of IL-4 secreting Th2 cells (Swain SL et al 1993).

Cytokines from each subset can regulate the development of the other. For instance, the Th2 cytokines IL-4 and IL-10 are able to regulate Th1 responses by inhibiting the expansion of Th1 cells (Fiorentino DF et al 1991, Hsieh CS et al 1992). Conversely, the Th1 cytokine, IFN-γ can down-regulate Th2-mediated allergic responses (Coffman RL et

al 1988, Abbas AK et al 1996, Cohn L et al 1999). However, it seems that antigen-specific Th1 cells do not inhibit pulmonary Th2 mediated inflammation but rather potentate the inflammatory response (Chaplan DD et al 1999). Similarly, Umetsu et al have reported that Th1 cells do not attenuate Th2 cell-induced airway hyperreactivity and inflammation in either SCID mice or in OVA-immunized (immunocompetent) BALB/c mice, but make the airway inflammation more severe (Umetsu DT et al 1999). However, immune deviation of Th2 cells toward Th1 responses, as a potential therapy for asthma and allergy is still a critical and important concept. Consistent with this hypothesis is the recent success that DNA vaccines have had in attenuating pulmonary eosinophilia (Broide D et al 1998).

Th1 $CD4^+$ T cells secretes IL-2 and IFN-γ, which are principal effector of cell-mediated immunity and expression of delayed-type hypersensitivity. IFN-γ activates macrophage, a promotes lysis of intracellular pathogens. The IFN-γ produced by Th1cells promotes isotype switch of B cells to produce IgG2a and IgG2b. IgG2 and IgM antibody are important in activating complement and opsonization of microorganisms by phagocytes. Interestingly, IFN-γ and TNF-α have been identified in bronchoalveolar lavage fluid and serum of asthmatic patients suggesting that Th1-like cells may contribute to the pathogenesis of this disease (Cembrzynska-Nowak et al 1993). Th2 cells, producing cytokines such as IL-4, IL-5, IL-10 and IL-13, were originally defined as factors that helped B-cell responses, but are now clearly implicated in allergic responses by their activation of mast cells and eosinophils (Resnick MB et al 1993). Type 2 CD4+ effector cells have been described in both mice and humans. However, human Th2 clones differ

from murine clones in a number of respects. In particular, both humans' Th1 and Th2 clones produce IL-10, whereas this is a Th2 cytokine in the mouse (Del Prete G et al 1993). The selective expansion of Th2 cells is thought to play a critical role in inducing the IgE synthesis and eosinophilia associated with allergic disease. IL-4 causes immunoglobulin gene isotype switching to IgE and IgG4 in humans and IgE and IgG1 in mice. IL-5 induces the proliferation and differentiation of eosinophils from bone marrow progenitors and IL-13 has been shown to be a chemotactic factor for eosinophils. Furthermore, IL-4 is mainly responsible for the Th2 effector cell. The ability of cytokines such as IL-10 and IL-4 to inhibit inflammation and Th1-type cell-mediated immune responses may in part explain why cell-mediated and humoral immune responses were often observed to be mutually exclusive, and implicate the Th2 subset as important regulators of cell-mediated immunity. Infection of mice with Schitosomes also caused the intrapulmonary and BAL eosionphilia via an IL-4-dependent mechanism (Lukacs et al 1994) possibly by altering the Th2/Th1 balance.

1.4.1 The Th1 cytokine Interferon-γ

IFN-γ was originally identified as a product of mitogen stimulated T lymphocytes that inhibited viral replication in fibroblasts (Gribando G et al 1991). Sources of IFN-γ include NK cells, $CD8^+$ cells and $CD4^+$ Th1 cells (Handa K et al 1983, Schreiber RD et al 1985). While NK and $CD8^+$ T cells produce IFN-γ following primary activation, $CD4^+$ T cells have to differentiate into Th1 cells, for which signals from IL-12 and Stat4 activation in addition to TCR ligation is clearly important. Cyclosporin-A inhibits TCR-induced IFN-γ production, but not IL-12/IL-18-induced IFN-γ production (Jianfei Yang

et al 1999). The IFN-γ receptor is expressed on T cells, B cells, monocytes/macrophages, dendritic cells, granulocytes, epithelial cells, endothelial cells, and platelets. IFN-γ has extensive and diverse immunoregulatory effects on various cells. It is produced by Th1 cells and exerts an inhibitory effect on Th2 cells. It inhibits antigen induced eosinophil recruitment in the mouse (Nakajima H et al 1993). It is also a powerful and relatively specific inhibitor of IL-4 induced IgE and IgG_4 synthesis by B cells (Snapper CM et al 1987, Mond JJ et al 1986). Moreover, IFN-γ upregulates class II molecules on monocytes/macrophages and dendritic cells and induces de novo expression on epithelial, endothelial and other cells, thus making them capable of antigen presentation.

There is reduced production of IFN-γ by T cells of asthmatic patients and this correlates with disease severity (Koning H, et al 1997). Intra-nasal administration of exogenous IFN-γ prevents the airway eosinophilia and hyperresponsiveness following allergen exposure in mice (Lack G et al 1996). In asthmatic patients nebulized IFN-γ reduces the number of eosinophils in BAL fluid (Martin D et al 1995). There is reduced production of IFN-γ by T cells (BAL and peripheral) of asthmatic patients. These studies indicate that IFN-γ has a potential modulating effect on allergic responses to allergen. In the mean time, corticosteroid treatment increases IFN-γ expression in asthmatic patients (Bentley AM et al 1996), but in corticosteroid resistant patients IFN-γ is unexpectedly reduced.

1.4.1.1 Factors that regulate IFN-γ expression: IL-12 and IL-18

IL-12 was first discovered as a factor that enhanced NK Activity (Kobayashi M et al 1989). IL-12 is capable of synergising with IL-2 to increase cytotoxic T lymphocyte responses, and also induces IFN-γ synthesis by resting human peripheral blood mononuclear cells *in vitro* (Trinchieu et al 1995). It is a heterodimeric molecule (IL-12 p70) composed of the p35 and p40 subunits (Wolf SF et al 1991). Its physiologic source *in vivo* is primarily antigen presenting cells including B-lymphocytes, monocytes/macophages, and dendritic cells. However, neutrophils and keratinocytes, have also been shown to produce IL-12 (Macatonia et al 1995). There are two distinct pathways for IL-12 production *in vitro* (Umetsu DT 1997, Kennedy MK 1996). The first pathway is direct induction of IL-12 in response to infectious pathogens. The second is a T cell-dependent mechanism in which CD40L expressed on T cells after activation interacts with its counterreceptor, CD40, on APCs, leading to production of IL-12. IL-10 is a potent negative regulatory cytokine capable of inhibiting many proinflammatory cytokines, including IL-12 (D'Andu' 1993). It should also be noted that IL-12 itself enhances IL-10 production from T cells (Gerosa F et al 1996, Jeannin P et al 1996). IL-12 receptors are expressed on T cells and NK cells, it has been shown in both mice and humans that Th1 cells express the IL-12 receptor β2 chain but Th2 cells do not. Consequently, this cytokine enhances the growth of activated T cells; NK cells and enhances cytotoxic T cells and NK activity. IL-12 promotes differentiation of mouse and human T cells into Th1 phenotype and inhibits the differentiation of T cells into IL-4 secreting cells (Manetti R et al 1993, Hsieh CS et al 1993, Manetti R et al 1994). IL-12 indirectly inhibits IL-4 induced human IgE responses by IFN-γ dependent and

independent mechanisms *in vitro*. Thus, IL-12 may play an important role in directing the development of Th1-like T cell responses against intracellular pathogens while inhibiting the development of Th2-like responses and IgE synthesis. The counter Th2 effects of this cytokine have been used in murine models of asthma were treatment with IL-12 during active sensitization reduced antigen induced influx of eosinophils in BAL fluid, inhibited IgE synthesis, and abolished antigen induced bronchial hyperresponsiveness (Kips JC et al 1996, Gavett SH et al 1995). These effects of IL-12 are largely mediated by IFN-γ (Bruselle GG et al 1997). The production of IL-12 and IL-12 induced IFN-γ release is reduced in patients with allergic asthma compared with normal subjects (Van der Pouw Kraan TC et al 1997). Interestingly, there is a reduction of IL-12 mRNA expression in airway biopsies from patients with allergic asthma compared with normal subjects. However, following treatment with oral corticosteroids, the levels of IL-12 mRNA increased and significant changes were observed in those with corticosteroid resistant asthma (Naseer T et al 1997). Also allergen immunotherapy results in an increase in the production of both IL-12 (Hamid QA et al 1997) and PGE_2 (Van der Pouw Kraan TC et al 1995, Panina-Bordignon P 1997).

Formerly called IFN-γ- inducing factor, IL-18 is a cytokine that plays an important role in the Th1 response, primarily by its ability to induce IFN-γ production in T cells and NK cells (Diarello CA et al 1999). IL-18 expression is observed in colonic specimens and isolated mucosal cell populations from patients with Crohn' disease, a prototypic Th1 mediated disorder has been observed (Pizarro TT et al 1999, J Immunol). Mice deficient in IL-18 have suppressed IFN-γ production despite the presence of IL-12. IL-18 is related to the IL-1 family in terms of both structure and function (Ghayur T et al 1997, Fantuzzi

G et al 1998). It is synthesized as a precursor molecule without a signal peptide, but requires the IL-1-converting enzyme (ICE, caspase-1) for cleavage into a mature peptide. Therefore inhibitors of ICE activity may limit the biologic activity of IL-18 and may be useful as Th1 immunosuppressive agents. In the presence of a specific inhibitor of ICE IL-12–induced IFN-γ from splenocytes was reduced by 85%. Using splenocytes from ICE-deficient mice, IL-12-induced IFN-γ was reduced 80%. Neutralizing anti-IL-18 IgG reduced IL-12-induced IFN-γ in splenocytes by 85%. These data demonstrate that endogenous, ICE-cleaved IL-18 significantly contributes to induction of IFN-γ by IL-12 (Fantuzzi G et al 1999). Nitric oxide may prevent IL-1β and IL-18 release from macrophages by inhibiting caspase-1 (Kim YM et al 1998). Human IL-1 receptor protein is a component of the IL-18 receptor (Torigoe K et al 1997). IL-18 receptors are expressed selectively on murine Th1 cells but not on Th2 cells (Xu D et al 1998). IL-18 and IL-12 have synergistic effects on Th1 development, which may be due to reciprocal upregulatiion of their receptors. Consequently, IL-12 is needed for IL-18-induced IFN-γ production and IL-18 induces IFN-γ only when its receptor is upregulated by IL-12 (Dinarello CA 1999). The culture of T cells with anti-CD3 and anti-CD28 in the presence of rIL-12 induced IL-18 receptor expression and IL-18-stimulated IFN-γproduction, which reached higher levels than that induced by IL-12 stimulation alone Combined stimulation of bone marrow-derived macrophages with IL-12 and IL-18 lead to the efficient production of IFN-γ protein, demonstrating the macrophage not only responds to IFN-γ but is also a potent IFN-γ producing cell (Munder M et al 1998). IL-4 is essential for Th2 development, while IL-12 induces Th1 development, which can be enhanced by IL-18. Paradoxically, IL-18 is also a potent coinducer of IL-13 in NK and T cells;

therefore, IL-18 may act as a strong coinducer of Th1 or Th2 cytokines (Hoshino T et al 1999). The combined treatment of murine T cells with IL-12 and IL-18 inhibits antigen-specific Th2-like cell differentiation, and consequently inhibits the development of airway symptoms in a mouse model of allergic asthma (Hofstra CL et al 1998). IL-12 and IL-18 also protects mice against cryptococcal infection in a synergistic manner by enhancing the local production of IFN-γ by NK and γδ T cells in the early phase of infection and by suppressing the production of IL-4 in lungs (Qureshi MH et al 1999). IL-18 is also important for the generation of protective immunity to mycobacteria, by inducing of IFN-γ expression (Sugawara I et al 1999).

1.4.2 The Th2 cytokines IL-4, IL-5, and IL-10

1.4.2.1 Properties of IL-4 and IL-13

IL-4 is produced by Th2 lymphocytes, certain populations of thymocytes, eosinophils and cells of the basophil and mast cell lineage. Crosslinking of CD40 ligand on human $CD4^+$ T cells from normal non-allergic subjects generates a co-stimulatory signal that increases IL-4 synthesis (Blotta MH et al 1996). Mast cells and basophils can produce IL-4 following engagement of the high affinity IgE Fc receptor (FcεRI) crosslinking in mast cells and basophil. IL-4, is a key cytokine to induce IgE synthesis. Interestingly, there is a requirement for IL-4 before Th2 differentiation can take place and a Th2 response develops. It is paradoxical the IL-4 should be required before Th2 differentiation can proceed and implies that *in vivo* a particular cell type may be responsible for priming Th2 differentiation. However, the primary source of IL-4 needed to mount an appropriate

allergen-specific Th2 response has not yet been identified. Possibly there may exist another population of IL-4-producing T cells that are the primary source of IL-4 at the onset of an immune response (Bendelac et al 1992; Arase et al 1993). Alternatively mast cells may provide this priming function. This phenomena has been clearly demonstrated using IL-4 knockout mice which were described to have an impaired Th2 type immune response as assessed by infection with Nipposstrongulus brasiliensis (Kopf et al 1993). In addition, levels of the Th1 cytokine IFN-γ were not altered in IL-4–deficient mice, and DTH reactions to infectious choriomeningitis virus appeared normal. These results provided the evidence that the Th2 response *in vivo* is critically dependent on the availability of IL-4.

Interestingly, the culture of naïve CD4+ T cells from αβ TCR transgenic mice in the presence of various APC which induced a Th2 response as assessed by the induction of IL-4 and by the inhibition of IL-2 and IFN-γ synthesis (Seder et al 1992, 1994; Hsieh et al 1992; Swain et al 1991; Tanaka et al 1993). It also promotes immunoglobulin synthesis by B lymphocytes and plays a central role in immunnoglobulin class switching of activated B lymphocytes to the synthesis of IgG4 and IgE in mice (Brunner T et al 1993). Interestingly, corticosteroids also enhance the capacity to induce IL-4 synthesis from CD4+ T cells (Blotta MH et al 1997). The IL-4 receptor is a multi-chain structure consisting of two chains, a high affinity IL-4 binding chain (α chain) which binds IL-4 and transduces its growth promoting and transcription activating functions and the IL-2R γ chain (the common γ chain) which amplifies signaling of the IL-4R. High affinity Il-4 receptors are abundant in activated B and T cells and is also present on haematopoietic

progenitor cells, mast cells, macrophages, endothelial cells, epithelial cells, fibroblasts, and muscle cells.

$CD4^+$ and $CD8^+$ T cell clones produce IL-13 in response to antigen specific or polyclonal stimuli. IL-13 shares many biologic activities with IL-4. This is due to the fact that there is a close similarity between IL-4 and IL-13 receptors. The IL-4R α chain, important for signal transduction, is a component of IL-13 R (Aman et al 1996). T cells do not express functional IL-13 receptors. Consequently, IL-13 in contrast to IL-4 fails to induce Th2 cell differentiation. However, IL-13 is a potent modulator of human monocyte and B cell function (Minty et al 1993). It has profound effects on human monocyte morphology, surface antigen expression, antibody dependent cytotoxicity, and cytokine synthesis (McKenzie et al 1993). Moreover, IL-13 is required for optimal induction of IgE synthesis, particularly in situations in which IL-4 production is low or absent. On the other hand, IL-13 inhibits proinflammatory cytokine and chemokine production in vitro and has potent anti-inflammatory activities *in vivo* (de Waal Malefyt et al 1993; Berkman et al 1995). Similar to IL-4, IL-13 decreases the transcription of IFN-γ and of IL-12 (Swain et al 1990; Le Gros et al 1990; Hsieh et al 1994). IL-13 diminishes monocyte glucocorticoid receptor binding affinity (Spahn et al 1996), activates eosinophils (i.e. induces the expression of CD69) and prolongs eosinophil survival (Luttmann et al 1996). IL-13 induces the expression of CD23 on purified human B cells and acts as a switch factor directing IgE synthesis, similar to IL-4. Interestingly, a potent receptor antagonist of IL-4 blocks the proliferation of B cells and IgE synthesis by antagonizing IL-13 action (Aversa et al 1993). Increased expression of IL-13 mRNA has been reported in the airway mucosa of patients with atopic and non-atopic asthma (Naseer et al 1997;

Humbert et al 1997). In asthma there is significant correlation between the eosinophil counts and the levels of IL-13. A cloned soluble fusion protein that specifically binds to and neutralizes IL-13 without affecting IL-4 has been shown to suppress mucus secretion, eosinophilia and bronchial hyperresponsiveness in a murine model of asthma (Wills-Karp et al 1998; Grunig et al 1998). Consequently, it is possible that IL-4 responsible for the initiation of Th2 responses and IL-13 plays a major role during allergic immune responses.

1.4.2.2 Properties of interleukin-5

IL-5 was first isolated from supernatants of activated murine spleen cells, which were shown to induce eosinophil colony formation from bone marrow cultures and was named eosinophil differentiation factor. IL-5 is produced by T lymphocytes and the increased expression of IL-5 mRNA has been demonstrated in $CD4^+$ T cells in asthmatic airways using *in situ* hybridization (Hamid et al 1991). Bronchoalveolar lavage $CD4^+$ and $CD8^+$ T cells can also secrete IL-5 (Till et al 1995). *In vitro*, IL-5 is chemotactic for eosinophils and *in vivo* eosinophilia as observed during helminth infections (Schweizer et al 1994) is abrogated by the administration of an anti-IL-5 antibody (Coffman et al 1989). Consistant with the hypothesis that asthma is associated with dysregulated Th2 response increased circulating levels of immunoreactive IL-5 have been measured in the serum of patients with an exacerbation of asthma and these levels fall with corticosteroid treatment (Corrigan et al 1993). In addition, IL-5 levels are raised in induced sputum following allergen challenge of asthmatic patients (Keatings et al 1997). IL-5 protein has also been localized by immunochemistry in mast cells in bronchial biopsy specimens of patients with asthma together with IL-4, IL-6 and TNF-α (Bradding et al 1994).

The human IL-5 receptor has been identified *in vitro* on eosinophils but not on neutrophils or monocytes. It consists of a heterodimer with two polypeptide chains, a low affinity binding α chain and a non-binding β chain shared with the IL-3R and GM-CSFR (Tavernier et al 1991). The expression of IL-5R is restricted to eosinophils and their immediate precursors. The number of cells in bronchial biopsy specimens from asthmatic subjects expressing IL-5 receptor is increased. Moreover, an increase in IL-5Rα expression by bone marrow progenitor cells ($CD34^+$) occurs following allergen challenge of atopic asthmatic subjects (Sehmi et al 1997).

IL-5 can influence the production, maturation, and activation of eosinophils. It acts predominantly at the later stages of eosinophil maturation and activation (Lopez et al 1988) and can also prolong the survival of eosinophils (Yamaguchi et al 1988). Administration of exogenous IL-5 causes eosinophilia in many *in vivo* models (Iwama et al 1992). However, IL-5 transgenic mice have large number of eosinophils with no evidence of degranulation and subsequent tissue damage (Dent et al 1990). Moreover, intratracheal administration of eotaxin leads to further eosinophil accumulation in the lungs with bronchial hyperresponsiveness, an effect not observed in wild type mice (Rothenberg et al 1996). IL-5 may cause eosinophils to be released from the bone marrow while local release of another chemoattractant, eg. eoxtain may be necessary to cause recruitment of eosinophils to the tissues (Collins et al 1995; Rothenberg et al 1997). Therefore, in addition to the effect of IL-5 in mobilizing eosinophils from the bone marrow, there is evidence for its effect as a regulator of eosinophil homing and migration into tissues in response to local chemokine release (Mould et al 1997). It is also likely that IL-5 and RANTES (Venge et al 1996) contribute to the eosinophilotactic responses

of BAL fluid from asthmatics during the pollen season. The involvement of IL-5 in the development of pulmonary eosinophilia is supported by the observation that pretreatment of mice with anti-IL-5 monoclonal antibodies can suppress allergen induced airway eosinophilia in mice (van Oosterhout et al 1993; Mauser et al 1995). Furthermore, in IL-5 *knockout mice* both allergen induced eosinophilia and airway hyperresponsiveness are abolished (Foster et al 1996). In patients with worsening asthma, systemic corticosteroids reduces the expression of IL-5 mRNA in the airways mucosa associated with an improvement in asthma (Robinson et al 1993).

1.4.2.3 Properties of interleukin-10

IL-10 was first described as a product of murine Th2 clones that suppressed the production of cytokines by Th1 clones in the presence of APC (Fiorentino et al 1989). In humans Th0, Th1, and Th2-like $CD4^+$ T cell clones, cytotoxic T cells, activated monocytes and peripheral blood T cells including $CD4^+$ and $CD8^+$ T cells have the capacity to produce IL-10 (Enk AH et al 1992; Spits et al 1992). Constitutive IL-10 secretion occurs in the healthy lung with the major source being the alveolar macrophage, however, the circulating moncyte elaborates more IL-10 than the alveolar macrophage (Berkman et al 1995). IL-10 is a pleiotropic cytokine that can exert either immunosuppressive or immunostimulatory effects on a variety of cell types. It is a potent inhibitor of moncyte/macrophage function, suppressing the production of a number of pro-inflammatory cytokines including TNF-α, IL1-β, IL-6, MIP-1α, IL-8 and the production of prostaglandin E2 (Firorentino et al 1991; deWaal Malefyt et al 1993; Niiro

et al 1994). One important mechanism by which IL-10 inhibits accessory function of macrophages/APCs for Th1 inhibition is by lowering expression of B7.1 (CD80) (Kubin et al 1994 J, Ding et al) MHC class I expression (de Waal Malefyt et al 1991) and IL-12 production (Murphy et al 1994). Accessory signals mediated by CD80 and CD86 by interacting with their receptor CD28 is essential for T cell activation. Expression of IL-10 by APCs may be a pathway for the induction of antigen specific tolerance. In conclusion, IL-10 inhibits the production of IFN-γ and IL-2 by Th1 lymphocytes and IL-4 and IL-5 production by Th2 cells by reducing CD28 stimulation. It has been proposed that this cytokine may be of therapeutic value in treating allergic asthma.

1.5 Inflammation

1.5.1 Allergic inflammation

Allergic reactions are the result of the production of specific IgE antibody to common, innocuous antigens. Such antigens normally enter the body at very low doses by diffusion across mucosal surfaces, and trigger a Th2 response. Naïve antigen-specific T cells are induced to develop into Th2 cells in the presence of an early burst of IL-4, which appears to be derived from a specialized subset of T cells. The allergen-specific Th2 cells drive allergen-specific B cells to produce IgE. The IgE binds to the high-affinity receptor (FcεRI) for IgE on mast cells, basophils, and activated eosinophils. IgE production can be amplified by these cells because upon activation they produce IL-4 and CD40L. Once IgE is produced in response to an allergen, re-exposure to the allergen triggers an allergic

response. Moreover, the resulting inflammation can be divided into early events, characterized by rapidly dispersed mediators like histamine, and later events that involve leukotrienes, prostanoids, cytokines, and chemokines, which recruit and activate particularly eosinophils, but also basophils. The late phase of this response can evolve into chronic inflammation, which is most clearly seen in allergic asthma.

1.5.2 Lipid mediators in allergic inflammation

Arachidonic acid is the obligate substrate for the biosynthesis of the eicosanoids, prostaglandin and leukotriene. Both posess a broad spectrum of biologic actions including effects on the tone of vascular and nonvascular smooth muscles cell motility, and glandular secretion.

1.5.2.1 The Prostaglandins

Prostaglandins (PG) are known be modulators or mediators of a variety of homeostatic biological functions in the body, in addition they are important mediators in inflammation and thrombosis. Arachidonic acid, a polyunsaturated fatty acid esterified to cell membrane glycerophospholipids, is released in response to various stimuli and then oxidized by lipoxygenase (LO), cyclooxygenase, cytochrome p-540 monooxygenase enzymes. During the cyclooxygenase pathway, prostaglandin H synthase (PGHS, cyclooxygenase), converts arachidonic acid to PGG_2, then reduces it to an unstable endoperoxide intermediate, PGH_2. In turn, PGH_2 serves as substrate for cell-specific isomerases and synthases to produce the PGD_2, PGE_2, $PGF_{2\alpha}$, PGI_2 (prostacyclin), and thromboxane (TX) A_2 (Figure 1.1). COX-1 is the predominant enzyme required for the

biosynthesis of PGE2 in the normal muse lung; COX-1 and COX-2 products limit allergic lung inflammation and IgE secretion and promote normal lung function. Moreover, airway inflammation can be dissociated from the development of airway hyperresponsiveness in COX-2 knock out mice (Stephen H et al 1999). Since the COX inhibitors exacerbate the development of an inflammatory exudate function in a rat model of pleurisy, the role of PGs in inflammation has become apparent. Recent evidences suggest that COX-2 may be pro-inflammatory during the early phase of a carrageenin-induced pleurisy, dominated by polymorphonuclear leukocytes but may aid resolution at the later mononuclear cells-dominated phase by generating an alternative set of anti-inflammatory PGs, PGD_2 and PGJ_2 (Derek W et al 1999). Also the COX-2 dependent arachidonic acid metabolites, especially possible PGE_2, are essential in the development and maintenance of intestinal mucosa homeostasis (Rodney D et al 1999).

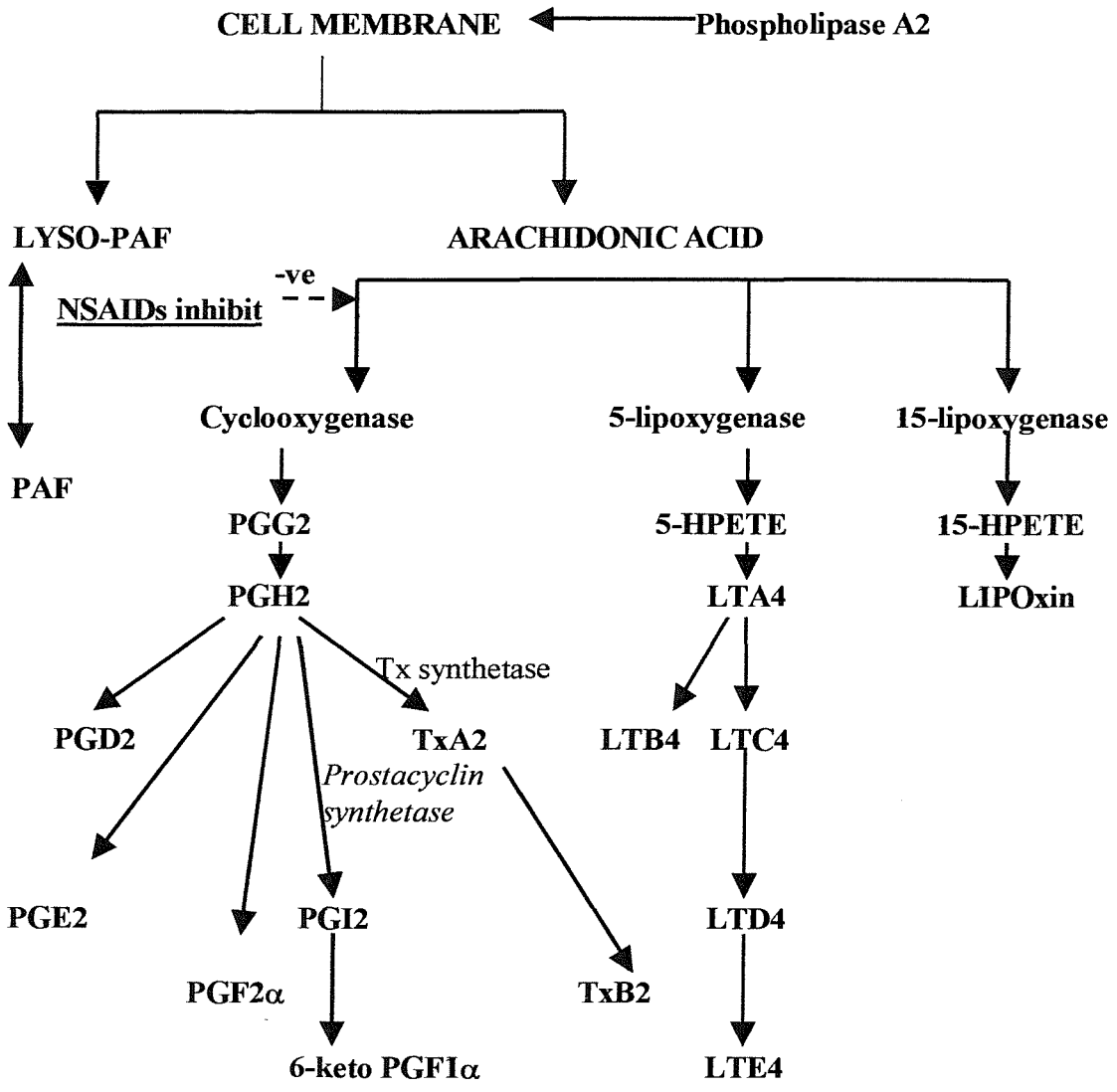


Fig.1.1

The spectrum of eicosanoids produced as a consequence of arachidonic acid metabolism

The prostanoids are most easily considered in two classes to evaluate their possible role in asthma. (i) The stimulatory prostaglandins, such as PGD_2 and $PGF_{2\alpha}$, TxA_2, which are potent bronchoconstrictors (Table 1.2). (ii) Inhibitory prostaglandins, such as PGE_2, PGI_2 which can reduce bronchoconstrictor responses and can attenuate the release of actetycholine from airway nerves (Table 1.2). How prostaglandins induce allergen-induced airway hyperresponsiveness still controversial. However, if they do, the most likely candidates are the stimulatory prostaglandins PGD_2, PGF2α or TxA_2.

Prostanoids	Mechanism of action	Function
PGD_2	DP receptor, TP-2 receptor Stimulating cholinergic nerve	Bronchoconstriction Increase airway hyperresponsiveness
$PGF_{2\alpha}$	TP-1 receptor Stimulating cholinergic nerve	Bronchoconstriction Increase airway hyperresponsiveness
TxA_2 (TxB_2)	TP-1 & TP-2 receptor Stimulating cholinergic nerve	Bronchoconstriction Increase airway hyperresponsiveness
PGE_2 tachyphylaxis	EP-1, EP-2, EP-3 receptor Inhibiting cholinergic nerve	Bronchodilatation Histamiine
PGI_2 (6-ketoPGF$_{1\alpha}$) tachyphylaxis	TI receptor Inhibiting cholinergic nerve	Bronchodilatation Histamine

Table 1.2
Role of Cyclooxygenase Products

1. PGD_2 is known to be released from stimulated dispersed human lung cells *in vitro* (Yen SS et al 1976) and from the airways of allergic human subjects that have been stimulated by allergen (Murray JJ et al 1986). Moreover, it has been proposed as a marker of mast cell activation in asthma (Liu MC et al 1990). PGD_2 elicits its biological actions through interaction with the PGD receptor, a heterotrimeric GTP-binding protein-couple, rhodopsin-type receptor that is specific for this prostaglandin (Hirata M et al 1994). PGD_2 causes bronchoconstriction, in part, directly through stimulation of specific receptor, TP1-receptor (Gardiner PJ et al 1989; Johnston SL et al 1995). Contraction also occurs indirectly through presynaptically stimulating release of acetylcholine from airway parasympathetic nerves (Beasley R et al 1987). Using a gene expression screen method, a gene encoding hematopoietic PGD synthase (hPGDS) was preferentially expressed in human Th2 but not Th1 clones (Kazuya et al 2000). Studies with anti-hPGDS mAbs confirmed the Th2-dominated expression of hPGDS protein. Therefore, PGD_2, which is well known as the major prostanoid produced by allergen-provoked mast cells, is also preferentially produced by antigen-stimulated human Th2 but not Th1 cells.

2. $PGF_{2\alpha}$ is highly effective at causing bronchoconstriction and airway hyperresponsiveness after allergen inhalation in human subjects. Since all contractile prostaglandins may act through a single TP1-receptor, differentiation of the relative importance of the contractile prostaglandins in causing asthmatic responses may prove be be extremely difficult. Newborn brain PGE_2 and $PGF_{2\alpha}$ concentration were significantly reduced by ibuprofen (COX-1 and COX-2 non-selective inhibitor), NS-398 and DUP-697 (COX-2 inhibitor) but not valerylsalicylate (COX-1 inhibitor). In newborn pigs treated

with DUP-697, NS-398, and ibuprofen, PGE_2 and $PGF_{2\alpha}$ receptor densities in brain microvessels were increased to adult levels. It indicates that COX-2 gernrated prostaglandins govern PGE_2 and $PGF_{2\alpha}$ receptor density and function in the cerebral vasculature of the newborn (Li DY et al 1997).

3. Thromboxane A_2 (TxA2) synthase catalyzes the conversion of prostaglandin H_2 to the unstable metabolite TxA2, which is a potent mediator of vasoconstriction and bronchoconstriction. TxA2 originally was described as being released by platelets but is known to be released from other cells, including macrophages and neutrophils (Higgs GA et al, 1983). It also causes platelet shape change and aggregation. As the biological half-life of TxA2 is very short (0.5-2.5 minutes), implicating TxA2 in disease processes has depended on measurement of its more stable metabolite thromboxane B_2 (TxB2) in biological fluid. The mechanism by which TxA2 causes airway hyperresponsiveness is not yet known, but possible mechanisms include presynaptic modulation of acetylcholine release or an effect plays on airway smooth muscle. The TxA2 synthetase inhibitor, OKY 046, administered orally, reduces acetylcholine airway hyperresponsiveness in stable asthmatic subject, while a lipooxygenase inhibitor had no effect in these subjects (Fujimura M et al 1986). Furthermore, the cellular localization of TxA2 synthase was examined by immunochistochemistry and in situ hybridization in human and rat lung tissues. Bronchial epithelial cells, bronchial smooth muscle cells, peribronchial nerve fibers, single cells of bronchus-associated lymphoid tissue, single cells located in the alveolar septum, and alveolar macrophages exhibited positive immunostaining for TxA2 synthase protein in lung tissue of both species. Also the pulmonary distribution of TxA2

synthase displays close similarity between rat and human lung tissues and matches well with the immunolocalization of COX-1 and COX-2 in this tissue (Ermert L et al 2000). In human monocytes, COX-1 activity appears to favour TxA2 production and COX-2 activity appears to favour PGE_2 production (Penglis PS et al 2000). Inhibition of TxA2 activity by the thromboxane receptor antagonists also inhibited the production of TNF-α and IL-1β (Caughey GE et al 1997). Therefore, therapeutically induced changes in eicosanoid ratios toward predominance of TxA2 may have unwanted effects in long-tern anti-inflammatory and anti-arthritic therapy (Penglis PS et al 2000).

4. Prostaglandin E2 (PGE2) is a potent lipid molecule with complex proinflammatory and immunoregulatory properties. PGE2 is a dominant cyclooxygenase product of airway epithelium and smooth muscle. It is produced in large quantities by monocytes/macrophages (Kurland JI et al 1978; Ferreri NR et al 1986) and fibroblasts (Elias JA et al 1987) and in lesser amounts by dendritic cells and follicular dendritic cell (Heinen E et al 1986; Phipps RP et al 1990). Many of these cells are professional APC, suggesting that PGE2 may have a role in modulating specific immune responsiveness. In vitro studies have shown that PGE2 inhibits many inflammatory events, including mast cell degranulation, leukotriene B4 production by alveolar macrophages, and eosinophil activation (Peters SP et al 1982; Christman BW et al 1990; Giembycz MA et al 1990). It has also been shown in vitro to have inhibitory effects on neurally mediated airway smooth muscle constriction (Walters EH et al 1984). Previous studies have provided evidence that PGE2 modifies lymphokine production and proliferation of T cells. It has been demonstrated that PGE2 inhibits mitogen-stimulated mouse and human T cell

proliferation (Goodwin JS et al 1983; 1977). It was reported that increased intracellular cAMP suppressed IL-2 and proliferation of Th1 cells, but did not affect IL-4 and proliferation of Th2 cells in mice (Novak TJ et al 1990; Munoz E et al 1990). Also working with murine cells, Betz and Fox subsequently demonstrated that PGE2 and the adenylate cyclase activator forskolin inhibited IL-2, and IFN-γ production by Th1 clones, but not IL-4 and IL-5 production by Th2 clones (Betz M et al 1991). On the other hand, PGE2 can shape the immune response by stimulating the production of IgE and IgG1 antibody by B lymphocytes while inhibiting production of Th1 cytokines (IL-12, IFN-γ) (Richard PP et al 1991). Recent analyses in nonhematopoietic cells have identified six PGE2 receptors (EP1, EP2, EP3α, EP3β, EP3γ, and EP4) (Ushikubi F et al 1995). This investigation examines quiescent B lymphocytes and reports that these cells express mRNA encoding EP1, EP2, EP3β, and EP4 receptors (Fedtyk ER et al 1996). The immunoregulatory functions of each receptor were investigated using small molecule agonists that preferentially bind EP receptor subtypes. The results showed that agonists bound EP2 or EP2 and EP4 receptor strongly stimulated class switching to IgE. Experiments employing inhibitors of cAMP metabolism demonstrate that the mechanism by which EP2 and EP4 receptors regulate B lymphocyte activity requires elevation of cAMP. Therefore, the antagonists of EP2 and EP4 receptors will be important for diminishing allergic and IgE-mediated asthmatic responses (Fedtyk ER et al 1996). The multiplicity of EP receptors leads to the multiplicity and complexity of PGE2 activity and, indeed, different concentrations of PGE2 can cause completely opposite reactions in the same tissues and different reactions in different species.

PGE2 main action on airway function is to relax airway smooth muscle (Kawakami U et al 1973; Mathe AA et al 1975). It is also known to inhibit early and late airway response to antigen inhalation (Pavord ID et al 1993) and to antagonize the contractile responses of other bronchoconstrictor agonists. In addition, PGE2 is extremely effective in inhibiting the release of acetylcholine from airway cholinergic nerves (Walters EH et al 1984). Since PGE2 aerosols inhibit the early and late asthmatic response to allergen inhalation and the bronchoconstriction to exercise or aspirin inhalation in sensitive asthmatic patients (Mastalerz L et al 1994; Melillo E et al 1994; Gauvreau GM et al 1999), some groups are using aerosolized PGE2 for the treatment of asthma.

5. PGI_2 is an unstable and hydrolysed rapidly to 6-keto-prostaglandin $F_{1\alpha}$. The half-live of this prostaglandin *in vivo* is typically 30 seconds, consequently measurement of PGI_2 production is usually following the concentration of the stable metabolite 6-keto-prostaglandin $F_{1\alpha}$, which detected first in vascular endothelial cells and macrophages. It is a potent inhibitor of platelet aggregation, relaxes vascular smooth muscle and increases cAMP concentration in platelets with and IC50 of 5 nM. Prostacyclin also plays an inhibitory role in the local control of vascular tone (Dusting GJ et al 1990). Moreover, it can mediate the bronchodilation effect on airway (Kawakami U et al 1973). Unfortunately, the absence of any prostacyclin receptor (IP receptor) antagonists has failed to obtain a therapeutic agent that would inhibit platelet activation without causing excessive vasodilatation. Also the value of agonist potency data has been lessened by complications relating to cross-species comparisons and to tissue-specific factors such as receptor-effector coupling. Prostanoid receptors have highly conserved amino acid

sequences and constitute a novel family of the rhodopsin-type receptor superfamily with seven transmembrane domains. Typically, specific (^{3}H) iloprost binding was displaced by unlabelled prostanoids in the order of cicaprost (PGI_2 analogue) $\geq$ iloprost > PGE_1 > carbacyclin >> PGD_2 = PGE_2 = 11a-carba-9_α, 11_α-thia-TXA_2 (STA_2) >$PGF_{2\alpha}$. These results confirm that PGE_1 is a moderately potent IP receptor agonist. In the prostacyclin receptor-deficient mice ($IP^{-/-}$), their susceptibility to thrombosis is increased, and their inflammatory and pain responses are reduced to the levels observed in indomethacin-treated wild-type mice (Takahiko M et al 1997). It showed that prostacyclin is an antithrombotic agent *in vivo* and provide evidence for its role as a mediator of inflammation and pain. Loss of the functional IP receptor in $IP^{-/-}$ mice was verified by the inability of a prostacyclin analogue, cicaprost, to inhibit ADP-induced platelet aggregation. Furthermore, intravenous injection of cicaprost caused hypotension in wild-type mice but not IP-deficient mice. On the other hand, intravenous administration of PGE_2 caused transient hypotension in both wild-type and IP-deficient animals, which further indicate that a single type of IP receptor mediates the actions of prostacyclin in both platelets and vascular smooth muscle. To use the acetic acid-induced writhing test to examine the involvement of IP in inflammatory pain, prostacyclin caused the writhing response in 60% of the wild-type mice and in none of the IP-deficient animals, whereas PGE2 induced the response in less than 25% animals in both type (Takahiko M et al 1997). In conclusion, prostacyclin not only plays the antithrombotic role but also is a mediator of inflammatory swelling and pain.

6. Prostaglandin J_2 (PGJ_2) and its metabolites 15-deoxy-deta prostaglandin J_2 (15d-PGJ_2) are naturally occurring derivatives of PGD_2 that have been suggested to exert anti-inflammatory effects *in vivo*. 15d-PGJ_2 is a high-affinity ligand for the peroxisome proliferator-activated receptor γ (PPARγ) and has been demonstrated to inhibit the induction of inflammatory response genes, including inducible NO synthase and TNF-α, in a PPARγ dependent manner. PGD_2 metabolites, PGJ_2 and 15d-PGJ_2, are major products of arachidonic acid metabolism in macrophages. PPARγ is markedly unregulated in activated macrophages, it can inhibit the expression of the inducible nitric oxide synthase, gelatinase B and scavenger receptor A genes in response to 15-PGJ_2 and synthetic PPARγ ligands. Therefore, disease such as atherosclerosis and rheumatoid arthritis in which activated macrophages exert pathogenic effects, PGJ_2 metabolite and synthetic PPARγ ligands may have therapeutic value. On the other hand, two mechanisms appear to be associated with anti-inflammatory actions of both 15d-PGJ2 and PPARγ agonists: 1) the direct inhibition of cytokine-and exdotoxin-stimulated iNOS and IL-1 transcription and 2) the inhibition of IL-1 signaling, an event associated with PPARγ induced activation of the heat shock response (Maggi LB Jr et al 2000). Moreover PGJ_2 inhibits platelet aggregation with an IC50 of about 5-10 nM and it has been shown to have anti-mitotic and anti-proliferative effect on a variety of cultured normal cells and tumor cell lines.

1.5.2.2 Properties of COX-1 and COX-2 isoforms

COX is a cytochrome b 'like' heme protein enzyme complex associated with the endoplasmic reticulum. It is now recognized that there are two related but distinct gene

products that possess COX activity termed COX-1 and COX-2. COX-1 is expressed constitutively in most tissues, and COX-2, whose expression is inducible and in a limited repertoire of cells, notably in monocytes, macrophages, neutrophils, and endothelial cells (Hla T et al 1992, Niiro H et al 1997). In contrast, COX-2 is not expressed by mature T lymphocytes (Smith WL et al 1996, Mosferrer JL et al 1994). Among the stimulants of COX-2 induction are bacterial lipopolysaccharides (LPS), growth factors, cytokines, and phorbol esters (OSullivan MG et al 1992, Jones DA et al 1993, Evett et al 1993). Increased expression of COX-2, but not COX-1, has been demonstrated in rheumatoid synovial tissues in vivo. Conversely, expression of this isoform is inhibition by glucocorticoids and by the IL-4 and IL-10 (Masferrer J et al 1990, Mertz PM et al 1994, Dworski R et al 1997).

1.5.2.2.1 Properties of COX-1

During the past decade investigators have identified two COX enzymes (COX-1 and COX-2) encoded by separate genes, located on human chromosome 9 and 1, respectively (Kraemer SA 1992; Fletcher BS 1992). The two genes for the two isoforms are differentially regulated (Funk CD 1991; Morham SG 1995). COX-1 is a continuously transcribed stable message, providing constant levels of enzyme. This is thought to be important in the gastric mucosa where the prostaglandin maintains the gastrointestinal mucusa integrity and limits acid secretion. COX-1 can also maintain the platelet normal aggregating function by the formation of thromboxane A_2 (TXA2), a platelet-activating and vesoconstricting eicosanoid, so gastrointestinal bleeding can be a problem following prolonged use of the COX-1 blocking agent (Table1.2).

COX-1	COX-2
Constitutive	Inducible
Present under basal conditions in, stomach, intestines, kidney, prostate, lung, liver, spleen, other tissue	Present under basal conditions in brain, kidney, platelets, testicles, uterus, tracheal epithelial cells
Housekeeping role	Expression enhanced by endotoxin, Cytokines (IL-1, TNF-α)
Regulates normal renal and gastric function and vascular homeostasis	Up-regulated at inflammatory sites: macrophages, synoviocytes
Inhibited by NSAIDs	Inhibited by glucocorticoids, IL-4, IL-13 NSAIDs and COX-2 slective inhibitors

Table 1.3
Comparison of COX-1 and COX-2 isoforms

1.5.2.2.2 Properties of COX-2

COX-2 is the product of an 'immediate-early' gene that is rapidly induced and tightly regulated during inflammation and other pathologic processes (O'Sullivan MG 1992; Masferrer JL 1994). COX-2 is 60% identical to COX-1, and the COX-2 isozyme appears to have roughly the same affinity and capacity to convert arachidonic acid to prostaglandin (Smith WL 1995). In animal models of inflammatory arthritis, COX-2 increases in parallel with prostaglandin production and clinical inflammation (Sarro H 1992). *In vitro* experiments have revealed stimulation of synoviocytes, endothelial cells, chondrocytes, osteoblasts and monocytes/macrophages with the proinflammatory cytokine IL-1 and TNF-α, increase COX-2 expression (OSullivan MG 1992; Ristimaki A

A 1994; Onoe Y 1996). Another distinguishing characteristic of COX-2 is, decreased expression in response to glucocorticoids (Anderson GD 1996) (Table1.2). Dexamethasone, could inhibit COX-2 induced in macrophages, but had no effect on basal production of prostaglandins. As COX-1 is localized in the endoplasmic reticulum (ER), COX-2 localized to the ER and nuclear membrane. It has been reported that COX-1 and COX-2 use different pools of arachidonate that are mobilized in response to different cellular stimuli for prostaglandin synthesis (Murakami M 1994; Reddy ST 1994).

1.5.2.3 Inhibitors of cycloxygenases: Nonsteroidal Anti-inflammatory Drugs (NSAIDs)

1.5.2.3.1 Clinical use of NSAIDs inflammatory disease

Over 35 million NSAID prescriptions and billions of over-the-counter aspirin and NSAID preparations are sold annually in the United States and greater than 1% of the US population uses these drugs on a daily basis.

Salicylic acid was chemically synthesized in 1860 Germany and was used as an antiseptic and an anti-rheumatic (Vane JR 1990). Almost 40 years later, aspirin was developed. Soon other drugs having similar action to aspirin were developed. The anti-inflammatory actions of NSAIDs are due to inhibition of COX-2 whereas the unwanted side effects are due to inhibiting COX-1. Aspirin, indomethacin and all aspirin like drugs can damage the stomach lining, causing bleeding, ulcers and damage the kidney. Also there were a dose-dependant inhibition of prostaglandin formation by the different NSAIDs, for instance, Indomethacin being the most and Sodium salicylate the least, potent. The identification of

selective inhibitor of COX-2 lead to advances in the treatment of inflammation without having the COX-1 induced toxicity. Moreover, selective COX-2 inhibitors bind reversibly to the enzyme. In contrast, aspirin, Indomethacin and other non-selective COX inhibitors transfer acetyl side groups to COX and inactivate it.

1.5.2.3.2 Clinical use of COX-2 Selective Inhibitors

A number of selective COX-2 inhibitors have been developed. Clearly, selective blockade of pain and inflammation without deleterious effects on diverse homeostatic functions is highly desirable. COX-2 is not only expressed in rheumatoid arthritis but also expressed constitutively in the kidney and brain, ensuring normal development and maturation of these organs. COX-2 may lead to increase renin secretion during sodium restriction in rats and, in the brain, may be associated with beta-amyloid protein deposition in neuritic plaque of Alzheimer disease. In addition, COX-2 is thought to be instrumental in supporting adenomas and colon tumors. COX-2 specific inhibitors have some activity on COX-1 and can cause inhibitory effects on gastric PGE_2 synthesis. Thus, for clinical use all NSAIDs including COX-2 selective inhibitors should be used cautiously.

1.5.2.4 Effects of prostaglandin on the immune response

The prostaglandins normally maintain a balance in the airways; both PGD2 and TxA2 are bronchoconstrictor, whereas PGE2 and prostacyclin are bronchoprotective (Wenzel SZ 1997). The PGE2 inhibits IL-2 and IFN-γ production by mitogen stimulated human peripheral blood lymphocytes and CD4+ T cells, although IL-4 production is not affected

and IL-5 production is even up-regulated (Snijdwint FG 1993). Therefore, PGE2 inhibits production of Th1 lymphokines but not of Th2 lymphokins (Belz M 1991). PGE_2 has been shown to promote Th2-like cytokine secretion profile in murine and human CD4+ T cells by inhibiting the production of IL-2 and IFN-γ and up-regulating the production of the IL-4 and IL-5 in a dose-dependent way (Hilkens CM 1995). PGE2 also inhibits IL-2 and IL-4 dependent proliferation of CTLL-2 and HT2 cells (Gurlo T 1999). PGE2 is a potent inhibitor of human IL-12 production and almost completely inhibits LPS-induced IL-12 production whereas IL-6 production was only partially inhibited. In contrast, the production of IL-10 was twofold enhanced at these conditions. However, the inhibitory effect of PGE2 on IL-12 production was not to be reversed by neutralizing anti-IL-10 antibodies (van der Pouw Kraan TC 1995). COX-2 has been found to be overexpressed in human lung cancer. Antibody directed against class II MHC molecules potentiated IgG2a production by LPS-stimulated B lymphocytes treated with PGE2 and IFN-γ (Stea SH 1992). Moreover, it has been proposed that PGE2 may play an important role in the modulation of the immune response to dietary antigen in the intestine (Rodney D 1999). Certainly the effects of prostaglandins on the inflammatory responses appear to be two-fold. COX-2 may be proinflammatory during the early phase of carrangeenin-induced pleurisy but may aid resolution at the later phase by generating an alternative set of anti-inflammatory prostanoids, PGD_2 and PGJ_2 (Derek W 1999).

PGE_2 is produced by several cells in the human airway, including epithelium (Churchill L 1999), smooth muscle (Haye-Legrand I 1988) and macrophages (MacDermit J 1994). It has a number of inhibitory effects in vitro, including inhibition of neurally induced

airway smooth muscle contraction (Ilo I 1990), mast cell degranulation (Peter SP 1982), LTB4 production by alveolar macrophages (Christman BW 1990), and eosinophil activation (Giembycz MA 1990). It has been shown to inhibit allergic inflammation in animal models (Rand J 1988) and the release of mediators after allergen challenge of passively sensitized human lung (Tauber AI 1973). These properties suggest that PGE2 may modulate the response to allergic and non-allergic bronchocontrictor challenge in asthma.

There is also evidence shown that PGE_2 attenuates allergen-induced airway hyperresponsiveness, and inflammation, when given immediately before inhaled allergen (Gaurean GM 1999). Inhaled PGE2 caused marked inhibition of the early and late response to allergen. The mean maximum fall in FEV1 from 0 to 2 hours after allergen was 37.8 and 7.7% with placebo and PGE2 pretreatment respectively and from 4 to 7 hours after allergen was 25.5 and 8.8% respectively (Pavord ID 1993). It has also been demonstrated that inhaled PGE2 markedly attenuates exercise bronchoconstriction in asthmatic subjects (Melillo E 1994).

1.5.2.5 The leukotrienes

Arachidonic acid may be metabolized by 5-lipoxygenase enzymes to yield unstable hydreoperoxyeicosatetraenoic acids (HETE) which is an intermediate in the production of leukotriene A4 (LTA4) (Dixon RA 1990). This product is relatively unstable and converted to LTB4 in the presence of LTB4 synthase enzyme in human leukocytes, erythrocytes and plasma. Under the control of LTC4 synthase, the LTA4 can convert to the sulfidopeptide LTC4 (Lam BK 1994). Sequential enzyme-mediated elimination of

glutamic acid and glycine form LTD4 and LTE4 respectively. Because LTC4, LTD4 and LTE4 all contain the amino acid cysteine, they are collectively referred to as cysteinyl leukoctrienes that were previously termed slow reacting substance of anaphylaxis (SRSA).

The leukotrienes exert their biologic action by binding to and activating specific receptors. Two subtypes of the receptor for cysteinyl leukotrienes, CysLT1 and CysLT2, have been identified (Coleman RA 1995). The receptor for the LTB4 is B leukotriene receptor (BLT) (Yokomizo T 1997). Most of the actions of the cysteinyl leukotrienes are mediated by the CysLT1 receptor (Piper PJ 1983; Serhan CN 1996). These actions include the contraction of human smooth muscle. Chemotaxis and increased vascular permeability (Drazen JM 1980; Coles SJ 1983). In human lung tissue *in vivo*, LTC4 or LTD4 have an equal capacity to stimulate smooth-muscle contraction by acting CysLT1 receptors. The CysLT2 receptor mediates contraction of pulmonary vascular smooth muscle, although the action is less well defined than those mediated by the CysLT1 receptor. The BLT receptor predominantly mediates chemotaxis (YokomiZ T 1997). Hence, one of the chief reasons for the interest in SRSA was its ability to stimulate smooth muscle contraction by a nonhistaminergic mechanism. It proved to be a potent stimulator of smooth muscle in animal and human tissue *in vitro* and *in vivo* (Drazen JM1979; Dahlen SE 1980). In addition to their potent brochoconstrictor properties, leukotrienes and other products of the 5-lipoxgenase pathway induce pulmonary tissue oedema (Hui KP 1991; Wasserman MA 1995) and migration of eosinophils (Laitinen LA 1993; Munoz NM 1995) and can stimulate airway secretion (Coles SJ 1983; Piacentini GL 1991). The leukotrienes also stimulate proliferation of both smooth muscle and

various hematopoietic cells (Cohen P 1995; Porreca Z 1996), these observations provide further evidence of a potential role of leukotriene in altering the biology of the airway wall in asthma.

1.5.2.6 Aspirin-induced Asthma and Rhinitis (AIAR)

Aspirin sensitive asthma and nasal polyposis were reviewed by Widal et al in 1992. The precipitation of asthma attacks and naso-occular reactions by aspirin are due to the suppression of cyclooxgenase and decline the prostaglandin production. It has been proposed that inhibition of COX activity by NSAID deviates the arachidonic acid metabolism through the uninhibited 5-lipoxygenase pathway and lead to the formation of the bronchoconstrictors sulphidopeptide leukotreines (LTC_4, LTD_4, LTE_4) or chemotactic products, LTB_4 or HETES. The general accepted prevalence of aspirin sensitivity is between 10% to 20% for all asthmatics. If nasal polyps and sinusitis are also present, the incidence increase is 34%. Patients can only be identified as aspirin sensitive after they have experienced a respiratory reaction to aspirin or NSAIDs, therefore, only ASA oral challenge and Lysine-aspirin inhalation challenge are used to identify this patient group. Normally, greater than a 20% decrease in FEV_1 associated with nasoocular reactions after the challenge represent a positive test. And typically, aspirin respiratory disease is acquired in adulthood with rare onset during early childhood. Corticosteroid continues to be the mainstay of therapy. It stimulates synthesis of phospholipase A_2 inhibitor protein (lipocortin) and blocking mRNA synthesis and post-transcriptional expression of phospholipase A_2 inhibition that offer significant therapeutic effect to AIAR. All AIA patients can undergo desensitization to aspirin until the patient can tolerate 650mg without any reaction. The mechanism is due to acetylation and

downregulation of phospholipase A_2 activity. However, poses two important questions. First, what is the role of prostaglandin in the regulation of pulmonary T helper cells as they are the critical cells responsible for the mucosal immune responses? Second, is the COX-2 selective inhibitor suitable for use in atopic or asthmatic patients?

1.5.3 Allergic inflammatory responses developing in response to inhaled antigen

1.5.3.1 The T cell response

Only a transient activation of the T cells in the lung has been observed in response to soluble antigens delivered intra-nasally. In animal models of allergic airway inflammation, it has been demonstrated that antigen challenge results in cytokine production in lung and cellular recruitment. IL-4 and IL-5 have been implicated in the recruitment of eosinophils and airway hyperreactivity. However, in the lung immunological suppressor mechanisms are operative, and at the antigen non-specific level, alveolar macrophages have been demonstrated to efficiently inhibit T cell activation by the production of mediators.

It has been suggested that allergic asthma, characterized by chronic airway inflammation and hyperreactivity, is driven by Th2 predominant responses in the lung. Mice in whom the IL-4 gene has been inactivated by homologous recombination do not develop a pulmonary eosinophilia, presumable as a consequence of their inability to develop a Th2 response (Kopf M et al 1993). Administration of anti-IL-4 antibody during the period of aerosol challenge abrogates the airway hyperreactivity but has no effect of the recruitment of eosinophils to the lungs (Corry DB et al 1996). Conversely, administration

of anti-IL-5 antibody suppresses the recruitment of eosinophils but has no effect on airway reactivity (Nakajima H et al 1992). IL-4 produced during immunisation is a prerequisite for generating airway hyperreactivity to inhaled antigens. Either IL-5 or eosinophils were involved in mediating increased airway hyperreactivity. Similarly, IL-5-deficient mice do not develop a pulmonary eosinophilia following aerosol exposure (Foster PS et al 1996). In contrast to the previous findings, the airways of normal mice become hyperreactive to β-methacholine, following challenge with aeroantigen, a response that is not evident in IL-5 deficient mice (Foster PS et al 1996). Pulmonary eosinophilia and airway hyperresponsiveness can be inhibited by the administration of IL-12 (Gavett SH et al 1995), perhaps as a consequence of IL-12 suppressing the expression of Th2 cytokines by inducing IFN-γ expression. IFN-γ administered in the form of an aerosol or perienteric injection inhibits eosinophil recruitment to the lungs (Nakajima H et al 1993, Lack G et al 1994). Local administration of IL-12 or IFN-γ may provide a novel immunotherapy for the treatment of pulmonary allergic diseases such as allergic asthma.

CD4+ T cells and Th2-derived cytokines are essential for the development of eosinophilic lung in inflammation, especially IL-4 and IL-5 which play an important role in initiating and sustaining an asthmatic response by regulating the production of IgE and the growth, differentiation and recruitment of mast cells, basophils and eosinophils. In addition, it has been demonstrated that the Th1-associated cytokines, IL-12 and IFN-γ, can inhibit allergic inflammation. Therefore, the ability of Ag-specific Th1 and Th2 cells to modulate allergic lung inflammation has been tested by passively transferred Ag-specific Th1 and Th2 cells either in SCID mice or Ag-immunised BALB/c mice

Randolph DA et al 1999, Hansen G et al 1999). However, it has indicated that Ag-specific CD4+ Th1 cells may not be protective in Th2-mediated allergic disease and asthma; rather they may augment the significant inflammatory responses.

Co-stimulatory signals have been found to play an important role in regulating Th1 and Th2. T cell activation in addition to TCR ligation requires the interaction of CD28 on T cells with its co-stimulatory ligands CD80 and CD86. CTLA-4 also binds both costimulatory ligand but serves to inhibit T cell responses. Using a mutant form of CTLA-4-Ig termed Y100F-Ig, which binds to CD80 but not CD86; it was observed that CD80 co-stimulation was not necessary for the induction of Th2 responses but rather for maintenance or amplification of lung inflammatory responses in mice (Harris N et al 1997). Using an ovalbumin airway immunization protocol, the selective blockade of CD80 had no effect on the systemic induction of Th2 responses but inhibited the infiltration of eosinophils into the lungs of intra-nasally challenged mice (Narris N et al 1997). These experiments are important since they implicate components of allergic inflammation that influence the airway function.

1.5.3.2 The B cell response

The level of serum IgE is a critical difference between atopic and normal individuals. Atopic asthmatic patients produce high levels of IgE in response to allergen, whereas the normal people do not. IgE antibody recognition of Ag elicits immediate hypersensitivity reactions. Two types of IgE receptors have been identified: high-affinity receptor FcεRI and low-affinity receptor FcεRII (Sutton BJ 1993, Gould H et al 1991). FcεRI is

expressed on mast cells, basophils and DCs, while FcεRII is expressed on B cells, macrophages and eosinophils. FcεRI polypeptides contain one α, one β and two identical γ chains. The α chain mediates binding of IgE and γ chains mediate the coupling of FcεRI to and intracellular signaling pathway.

The humoral response to inhaled antigen is initiated in the draining lymph nodes of rats but subsequently moves to the lung tissue (Sedgwick JD et al 1983, McMenamin C et al 1992). Antigen-specific antibody IgE, IgG1 and IgG2a can be detected in serum or BAL. The role of B cells and antigen-specific antibody responses has been examined by studying the histopathologic and physiologic responses of B cell-deficient mice and wild-type mice (MacLean JA et al 1999). Following systemic immunization and airway challenge with OVA, there are no significant differences in either the pathologic or physiologic responses in the B cell-deficient mice compared with wild-type mice. These include airway hyperresponsiveness, pulmonary inflammation and infiltration of the lungs with activated T cells and eosinophils. These data indicate that B cells and antigen-specific antibodies are not required for the development of airway hyperresponsiveness, eosinophilic pulmonary inflammation and chemokine expression in sensitized mice following aerosol challenge with antigen.

The production IgE to aerosol antigens occurs within 10 days and this response is dependent on CD4+ T cells. Following repeated intra-nasal exposure, the levels of IgE in the serum fall, which is not associated with CD8+, γδ T cells or IFN-γ (Brian W et al 1998). However, in some other systems this appears to coincide with the induction of CD8+ γδ or CD8+ T cells that produce IFN-γ (McMenamin C et al 1993, 1994). The loss

of IgE production on prolonged aerosol exposure may be primarily a consequence of immune deviation caused by a shift in cytokine profile from IL-4 to IFN-γ, rather than induction of tolerance (Renz H et al 1994).

1.5.3.3 The inflammatory response

Chronic airway mucosal inflammation is a characteristic of asthma and plays a role in the generation of airway hyperresponsiveness. Many pathophysiological manifestations of asthma are associated with airway infiltration by activated mast cells, eosinophils, neutrophils and lymphocytes. Infiltration of CD4+ T cells and eosinophils is a central feature of the inflammatory process and correlates with disease severity (Corrigan CJ et al 1990). T cells have also been suggested to play a role in orchestrating the inflammatory response, through the production of cytokines, which are known to prime and activate other cell types, stimulate eosinophil maturation, activation and survival, or to modulate the expression of adhesion molecules. Therefore, the use of animal models to prevent both lung eosinophilia and bronchial hyperreactivity has been intensively studied.

Asthma is associated with an increased number of eosinophils in blood, sputum and BAL. The extensive infiltration of eosinophils into the airways of patients who have died of acute, severe asthma, indicate that eosinophils play a central role in the inflammatory response in this disease. Eosinophils are capable of responding to a variety of chemoattractant factors, including C5a, LTB4, PAF, IL-5 and IL-2 (Rand TH et al 1991). IL-16 is a 14kDa protein that is released from CD8+ T cells after exposure to histamine and has been shown to interact with the CD4+ protein, which is expressed by both T cells

and eosinophils. IL-16 has been found in lavage fluid and promotes eosinophil chemotaxis (Rand TH et al 1991). The survival of eosinophils in the airways is dependent on numerous factors, most notably the presence of the cytokines IL-3, GM-CSF and IL-5 (Tai PC et al 1991). T lymphocytes are a likely source of IL-5, whereas GM-CSF is likely to derive from both the monocyte/macrophage and the bronchial epithelium. Eosinophil survival in vitro is prolonged by culture with fibroblasts, as a consequence of their production of GM-CSF (Owen Jr WF et al 1987).

The advantages of using murine systems to study asthma include a detailed knowledge of murine immunology, availability of reagents, a short reproductive life cycle and well-characterized genetics (Tu YP et al 1995). Experiments using mice have greatly contributed to our knowledge of the cellular mechanisms that mediate allergic inflammation in the lung tissue in response to inhaled antigens. Exposure of mice to aerosolized antigens has been used as an approach to induce pulmonary allergic responses. Mice that have initially been immunized with OVA using an alum adjuvant, and subsequently rechallenged with aerosols of OVA, develop a pronounced pulmonary eosinophilia and airway hyperresponsiveness (Guvett SH et al 1994). The eosinophilic inflammation was dependent on CD4+ T cells and was evident in B cell-deficient mice (Korsgren M et al 1997, MacLean JA et al 1999) demonstrating that neither IgE nor B cells were required. The accumulation of eosinophils at sites of allergic reactions is associated with T cells, T cell-derived cytokines, chemokines and adhesion molecules. The traffic of eosinophils to sites of allergic responses is proposed to be regulated at distinct levels: (i) adhesion receptors, e.g., selectins and integrins, that mediate transient or firm adhesion to inflamed vascular endothelium; (ii) activating factors such as

cytokines, chemokines and chemoattractants that induce expression of selectins and their ligands.

1.6 Animal models of lung injury

1.6.1 Sepsis

The clinical manifestations of sepsis are usually the consequence of intense cellular interactions that often result in tissue injury and organ dysfunction (Ghosh S et al 1993). A primary organ targeted for injury is the lung. Septic conditions often lead to pulmonary complications, including adult respiratory distress syndrome (ARDS), impaired lung function, and death. The key to improving morbidity and mortality requires an in-depth understanding of endogenous mediators that both initiate and maintain or regulate the responses. Thus, the development of applicable animal models of sepsis may prove to be useful for determining the mechanistic and activation pathways that control the septic responses.

In a rabbit model of septicemia, C5a level has been correlated with the number of neutrophils migrating into the lung and with the degree of lung damage (Bergh K et al 1991). Moreover, inhibition of the complement system either by complement depletion or infusion of the soluble complement receptor significantly reduces neutrophil recruitment, adherence, and degranulation, thus blocking release of destructive proteases and oxygen radicals (Bone RC 1992). Using a Fischer rat model of endotoxemia, inhibition of complement obtained a similar result (Witthaut R et al 1994). A corresponding

respiratory burst in neutrophils is important in development of septicemia (Trautinger F et al 1991). Formation of toxic oxygen products from neutrophils was attenuated using cyclooxygenase inhibitors in a swine model of LPS-induced lung injury (Carey PD et al 1992). In rat model, in addition to the infiltrating neutrophils, alveolar and interstitial macrophages were both found to produce significant levels of reactive oxygen intermediates (Wizemann TM et al 1994). The release of reactive oxygen intermediates not only damages the local cell populations and connective tissue matrix, but have an activating effect due to increased generation of inflammatory and chemotatic cytokines. In animal models of sepsis, it has been shown that the ability to recruit neutrophils into lung requires expression of adhesion molecules on the vascular endothelium, but the recruitment also appears to rely on increased avidity of β2-integrin molecules for ICAM-1.

The production of inflammatory and chemotactic cytokines, TNF-α, IL-1, and IL-8 levels in plasma correspond with those of the clinical outcome of patients with the septic syndrome. In a study of endotoxin-induced mortality, an IL-1 receptor antagonist (IL-1ra) significantly reduced mortality in rabbits (Ohlsson K et al 1990). However, when recombinant IL-1 and TNF-α were injected into canine lungs, only TNF-α induced lethal injury (Eichacker PQ et al 1991), suggesting minor role for IL-1 in this model. IL-8 produced later in the septic response involved in induction of recruitment of leukocytes into the lung. Macrophage inflammatory Protein-2 (MIP-2) is homologue for IL-8 in murine. Inhibition of MIP-2 by antibody has demonstrated significant attenuation of mortality in mouse model of endotoxemia (Standiford TJ et al 1995). Moreover, inhibition of MIP-1α (Shanley TP et al 1991) and RANTES (Standiford TJ et al 1995) in

vivo attenuated leukocyte infiltration into the lungs and blocked the lethality associated with endotoxemia. IL-10 is a potent suppressive agent for monocyte/macrophages and downregulates TNF-α, IL-1, IL-6, and chemokines. Administration of anti-IL-10 antibody significantly increased lethality, TNF-α and MIP-2 levels, and myeloperoxidase content in lungs, suggesting a role for endogenous IL-10 in regulation of the endotoxin-induced responses (Standiford TJ et al 1995). Moreover, in mouse model, using a liposome transfer system 48 hr prior to an endotoxin challenge, IL-10 intratracheal gene transfer reduced pulmonary TNF-α levels by 62% and neutrohil infiltration by 55% and blocked the lethality of the response (Rogy MA et al 1995).

1.6.2 Allergic airway inflammation

Allergic pulmonary diseases include asthma, pulmonary eosinophilia, and bronchopulmonary mucomycosis (Kay AB et al 1988). The inflammation induced during allergic airway inflammation is mediated by the coordination of several immune-specific events. The initial induction of IgE-mediated mast cell degranulation, upregulation of adhesion molecules and production of inflammatory cytokines resulting in infiltration of specific leukocytes are orchestrated in a sequential manner. Immune responses associated with asthma produce histopathological features of a chronic, cell-mediated immune reaction characterized by the infiltration of the bronchial mucosa with neutrophils, basophils, eosinophils, macrophages, and lymphocytes (Kay AB et al 1992, Gleich Gj et al 1986).

Eosinophils are considered to be the cells chiefly responsible for the production of bronchial mucosal injury and bronchial dysfunction associated with the asthmatic (Corrigan CJ et al 1992). Also in atopic asthmatic inflammation, a corresponding production of Th2 cell-type cytokines (IL-4, IL-5) has been observed that correlates with disease intensity and eosinophil infiltration (Robinson DS et al 1992, Romagnani S et al 1991, Walker C et al 1991). Depletion of CD4+ T cells in a mouse model of allergic airway inflammation results in an abrogation of esoinophilia and a reduction in airway hyperreactivity (Gavett SH et al 1994), verifying the importance of T cells in allergic inflammation. A primate model of ascaris antigen-induced airway inflammation showed that ICAM-1 is an important adhesion molecule leading to eosinophil recruitment into the airway (Gundel RH et al 1992). In a Brown Norway rat model of ovalbumin-induced airway inflammation, anti-ICAM-1 treatment demonstrated significantly reduced airway responsiveness without a decrease in eosinophil or lymphocytes recruitment (Sun J et al 1994). Interestingly, treatment of the mice with anti-ICAM-1 and anti-LFA-1 did not inhibit eosinophil recruitment in the lung interstium, possibly suggesting species differences and/or the specificity of the antigen used to induce the response. Using rat and guinea pig models that are either sensitized with alum-precipitated ovalbumin and challenged with nebulized aerosols containing high concentrations of dissolved ovalbumin or challenged directly into the airway with a known airway irritant resulting immediate and delayed airway hyperreactivity, peribronchial inflammation, and associated late-phase eosinophil recruitment. In particular, IL-5 has been shown to be involved in the recruitment of eosinophils to the airways (Van Oosterhout AJ et al 1993). The use of mouse models of allergic airway inflammation has the advantage of defining mechanisms of inflammation, in part because of the availability of a wide array of

reagents. Studies have demonstrated a role for IL-4 and IL-5 in eosinophil recruitment and airway hyperreactivity (Brusselle G et al 1994, 1995, Nagai H et al 1993). The sensitization of mast cell-deficient mice demonstrated a decreased eosinophil as well as a diminished airway hyperreactivity response to allergen, suggesting an important mechanism in allergic responses mediated via mast cell activation. In a model, using Schistosoma mansoni soluble egg antigen to induce allergic responses in lungs, has demonstrated the requirement for IL-4 (Lukacs NW et al 1994) and TNF-α (Lukacs NW 1995) as early response cytokines for the inflammatory reaction in the lung and airway responsible for eosinophil recruitment. In addition, the SEA-induced model has identified MIP-1α (Lukacs NW et al 1995) and RANTES, which together mediated eosinophil recruitment and accumulation around and within the airway. Interestingly, MIP-1 and RANTES appear to have a specific effect on eosinophil recruitment. The use of the mouse models of airway inflammation appears to have many advantages over that of the other models of inflammation; however, as with all animal models, their relationship to asthmatic responses in patient populations has been questioned.

1.6.3 Pulmonary granuloma formation

Granulomatous inflammation is characterized by an accumulation of leukocytes around infectious or noninfectious agents (Knuckle SL et al 1994). The cellular constituents of granulomas include immune as well as nonimmune cell populations including macrophages, lymphocytes, mast cells, epithelial cells, and fibroblasts. In addition, multinucleated giant cells are often common in delayed-type hypersensitivity granulomas; eosinophils are often found in chronic parasite-induced granulomas. The residual fibrosis

that accompanies the resolution phase of the granuloma may result in irreversible tissue damage and organ dysfunction. Environmental or industrial agents can induce noninfectious foreign body-type granulomas, whereas tubercle bacilli, fungi, viruses, or parasites induce infectious granulomas.

Reactive oxygen metabolites appear to play an important role in the development of granulomas. Studies of TNF-α production during schistosome egg and tuberculoid granuloma formation indicate that the production of inflammatory cytokines correlates well with granuloma development (Kindler V et al 1989, Joseph AL et al 1993). Most convincingly, *in vivo* TNF-α blockade inhibits development of mycobacterial granulomas (Tumang MC et al 1994), whereas exogenous administration of TNF-α into nonresponsive SCID mice reconstitutes the ability of these mice to mount a circumovum schistosome egg granulomatous response (Amiri P et al 1992). Also, regulation of IL-1 is crucial for controlling the size of granulomatous lesions (Chensue SW et al 1992, Curry AJ et al 1992). Other cytokines such as IFN-γ, IL-4, IL-10, and IL-12, have a striking ability to regulate the granuloma formation, leukocyte infiltration, chemokine production, and possibly end-stage fibrosis of the developing lesions. In intracellular infections, the ability of produce Th1 cytokines dictates the success of clearance of the agent and granuloma resolution (Huygen K et al 1992). The Th2-related cytokines are associated most closely with parasite-elicited responses, such as schistosomiasis (Grzych JM et al 1991) and leishmaniasis (Chakkalath HR et al 1994). The production of Th2 cytokines during intracellular infections may promote prolonged granuloma formation and increased lesion size leading and exacerbated fibrotic responses. In an glucan-induced foreign body granulomatous inflammation in rat, a significant mononuclear cell infiltrate

has been associated with the expression and production of MCP-1 (Jones ML et al 1992, Flory CM et al 1993). The expression and production of MCP-1 were significantly attenuated when IL-1 and TNF-α were neutralized *in vivo*, suggesting the presence of cytokine cascades leading to the accumulation of mononuclear cells.

1.6.4 Immune complex-mediate lung inflammation

Acute lung injury as well as chronic disease, such as Wegner's granulomatous, sarcoidosis, and several other pulmonary and non-pulmonary diseases (SLE), may in part be linked to the presence of immune complexes (Shasby DM et al 1982). The injury that is induced by immune complex deposition in lung includes a vascular leak syndrome, significant recruitment of leukocytes, leukocyte activation, and damage of alveolar walls. Intrapulmonary deposition of IgG or IgA immune complexes in rats leads to acute lung injury to vascular and alveolar epithelium (Johnson KJ et al 1979, 1986). The deposition of IgG facilitates the local accumulation of neutrophils, whereas deposition of IgA immune complexes appears to activate residential macrophages. Regardless of the type of leukocyte associated with the injury, the damage induced during the inflammatory response appears to be mediated by toxic oxygen radical release from phagocytic cells (Johson KJ et al 1981, Mulligan MS et al 1991). In both the IgG and IgA immune complex models of lung injury, complement activation occurs. When rats with IgG or IgA immune complex-induced inflammation in lungs were pretreated with soluble complement receptor-1, a marked attenuation of the inflammatory damage was observed (Mulligan MS et al 1992).

In the IgG immune complex model, the activation and degranulation of migrated neutrophils and activation of residential macrophages releases multiple oxidants and proteases (serine proteases and metalloproteases), leading to structural damage of cells and connective tissue matrix. In the IgA immune complex model, the activation of resident pulmonary macrophage populations leads to the production of MCP-1 followed by the generation of oxidants and release of metalloproteases which in turn damage cells and connective tissue matrix. In a study of the IgG immune complex model of lung injury, the use of IL-10 was highly suppressive in a manner associated with reduced levels of BALF TNF-α as well as diminished influx of neutrophils (Mulligan MS et al 1993). In addition, administration of IL-4 also suppressed the IgG immune complex-induced inflammation.

1.7 Aim of the project.

The critical role that T cells play in the allergic inflammatory response in asthma is well documented but little is known as to how this response is regulated. Consequently, the delineation of how mucosal T cell responses are regulated forms a prerequisite to understanding of how chronic inflammation diseases of the airway develop. In our previous study, DO11.10 transgenic mice that expressing an OVA-specific TCR, were used to study pulmonary T cell responses to inhaled antigens. The observations demonstrate that clonal expansion of T cells in the lung compartment is prevented following the onset either Th1- or Th2-mediated inflammation. This form of immune

regulation, which appears as a selective defect in IL-2-driven proliferation, may serve to prevent the development of pulmonary lymphoproliferative responses. Since COX-2 responses to prostanoids production and has been proposed to promote immune regulation in the intestinal T cell responses, we plan to monitor the effect of NSAIDs on the production of prostanoids to inhaled antigens. Moreover, treatment of DO11.10 Th2-adoptive transfer BALB/c mice with a COX-2 inhibitor results in increased pulmonary eosinophilic inflammation in our primary study. We believe that targeting COX-2 and the underlying T cell response will provide opportunities to intervene in the inflammatory process in allergic asthma. Prostanoids produced in the lung following antigen inhalation influence the lung mucosal T cell responses are hypothesized.

Chapter Two

Materials & Methods

2.0 Materials and Methods

2.1.1 Animals

BALB/c mice were obtained from Charles River (Maidstone, UK). DO11.10 transgenic mice express a TCR recognizing OVA peptide 323-339 and backcrossed to BALB/c mice in the University of Texas Medical Branch facility were kindly provided by Dr. D.Y. Loh (Howard Hughes Medical Institute, St. Louis, MO). They were bred in a specific aseptic condition in a barrier facility. All mice used in these experiments were 4 – 8 weeks of age and were housed in our pathogen-free conditions with food and water provided ad libitum.

2.1.2 Media

CM-RPMI consisted of RPMI 1640 (Gibco BRL, Paisley, UK) supplemented with penicillin (50μ/ml), streptomycin (50μg/ml), 2mM glutamine, 2 mercapto-ethanol (5μM), 5mM HEPES and 5% foetal calf serum was used.

2.1.3 Antigen

Whole OVA

OVA peptide 323-339 (H-ISQAVHAAHAEINEAGR-OH) was purchased by Chiron Technologies. Peptide was dissolved in PBS and purified using Sephadex G10 column (Pharmacia Biotech). PBS was used to elute the peptide. The protein was quantified by

BCA protein assay (Pierce). The final concentration used for proliferation assay was 1μg/ml, by adding 50μl of 4μg/ml of OVA peptide into each well having a total volume of 200ul.

2.1.4 Antibody

The antibodies used in this study were the following :

Specificity	Clone	Isotype	Conjugated	Company
αβTCR	H57-597	Hamster IgG		ATCC, HB-218
DO11.10 TCR	KJ1-26	Mouse IgG	FITC	P. Marrack (Den Loh, USA)
CD3ε	2C11	Hamster IgG	FITC	ATCC, CRL-1975
CD4	GK1.5	Rat IgG2b	PE	Pharmingen
CD8a (Ly-2)	5367 YTS1634	Rat IgG2a		ATCC, TIB-211 M. Glinnie, Tenovus
Anti-IL-4	11B11	Rat IgG1		W. Paul
Anti-IL-12	Polycolonal	Rat		D. Presky (Hoff, LA Roche, USA)
Anti-IFN-γ	XMG1.2 HB170	Rat IgG1		ATCC, M. Glinne Tenovus
Anti-IFN-γ	XMG1.2	Rat IgG1	Biotin	Pharmingen
Anti-mouse IgE	R35-118	Rat IgG1		Pharmingen
Anti-mouse IgE	R35-72	Rat IgG1	Biotin	Pharmingen
Ia	M5/114 N22	Rat IgG2b		ATCC, TIB-120 M. Glennie, Tenovus
F4/80	C1:A3-1	Rat IgG2b	PE Biotin	P. Marrack (Den Loh, USA)

2.2 Preparation of OVA-specific $CD4^+$ Th1 and Th2 cells

OVA specific DO11.10 Th1 and Th2 lines were generated from pooled peripheral and mesenteric lymph node cells from DO11.10 mice. T cells with APCs were cultured at $5x10^5$ /ml and OVA peptide (0.5μg/ml). To generate Th1 or Th2 cells, either rIL-12 (5μg/ml, R+D Systems), anti-IL-4 mAb (11B11-10% hybridoma culture supernatant) or rIL-4 (2ng/ml, R+D Systems), anti-IFN-γ mab (HB170-10% hybridoma culture supernatant) were added to the culture. After the initial stimulation for 4 days, cells were washed, counted and re-stimulated under the same conditions used for the first stimulation at a cell concentration of $2x10^5$/ml) with the exception of additional rIL-2, (50μ/ml, CETUS) in both Th1 and Th2 cell lines. After 3 days cells were washed and any $CD8^+$ cells were depleted by panning. To pan petri dishes were pre-cultured the mouse anti-rat Abs 20μg/mg (Mar 18.1) with 10 ml PBS at 37C in the incubator overnight.Th1 and Th2 cultured cells were resuspended in 10 ml CM-RPMI. Th2 cells was tended to be more adherent than Th1 cells and were harvested with the aid of a cell scraper. Two ml culture supernate from the 5367 hybridoma (anti-CD8) was added to $100x10^6$ cells and incubated at 4C for 30 minutes. Cells were then washed with CM-RPMI once. Coated petri dishes were washed twice with PBS. Twenty-five x 10^6 Th1 or Th2 cells were added to each petri dish and incubated at room temperature for 1 hour. Non-adherent T cells were removed washed and used for adoptive transfer. The cytokine profiles of the Th1 and Th2 lines were confirmed, by direct examination of supernatants generated from these lines following anti-CD3 stimulation, using CTLL bioassay for IL-2 and ELISA for IL-4, IL-5, IL-12, IL-18, TNF-α and IFN-γ measurements.

2.3 Isolation of Lung Mononuclear Cells (LMC)

Lung tissue was cut into small pieces and washed extensively in CM-RPMI. The tissue was then incubated for 90 minutes at 37^0C in CM-RPMI containing 0.1% collagenase (Type IV, Sigma) and 0.01% hyaluronidase (Sigma). Shaking lung fragments vigorously half way and at the end of incubation period. After digestion, cells were filtered through 70um gauge cell strainers (Falcon) and washed in media by centrifuging at 438 g at 19^0C for 10 minutes.

The viable mononuclear cells were isolated over a Percoll density gradient (41.7% and 67.6%). To prepare the Percoll density gradient, the stock solution of Percoll (Pharmacia) was made by adding 2ml of 10X PBS to 20ml Percoll. The heavy (67.6%) and light (41.7%) density Percoll were made by mixing 10ml stock Percoll with 4.8ml and 14 ml CM-RPMI respectively.

One universal tube (Sterilin) of Percoll density gradients was prepared for lung tissue prepared from 3 mice. The digested lung tissue was resuspended in 14 ml of light density Percoll. The gradient of Percoll was made by first adding 7 ml of high density Percoll into a universal tube and 7 ml of light density Percoll containing cells was carefully layered over the high density layer. Then the tubes were spun at 800 g at 19^0C for 30 minutes. The lung mononuclear cells present at the interface were removed using a Pasteur pipette (Alpha Laboratories LTD) and were transferred into a new universal tube.

Cells were resuspended in CM-RPMI after washing it with HBSS (Life Technologies LTD) at 500 g at 19^{0}C for 10 minutes.

2.4 Isolation the Epithelial Cells from Trachea

The tracheas were dissected and removed from the mice. They were placed in a petri dish containing 2 ml CM-RPMI. The trachea was split longitudinally and cut into 5mm sections before putting it in 10 ml 37^{0}C pre-warmed CM-RPMI. The tracheal fragments were stirred for 2-3 minutes and then shaken vigorously for 20 seconds. This step was repeated again and the mixture passed through a strainer. Using a discontinuous Percoll density gradient as before (2.4) cells were separated. The top layer was collected, which contained the epithelial cells and fibroblasts, which were washed in HBSS and plleted by centrifugation at 500 g, 19^{0}C for 10 minutes. Cells were resuspended spun onto glass slides (cytospin 3, Shannon) and stained with giemsa to confirm the epithelial cells by the light microscopy.

2.5 Isolation of the Lymph Node Cells

Peripheral lymph nodes (brachial, axillary, and inguinal LNs) were taken from mice and pooled with mesenteric nodes and transferred to CM-RPMI. Lymph nodes were disrupted using two 23-gauge needles and the suspension then passed through a cell strainer. The

cell suspension was centrifuged at 503 g, 4^0C for 10 minutes. Cells were counted and resuspensed in CM-RPMI.

2.6 Anatomical Location of the Adoptive Transferred Cells by Flow Cytometer

Lungs or lymph nodes were removed from the experimental mice and the cells as prepared as described previously. The cells were counted and resuspended at 15-20x10^6/ml, then the B cells and macrophages were depleted by complement lysis method using M5-114 mAb and rabbit anti-mouse complement. The cells were counted and adjusted to a final concentration of 5x10^5/100ul. Cells were stained by adding monoclonal antibodies (Kj1-26-FITC, CD4-PE, CD-8-Biotin, PE-Avidin etc.) and incubating on ice for 30 minutes. The cells were washed twice with PBS staining buffer (PBS containing 5% foetal calf serum and 0.5% sodium aside). The cells were resuspended in 1ml PBS staining buffer and analyzed by the FACScan (Becton Dickinson), 50 ul of 4mg/ml PI solution (Sigma) was added to each tube. The live cells in FITC-labelled tubes were gated and 10000 events were measured

2.7 Adoptive Transfer of Cells

$CD4^+$ effector cells were transferred into histocompatible BALB/c mice by intravenous injection via the lateral tail vein. Tenx10^6 Th1 or Th2 cells in 200µl HBSS were adoptively transferred intravenously into each BALB/c recipient.

2.8 Animal Sensitization

Mice were exposed to aerosolized solutions of 0.5% ovalbumin solution (Grade V, Sigma, Poole, UK) for 20 minutes a day for 6 consecutive days using a Wright's nebulizer. On day 7, mice were sacrificed by using Diethyl ether inhalation anesthetized until dead. Bronchoalveolar lavage was performed and the lung cells peripheral lymph nodes cells were isolated and harvested for analysis.

2.9 Bronchoalveolar Lavage (BAL)

An open tracheotomy method was used. Lungs were lavaged through a tracheal tube by cannulating the trachea of the mice and injecting with 0.5ml PBS at 37^0C through the cannula and retrieving the lavage. This procedure was repeated three times for each mouse. The volume and number of cells in the BALF was assessed. The BALF was placed on ice and centrifuged at 503 g for 10 minutes at 4^0C. The BAL cells in each group were resuspended in 1 ml PBS and prepared for cytospin slide staining and

eosinopil peroxidase (EPO) analysis. Aliquots of BALF supernatants were kept at -80^0C for biochemical analysis.

2.9.1 BAL Cells Staining and Differential Count

BAL cells were cytospin onto glass slides using $5x10^5$ cells at 280 g (Cytospin 3, Shannon). Slides were air-dried and stained using modified Wright stain, differentiated by the same examiner blinded to sample identity counting at least 300 cells with a light microscope.

2.9.2 EPO Assay of BALF and Cytochemical Evaluation of Eosinophil infiltration into BAL

A colorimetric method was also used to determine the eosinophil peroxidase (EPO) activity in BALF. Routinely EPO measurements were made from both cells and supernatants. EPO assays were performed in 96 wells, flat bottomed ELISA plates (NUNC). 100 µl of undiluted cell suspension and a 1 in 3 serial dilution was used. Similarly, the cell-free BALF was used to measure released EPO using 100 µl for different group. 100 µl substrate solution, which comprised of 0.1mM orthophenylene diamine dihydrochloride (OPD, Sigma), 50mM Tris-HCl (BDH), 0.1% Triton X-100 (Sigma) and 1mM hydrogen peroxide (Sigma), was added. The plates were then incubated in the dark at room temperature for 30 minutes. Finally, 50 µl of 4M sulfuric acid (BDH) was used to stop the reaction. An automatic plate reader determined the

absorbency at 495nm. The results indicated the number of eosinophils present in the cell suspension and also the EPO released into the supernatant.

2.10 Cytokine Measurements

To measure cytokine production, cells were added to 24 well cluster plates (Costar LTD). Each well contained $2x10^6$ cells in 2 ml of medium. Some wells were precoated with KJ1-26 or 2C11 mAb (Typically 5μg/ml in PBS overnight at 37C). In some instances, cells were stimulated with OVA323-339 peptide (1μg/ml). Cultures were maintained for 48 h after which supernates were harvested and frozen (-20°C) prior to analysis. For IL-2 measurement, CTLL bioassay was used. TheIL4, IL-5, IL-12 and IL-18 levels were measured by ELISA.

2.10.1 IL-2 Measurement using the CTLL Bioassay

The IL-2 dependent cell line, CTLL was maintained in CM-RPMI with exogenous IL-2 (10U/ml) at 37^0C, 5% CO_2. CTLL cells ($5X10^3$/well) were added to a 96-flat bottom plate. To assay supernates, 50 μl of sample was added to wells and the well volume adjusted to 200 μl. To calibrate the assay recombinant human IL-2 (CETUS) was added to wells at known concentrations. The proliferation of the cells was determined by adding 1uCi of ^{3}H-thymidine to each well after 24 h. After a further 18 h, the plate was

harvested using the Dynaltech Harvester and the ^{3}H-TdR incorporation was measured by the β-counter.

2.10.2 Anti-IL12 and/or Anti-IL18 Effect on IFN-γ Production

Although T cells are the major source of IFN-γ, IL-12 and IL-18 act synergistically to increase IFN-γ production from murine bone marrow derived macrophages. After Th1 or Th2 cells were adoptively transferred to the BALB/c recipients. The mice were giving OVA aerosol challenge as described above for 6 days. Murine anti-IL12 and anti-IL18 were purchased from R&D System. Lung mononuclear cells were isolated after OVA challenge and stimulated by OVA peptide (1μg/ml) and with either anti-IL12, anti-IL18 alone or combined together for 48 hours. Supernatants were collected separately and prepared for the IFN-γ ELISA assay.

2.11 The Measurement of Cytokines by ELISA

2.11.1 Murine IFN-γ Assay

The IFN-γ assay was by antibody-sandwich ELISA. A primary capture Ab (HB170 ATCC) at 5μg/ml in carbonate buffer (1 mM, pH9.6) was used to coat a 96-well ELISA plate by incubating at 4^{0}C overnight. The next morning, excess solution was flicked off and the plate washed twice in PBS/0.1% Tween (250μl/well). The plate was pounded dry

after the last wash, then blocked using 200ul blocking buffer (1% BSA which had been heat-treated, Sigma) at room temperature for 2 hours. The plate was washed twice in PBS/Tween-20 ensuring it was thoroughly dry after the last wash. During the blocking period, samples or standards were thawed. Culture supernatants were diluted 1/10 using blocking buffer. Samples were added to the plate using 50µl/well and determinations performed in triplicate. Standands added were added at 50µl/well also in triplicate. The plate was incubated at 4^0C overnight and then washed the 4 times in PBS/Tween and shaken dry. Biotinylated anti-IFN-γ mAb (XMG1 Ab, PharMingen) at a concentration of 2.5 µg/ml in diluent buffer was added (50µl/well) and left at room temperature for 45 minutes. The plate was then washed 6 times in PBS/Tween and shaken dry. One hundred µl of Streptavidin-alkaline phosphatase (1:2000 dilution) (2.2µl in 11ml) was added per well and the plate incubated for 30 minutes at room temperature. The plate was washed with final buffer (Tris-saline, pH7.5) 5 times. An enzyme amplifying kit (Cat No: 19589-091 GIBCO BRL) was used to increase the sensitivity. Amplifier substrate (GIBCO BRL, Paisley, Scotland) was added (50µl of substrate per well) and incubated the plate at room temperature for 30 minutes. To complete the assay 50µl/well of stop solution (0.3M H_2SO_4) was added and the plate was read at 495nm using a microter plate reader.

2.11.2 Murine IL-4, IL-5, IL-12 and IL-18 Assay

To measure IL-4 or IL-5 in culture supernatants antibody pairs were used as follows:

IL-4: capture 11B11 detection 18042D (Pharmingen)

IL-5: capture TRFK 5 detection 18062D (Pharmingen)

The protocol used was as detailed in 2.11.1. The levels of IL-4, IL5, IL-12 and IL-18 cytokine levels were calculated by comparison with known cytokine standards that with detection limit of less than 5pg/ml for each cytokine.

Since the sensitivity of the commercial kits were superior to our assays. The detection of IFN-γ, IL-4, and IL-5 in BALF commercial kits were used. These were obtained from R + D Systems and assays performed as instructed. Similarly kits were also used to assay IL-12 and IL-18 (R+D System).

2.12 In vivo Methods for Elimination and Suppression of Macrophages

Carrageenan Type V (Sigma) and Gadolinium chloride (Sigma) were used to deplete the pulmonary macrophages. The elimination of the macrophage by using Carrageenan is based on its cytotoxic effect to macrophages. Gadolinium chloride is also able to suppress the phagocytic capability of macrophages. For depletion, Carrageenan 100ug per 100ul PBS or Gadolinium chloride 10mg per kg was given intraperitoneally to each mouse daily during OVA inhalation challenge for 6 days. Intra-nasal aerosol route of Carrageenan has also been tried.

2.13 T Cell Proliferation Assay

2 X 10^5 cells per well were placed into a 96-flat bottom plate (Falcon) with either OVA peptide (1μg/ml), anti-CD3 (10μg/ml), or KJ1-26 (10μg/ml) for 3 days. After 48 hours, 1uCi of ^{3}H-thymidine (Amersham International) was added to each well. The plate was harvested onto a glass-fibre filter (Whatman International) using a Dynateck harvester 18 hours later. Dry filter paper discs were transferred to scintillation vials (Packard) and 2 ml of Optiscint scintillation fluid (Wallac) was added. ^{3}H-thymidine incorporation was determined using a β-counter (2000 CA, Packard).

2.14 Treatment of Mice with Prostaglandin E2 (PGE_2)

PGE_2 10μg was dissolved in 10ml of PBS. Mice were exposed to an aerosolised solution of PGE_2 for 10 minutes before commencement of an OVA aerosol challenge. Mice were treated with aerolised PGE_2 for 6 days. Following challenge the bronchoalveolar lavage was harvested and level of prostaglandinE2, leukotreine B4 and the EPO in BALF determined as described below.

2.15 Treatment of the Mice with Corticosteroids

Dexamethasone (Sigma) was dissolved in PBS at a concentration of 25mg per ml. Mice were treated with a dose of 1mg/kg by injecting 100μl of dexamethasone solution intravenously through the lateral tail vein. After aerosol challenge, BALF was harvested and used for EPO assays and cytospin slide staining examination.

2.16 Treatment of Mice with Non-Steroid Anti-inflammatory Drugs (NSAIDs)

Indomethacin and lysine acetylsalicylate and the cyclooxygenase-2 selective inhibitor (NS-398) were used in this study

A stock solution indomethacin (Sigma) was made by adding 40mg of the drug to 5ml absolute alcohol. Five ml of stock solution was added to 1 liter of drinking water. The mice were then allowed to drink the solution ad libium for the duration of the experiment. The indomethacin drinking solution was replaced daily. In some experiments, intraperitoneal administration of the drug was tried. Using a dose of 5mg/kg was given to each mouse daily.

One vial (0.5g) of Lysine acetylsalicylate (Aspegic, Laoratories Synthelabo, France) was dissolved into 5 ml water to make a stock solution. A dose of 100mg/kg of the drug was used by daily intraperitoneal injection for the course of the experiment.

Five mg of NS-398, N- (2-Cyclohexyloxy-4-nitrophenyl) methane sulfonamides (Calbiochem) was dissolved in 0.5ml DMSO to form a stock solution. Mice were treated with either 1 mg/kg or 10 mg/kg doses intraperitoneal injection. Experimental mice were treated daily for the duration of the experiment.

2.17 Prostaglandins and Leukotrienes Measurement

The commercial kits (R&D System) for the measurement of PGE2 and LTB4 and cayman for the measurement of PGD2, PGF2α, TXB2, 6-keto PG1α were used. Method was described briefly as follow.

2.17.1 Prostaglandin Measurement by EIA (PGD2, PGE2, PGF2α, TXB2, and 6-ketoPGF1α)

All reagents and samples were at room temperature before use. The BALF samples required at least a 10-fold dilution in PBS before assay.

Assay for prostaglandin was by the competitive ELISA technique (EIA). All reagents, working standards and samples were prepared as described in the instructions. One

hundred µl of standard or sample was added to the wells coated with anti-mouse Ig antibodies. Fifty µl of anti-PGE2 (etc) mouse Ig was added to the wells. The plates were incubated for 2 h at room temperature and then washed with wash buffer 3 times. The PGE2 (etc) conjugate was added to wells which were incubated for 2 h at room temperature, washed and then substrate (pNPP) added for 1 h. Stop solution was added and plated read at 405nm using a microplate reader. Controls comprised of wells to determine non-specific binding and total activity.

2.17.2 LTB4 Immmoassay

The principle is similar to the PGE2 method described above. It is based on the competitive binding technique in which LTB4 present in a sample competes with a fixed amount of alkaline phosphatase labeled LTB4 for sites on a rabbit polycolonal Ab. During the incubation, the polycolonal Ab becomes bound to the goat anti-rabbit Ab coated onto the microplate. Following a wash to remove excess conjugate and unbound samples, a substrate solution was added to the wells to determine the bound enzyme activity. The color development was stopped and the absorbance is read at 405nm. The intensity of the color is inversely proportional to the concentration of LTB4 in the samples.

2.18 Determination of total IgE antibody level in serum

Total serum IgE antibodies were determined by ELISA. A 96-well ELISA plate was coated at 4 C over night with monoclonal rat anti-mouse IgE antibody that was diluted in carbonate buffer (pH9.6) at 2.5μg/ml concentration. The next morning, the plate was washed 3 times in PBS/Tween 20 and then blocked with 200μl of a 1% BSA solution and incubated at 37 C for 1 hour. Standard IgE and samples were added using 100μl/well, in triplicate and incubated at 4° C over night. Plates were washed 3 times with wash buffer and shaken dry, the detecting antibody for IgE (biotinylated rat anti-mouse IgE) was added to wells at a concentration of 2.5μg/ml and incubated for 1 hour at room temperature. The plate was washed 5 times with wash buffer and shaken dry. Subsequently, the plate was incubated with peroxidase-conjugated streptavidin (1:1000) dilution in PBS/Tween 20. Finally, TBS (final wash buffer) was used to wash the plate 5 times, and the plate developed using the amplification kit (Gibco) as detailed previously and plates read the absorbance at 492nm.

2.19 Statisticial tests

Unless stated otherwise, data are expressed as means ± standard error of the mean and evaluated using a two-way analysis of variance, followed by Student's *t* test for comparison between two groups. A probability value of $P<0.05$ was considered statistically significant.

Chapter Three

Development of Models of Th1- and Th2-induced Pulmonary Inflammation

3.1 Introduction

$CD4^+$ T helper type 2 cells are thought to orchestrate pulmonary inflammation in asthma (Foster PS et al 1996; Corry DB et al 1996; Gavett SH et al 1994). Several laboratories have shown that CD4+ T cells bearing a Th2 phenotype can elicit pulmonary allergic inflammation in mice. Interestingly, both IL-4 deficient and class II MHC-deficient mice do not develop pulmonary inflammation when exposed to aerosolized OVA as determined as a BAL lymphocytosis and eosinophilia (Brussells et al; 1994). Type 1 (Th1) and Type 2 (Th2) $CD4^+$ Th cells are distinguished by the pattern of cytokines that they produce. Th1 cells produce IFN-γ, IL-2, IL-3 and TNF-β (Mosmann TR et al 1986) and play a critical role in directing cell-mediated immune responses, important for the clearance of intracellular pathogens (Steven TL et al 1988). Th2 cells produce IL-3, IL-4, IL-5, IL-6, IL-9, IL-10 and IL-13 (Mosmann TR et al 1989) have been associated with allergy and are important for humoral responses(Coffman R et al 1986; Finkelman FD et al 1989).

The principle objective of this project was to analyze lung mucosal T cell responses and associated inflammation elicited in response to inhaled antigen. The critical requirements in setting up this model were:

1. To drive differentiation of DO11.10 T cells into a Th1 or Th2 phenotype.

2. To adoptively transfer effector Th1 or Th2 cells into naive BALB/c recipient's mice and determines whether they migrated to the lung.

3. Characterising the inflammatory response that developed in the lungs following antigen inhalation.

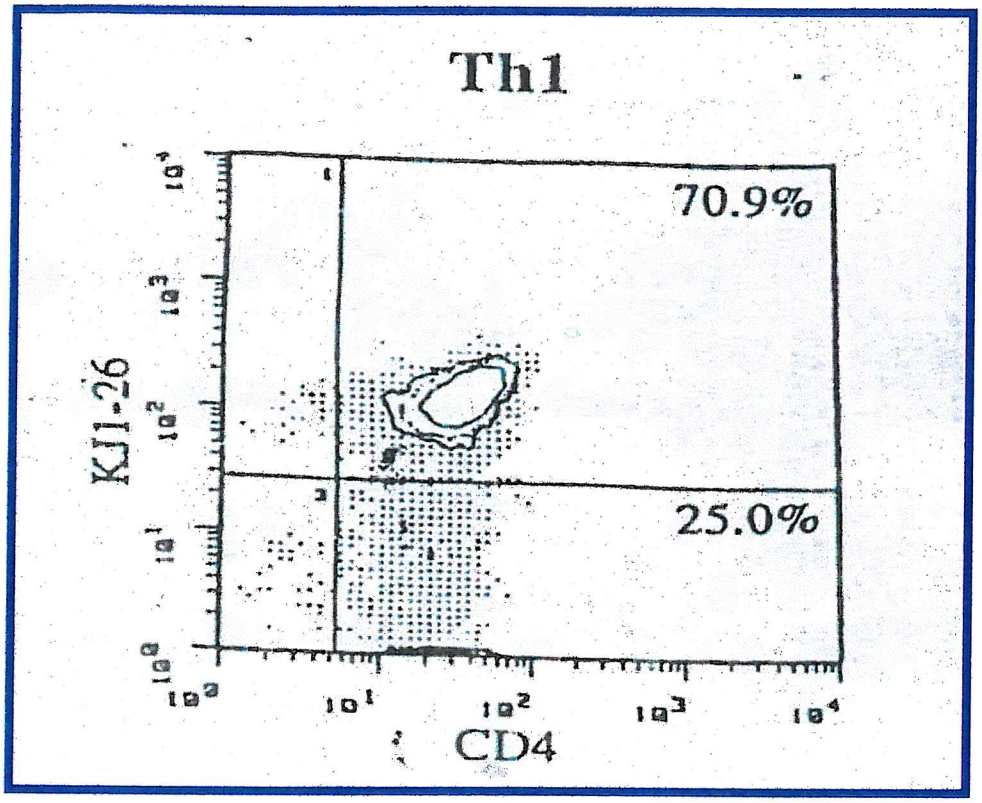
3.2 Generation of Th1 or Th2 Cell Lines

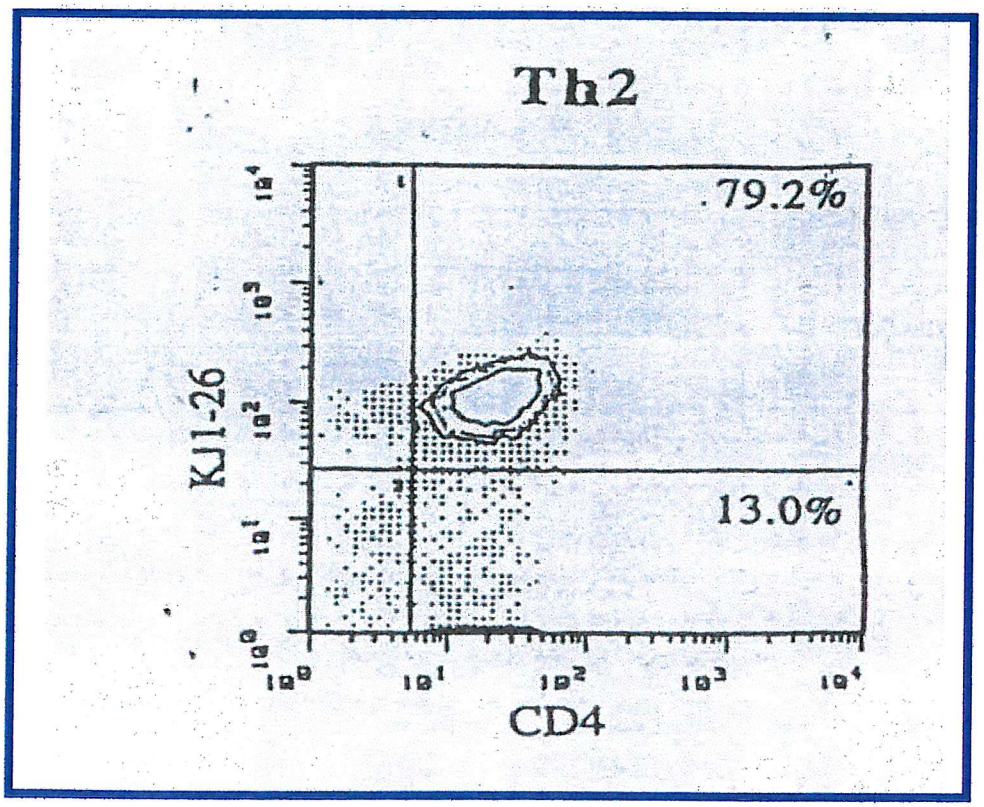
To investigate the ability of CD4+ effector cells to elicit lung inflammation, we have used an OVA based murine model. The reason for using TCR transgenic mice is that in using this approach it is possible to identify antigen specific T cells *in vitro* and *in vivo*. In this study DO11.10 mice were used which express a transgenic TCR specific for OVA peptide 323-339 (Murphy KM et al 1990). The DO11.10 mice used have been extensively backcrossed with BALB/c mice (Iadc) haplotype. Antigen specific DO11.10 T cells could be identified using the anti-clonotypic antibody KJ1-26. These mice contain primary CD4+ T cells, however, low numbers of CD8+ T cells are present (<5%). Since it is impossible to fully control T cell differentiation '*in vivo*', we decided to inject effector Th1 or Th2 cells into naïve BALB/c mice. It has been shown by several laboratories that culture of T cells with the appropriate cytokines can promote differentiation into Th1 or Th2 effector cells. Consequently, the simplest way to study Th1 and Th2 responses to inhaled antigen was to produce OVA specific CD4+ effector cells *in vitro* and then adoptively transfer them into a normal BALB/c mouse. Recipients were then exposed, to aerosolized OVA over several days. To produce fully differentiated Th1 or Th2 cells pooled peripheral and mesenteric lymph node cells from the mice were cultured at $5x10^5$/ml and OVA peptide (0.5mg/ml). To generate Th1 cells, rIL-12 was added at 5ng/ml and the anti-IL4 (11B11). To generate Th2 cells, cultures contained rIL-

4 at 2ng/ml and anti-IFN-γ mAb HB170. Cultures were maintained for 4 days after which the cells were washed, counted and re-stimulated at $3x10^5$/ml under the same conditions used for the initial stimulation with the exception of additional rIL-2 at 50u/ml in both Th1 and Th2 cell lines. After the second stimulation cycle (3 days) cells were washed, and CD8+ cells depleted by panning using a rat anti-CD8 mAb (5367) and plate bound mouse anti-rat antibodies. DO11.10 lymph node cells were cultured with OVA peptide and either IL-12 or IL-4 proliferated vigorously over the 7 days period. Cells analyzed at day 7 were >80% KJ1-26+CD4+ T cells (Figure 3.1.a). Both Th1 and Th2 cells expressed CD25 and low levels of CD69 and OX-40. Interestingly, Th2 cells expressed higher levels of CD30 than the Th1 cells (Figure 3.1.b). The principle concern was that the lines generated have a good Th1 or Th2 profile. Specifically that the Th2 cells generated this way might continue to produce Th1 cytokine (most notably IFN-γ). It has been shown by several laboratories that the commitment of T cells to a Th2 phenotype can occur in 4 days (Seder RA et al 1992, 1993) or 8 days (Murphy E et al 1996). Initial experiments used a 4 day culture period, however Th2 cells cultured in this way continued to produce low levels of IFN-γ on restimulation with anti-CD3 (Figure 3.2). Consequently, an 8 day culture period was subsequently adopted unless stated differently. The cytokine profiles of the Th1 and Th2 cell lines were confirmed, by direct examination of supernatants generated from these lines following stimulation with anti-CD3, using CTLL bioassay for IL-2 and ELISA for IL-4, IL-5 and IFN-γ measurements. The CD4+ cells stimulated to differentiate into Th1 cells produced high levels of IL-2 and IFN-γ and minimal IL-4, while the cells stimulated to differentiate into Th2 cells secreted high levels of IL-4 and minimal IFN-γ and IL-2 (Figure 3.3). Th2 cells generated

this way when cultured with either IL-12 or IL-18 alone failed to produce IFN-γ. However, treatment with both IL-12 and IL-18 did induce the production of significant levels of IFN-γ (Figure 3.4).

(a)





(b)

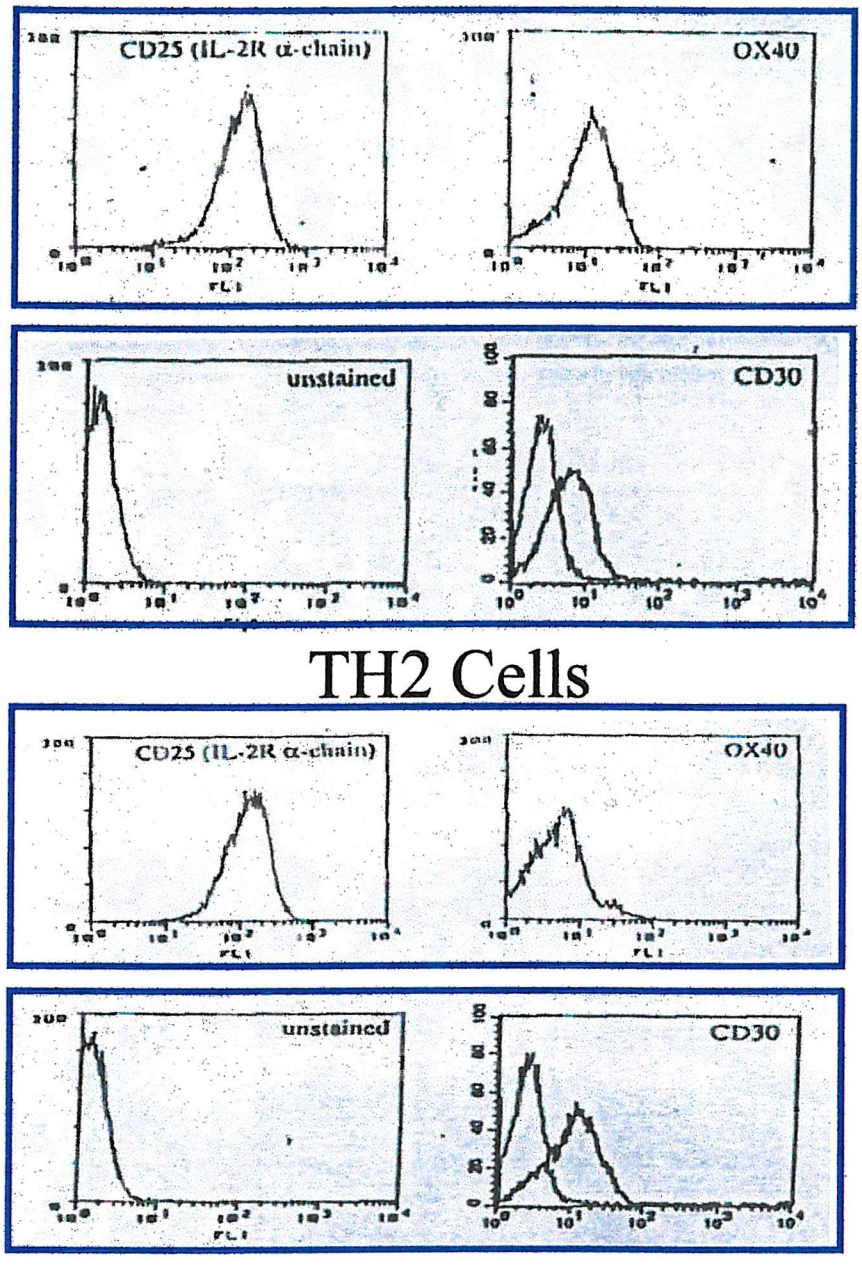


Fig 3.1
Phenotype of cultureed DO11.10 T cells
DO11.10 lymph node cells were cultured with 0.5μg/ml OVA peptide and either (i) IL-12 and anti-IL-4 mAb, or (ij) IL-4 and anti-IFN-γ mAb for 7 days. (a) Cells were stained with FITC labeled KJ1-26 and PE labeled anti-CD4. Flow cytometric analysis of the cells demonstrated that 70.9% of the Th1 cells and 79.2% of the Th2 cells expressed the transgenic TCR respectively. (b) Cells were stained by indirect immunofluorescence using 7D4 (Rat anti-CD25), H1-2F3 (Hamster anti-CD 69), rat anti-OX-40, and rat anti-CD30 (CD30Li-CD4 fusion protein + αCD4 Ig-FITC). Both Th1 and Th2 cells expressed CD25 and low levels of CD69 and OX-40 but higher levels of CD30 were expressed on Th2 cells.

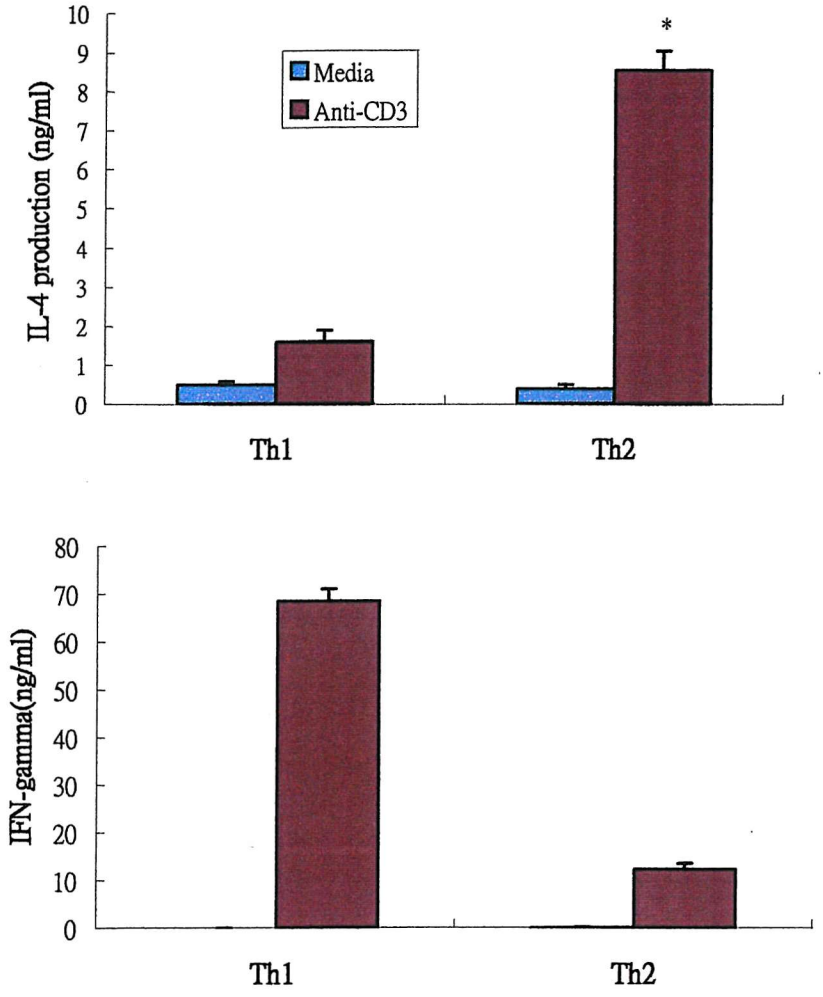


Fig. 3.2

Cytokines produced by day 4 DO11.10 Th1 and Th2 cells

To generate DO11.10 T cells bearing a Th1 or Th2 phenotype, PLN cells were cultured 4 days in the presence of 1μg/ml OVA 323-339 peptide and either (i) 400ng/ml mouse IFN-γ and 10μg/ml anti-IL-4 Ab (11B11) or (ii) 2ng/ml mouse IL-4 and 10μg/ml anti-IFN-γ. The phenotype of the T cells was evaluated by examining the production of IL-4, and IFN-γ when stimulated with plate-bound anti-CD3 (n=3, *$p<0.05$).

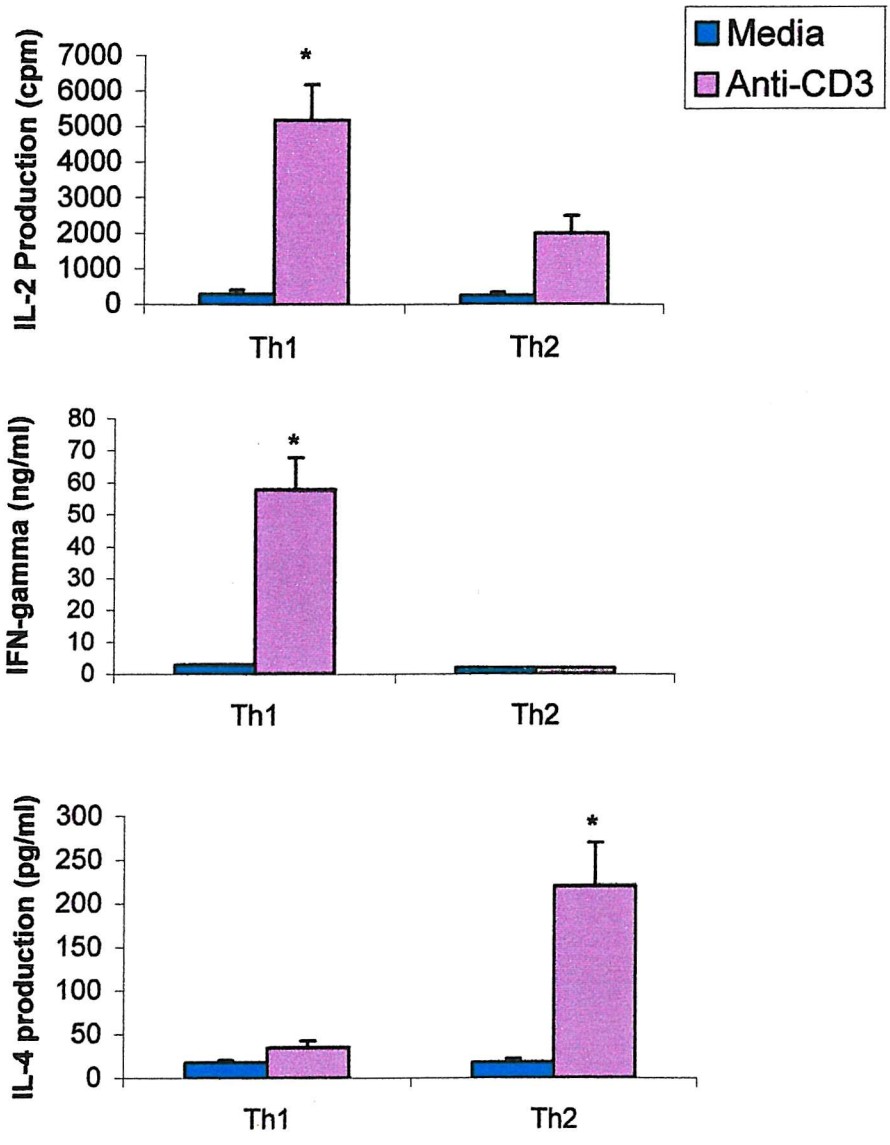


Fig. 3.3

Cytokines produced by day 8 DO11.10 Th1 and Th2 cells
DO11.10 T cells after 8 days in culture were washed and resuspended at 10^6/ml cells were then stimulated for 48 hours on plates pre-coated with anti-CD3 Ab (10μg/ml) and cytokine present in the supernate determined by ELISA. Data are means from 3 separate experiments (*$p<0.05$).

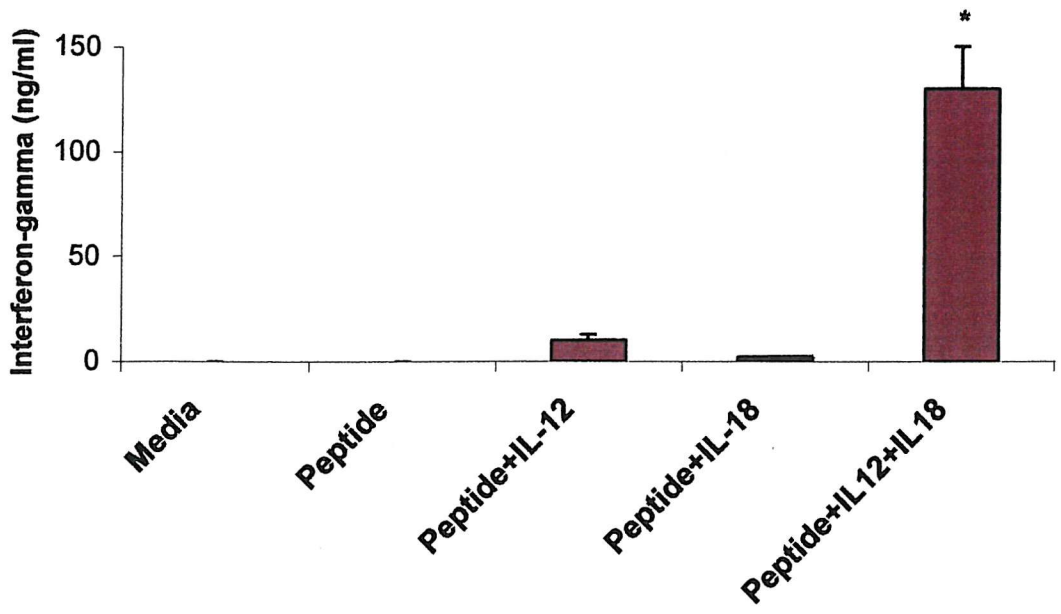


Fig. 3.4

Interferon-γ production by DO11.10 Th2 cells
After two cycles' stimulation (8 days) DO11.10 Th2 cells were cultured with either IL-12, IL-18 alone or with both together for 48 hours with OVA peptide stimulation. (n=3, *$p<0.05$).

3.3 The adoptive transfer of DO11.10 T cells into BALB/c mice

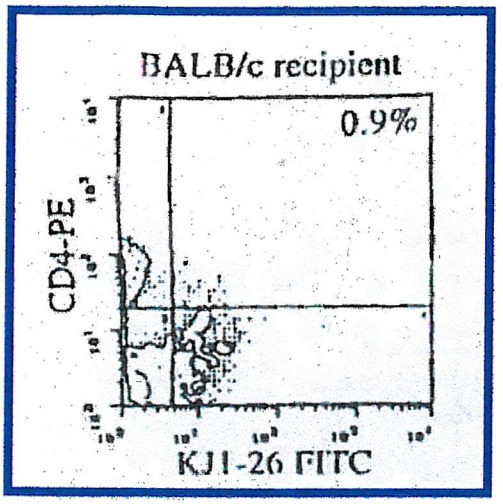
The next stage was to inject the Th1 and Th2 cells into naive BALB/c recipients. The principle concern was whether the cells would migrate to the lung tissue and mediate an inflammatory response to inhaled antigen. To resolve this issue we investigated whether the DO11.10 T cells injected could be identified in the lung tissues using the KJ1-26 mAb, the cytokines they expressed and whether they proliferated in response to antigen.

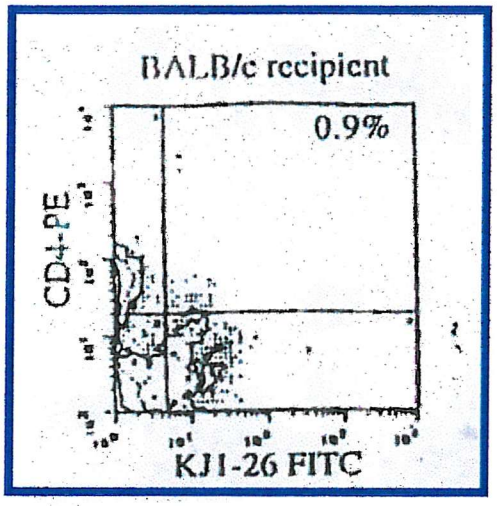
3.3.1 The number of DO11.10 T cells entering the lung

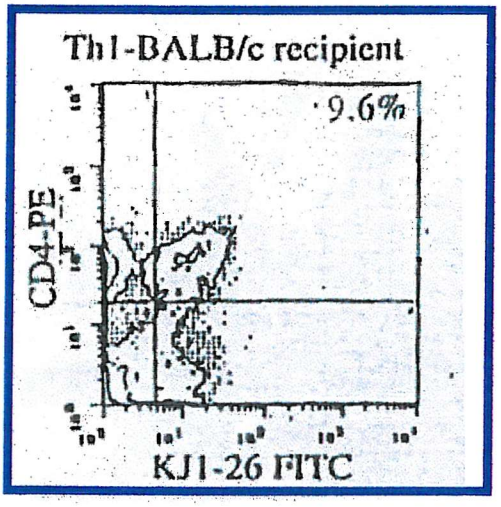
To investigate whether the CD4+ T cells that had been injected entered the lungs, the numbers of KJ1-26 + T cells present in the lungs was determined over a 10 day period. The effect of OVA inhalation on the number of DO11.10 T cells was also assessed. Mice were given $10x10^6$ cultured Th1 or Th2 cells since this has been shown to be sufficient to elicit a pulmonary eosinophilia. Mice were either left or exposed to OVA aerosols for 3, 6 and 10 consecutive days. After these times lung tissue was harvested dispersed in collagenase and the number of DO11.10 T cell present determined by flow cytometry using the KJ1-26 and anti-CD4 antibodies (Figure 3.5 a,b).

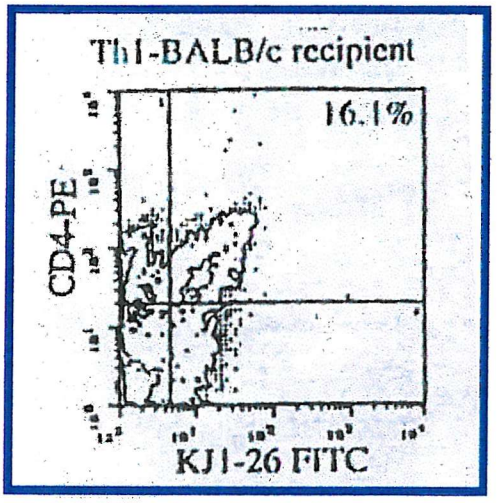
In both Th1 and Th2 driven inflammatory responses the number of DO11.10 transgenic T cells present in the lung tissue doubled following aerosol exposure. These data demonstrated that DO11.10 T cells do enter the lung tissue after injection and that their numbers are increased following exposure the animals to aerosolized OVA (reaching 16.1% and 12.2% for Th1 and Th2 cells respectively). By 10 days of aerosol challenge the number of DO11.10 T cells present in both Th1 and Th2 recipients had reached a maximum. DO11.10 T cells could also be found in the BALF of mice that had received either Th1 or Th2 cells and been exposed to OVA aerosols, however their numbers were typically low.

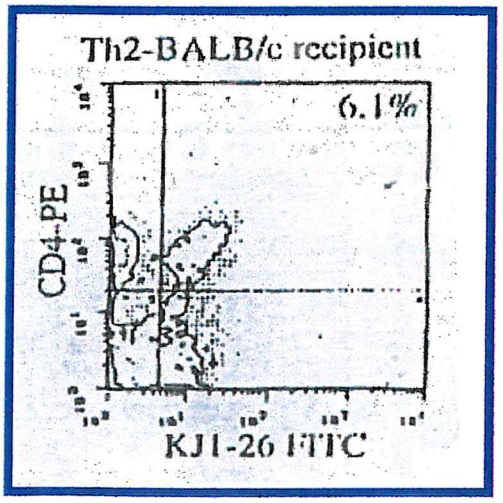
(a) NO OVA INHALATION CHALLENGE OVA INHALATION CHALLENGE

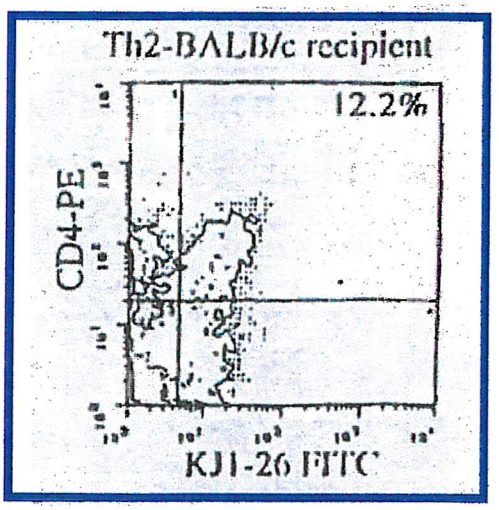












(b)

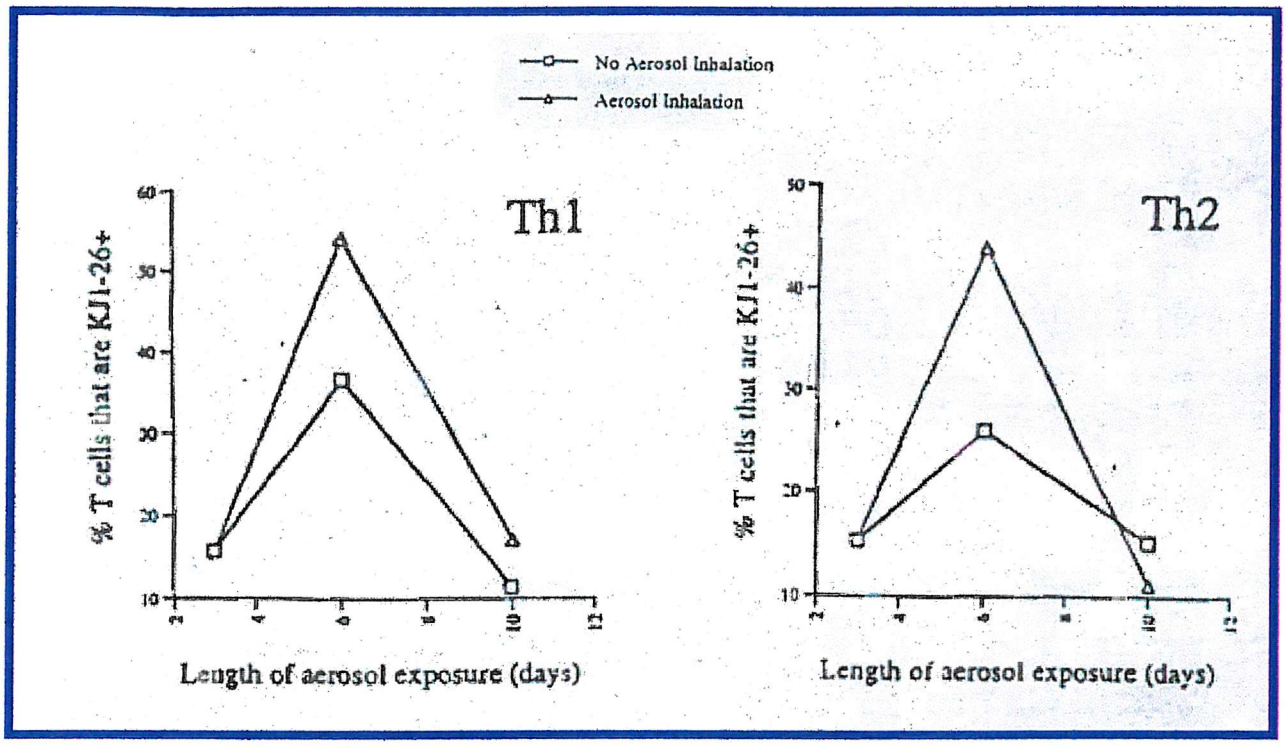


Fig.3.5

Enumeration of DO11.10 T cells in the lungs

DO11.10 T cells bearing either a Th1 or Th2 phenotype were injected into the tail veins of BALB/c mice (10^7/mouse). Mice were then either left repeatedly exposed to OVA aerosols. After 3, 6 and 10 days, mice were lavaged and the lungs dispersed in collagenase. LMCs were stained with PE labelled anti-CD4 antibody and FITC labelled KJ1-26 (anti-clonotypic antibody) and examined by flow cytometry. In (a) Cells were prepared from the lungs of BALB/mice and BALB/c mice that had received DO11.10 T cells bearing a Th1 or Th2 phenotype. The effect of OVA inhalation on the frequency of DO11.10 T cells in the lungs was examined. In (b) the proportion of CD4+ T cells in the lung that were clonotype positive over a 10 day period of OVA inhalation was examined.

3.3.2 Cytokine production by DO11.10 T cells entering the lung

Cultured Th1 or Th2 cells were harvested after two stimulation cycles over 7 days washed with PBS and $10x10^6$ cells were injected intravenously into the tail vein of BALB/c recipients. To monitor the pulmonary Th2 response we adopted three different approaches to measure cytokine expression:

1. Assay for cytokines in the BALF
2. Separation of LMC and evaluating the range of cytokines produced after stimulation with OVA, OVA peptide or anti-CD3.

In two separate experiments no detectable IL-5, IFN-γ or TNF-α was detectable in the BALF by ELISA (data not shown, R+D System). Consequently, to monitor cytokine production in the lung we determined which cytokines were produced by enzymically dispersed tissue on stimulation with OVA. LMC produced large amounts of IL-4 following stimulation with OVA peptide in mice that had received DO11.10 Th2 cells and been exposed to OVA aerosols (Figure 3.6). LMC from mice that had not received DO11.10 T cells or Th1 DO11.10 T cells did not produce IL-4 in response to OVA peptide (Figure 3.6). These data demonstrate that not only do DO11.10 Th2 cells enter into the lungs but that then also retain a capacity to produce IL-4 despite inhalation of OVA for 6 days.

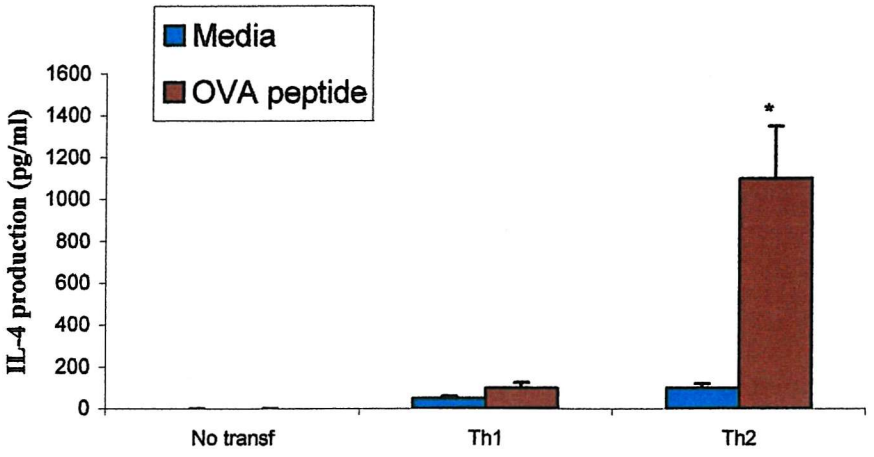


Fig. 3.6
IL-4 production by LMC in mice that had received DO11.10 T cells and then exposed to OVA aerosols
The pulmonary tissue of BALB/c mice which had received Th1 or Th2 cells and had been exposed to OVA aerosols for 6 days were dispersed in collagenase and IL-4 production was measured following stimulation with $OVA_{323-339}$ peptide for 48 hours. ELISA technique was used to assess IL-4 levels (n=3, *$p<0.05$).

A problem was encountered when using Th2 cells that had been cultured for only 4 days since LMC from mice that had received such Th2 cells, and inhaled OVA, produced large amounts of IFN-γ in addition to producing IL-4 and IL-5 (Figure 3.7). In contrast, LMC from mice that had received Th2 cells and had not inhaled OVA produced less IFN-γ (Figure 3.7). Since cytokine production was elicited by the OVA peptide 323-339, it seems highly likely that these cytokines were produced by the DO11.10 cells that were injected into these mice. This was confirmed by stimulating LMC with " plate-bound " KJ1-26, which also induced LMC from the challenged-Th2 recipients to produce IFN-γ (Figure 3.8).

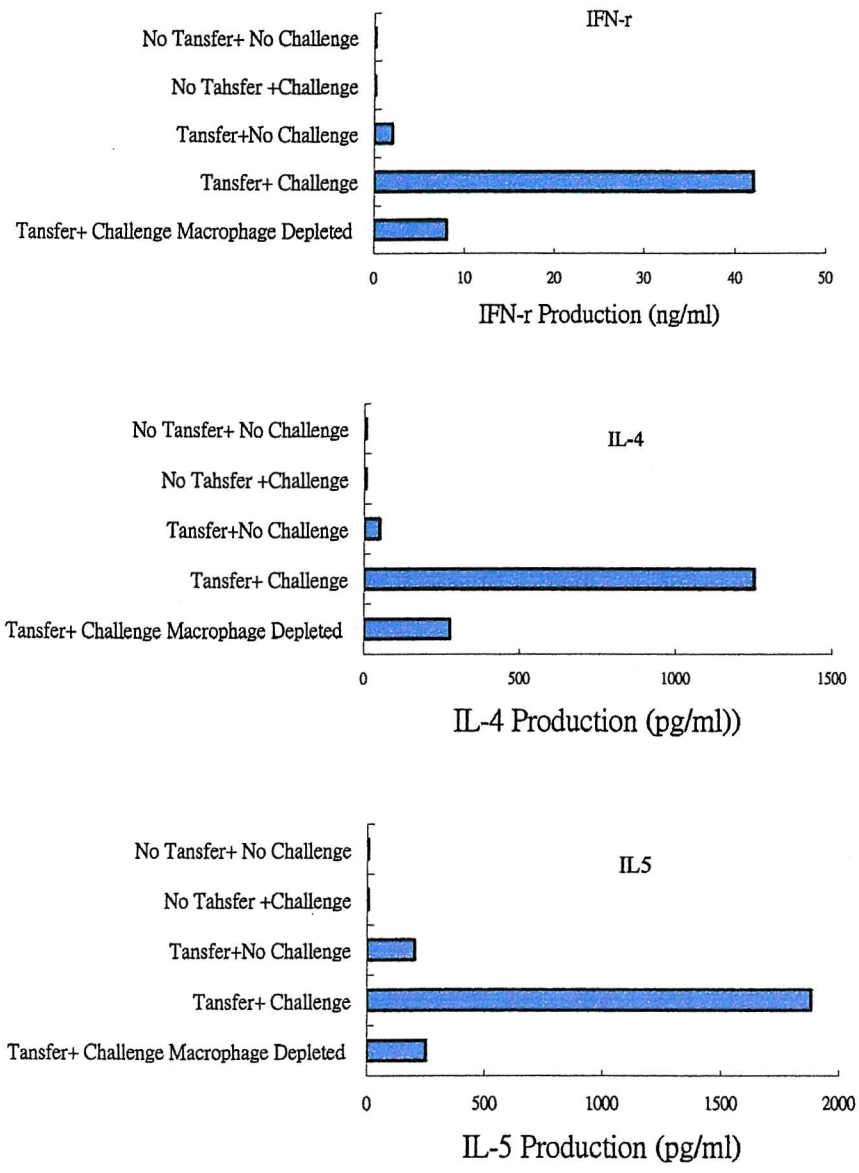


Fig. 3.7

Cytokines produced by LMC in mice that had received 4 days cultured Th2 cells and inhaled OVA

DO11.10 Th2 cells (cultured for 4 days) were transferred to BALB/c recipients which were then exposed to OVA aerosols. Controls comprised of mice that had received cells but were not exposed to OVA aerosols or had not received cells but inhaled OVA. IL-4, IL-5, and IFN- γ measurement were assessed by using the ELISA method.

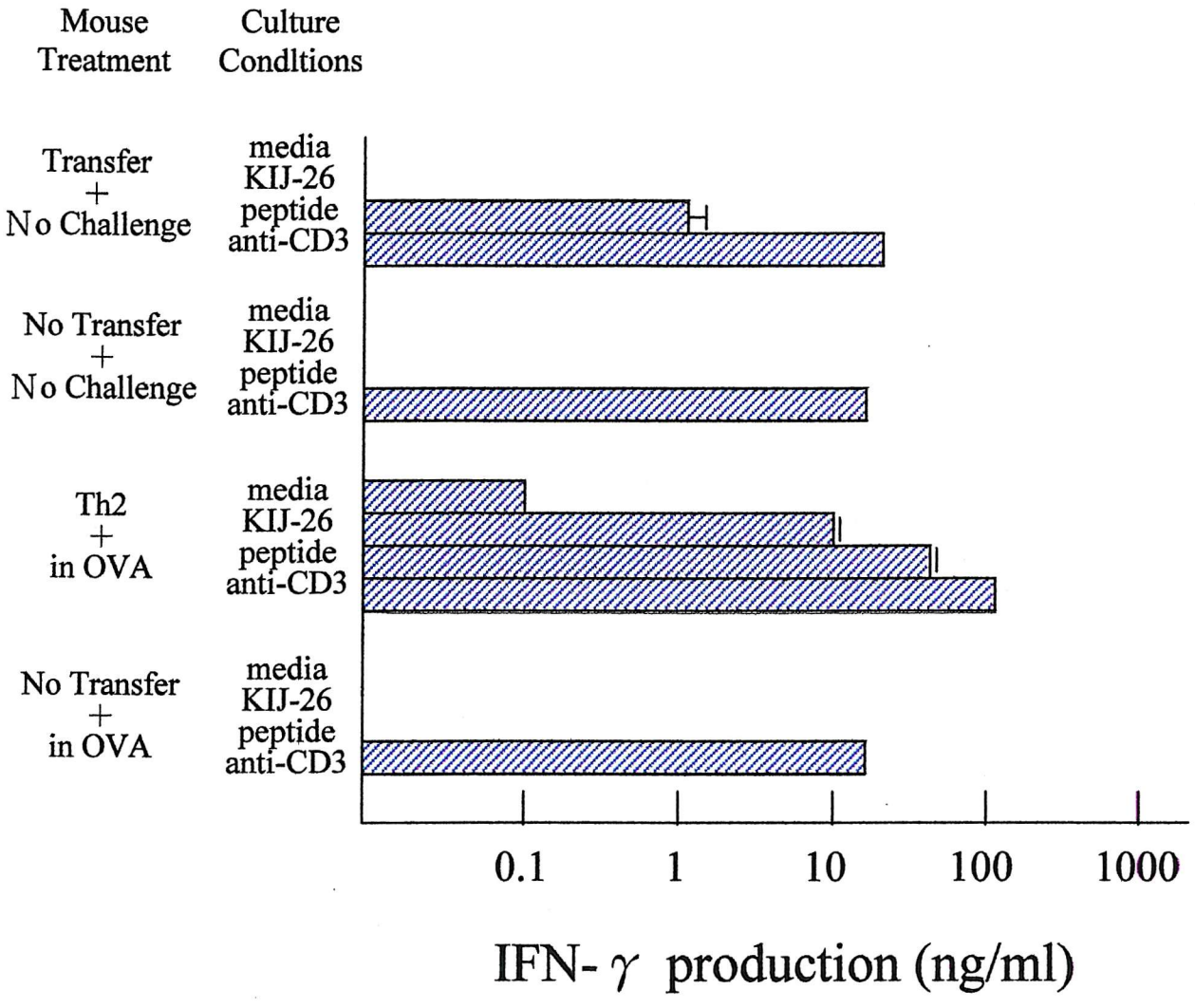


Fig. 3.8

IFN-γ production by LMC after adoptive transfer of 4 days of Th2 cells

Th2 cells cultured for 4 days were adoptively transferred to BALB/c recipient mice which were exposed to OVA aerosols. Controls comprised of mice that had received cells but were not exposed to OVA aerosols. IFN-γ level was assessed following stimulation of LMC with KJ1-26 mAb, OVA peptide 323-339, or anti-CD3 for 48 hours. IFN-γ was measured by using ELISA assay (n=3).

To circumvent the problem of IFN-γ production by Th2 cells in vivo the duration of polarisation of DO11.10 cells in culture was extended from 4 to 8 days. LMC from mice that had received Th2 cells that had been cultured for 8 days and had exposed to OVA aerosols did indeed produce only IL-4 and IL-5 but no IFN-γ in response to OVA peptide (Figure 3.9). In contrast, IFN-γ was produced by LMC from Th1 recipients. These data imply that the production of IFN-γ by Th2 cells was a consequence of adoptive transfer of cells that were not completely polarised.

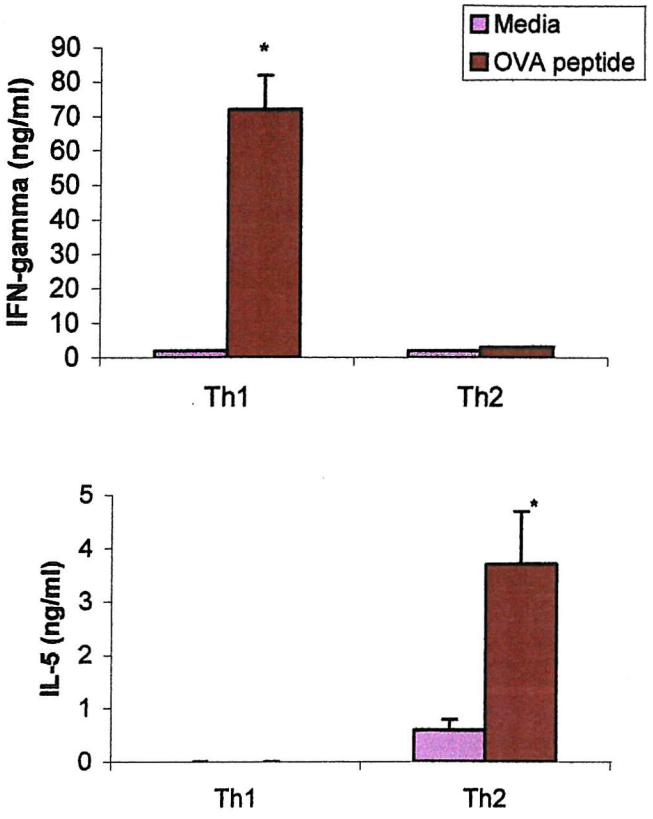


Fig. 3.9
Cytokine production by LMC from animals that had received 8 days DO11.10 Th2 cells
DO11.10 Th2 cells (8 days) were transfered into the BALB/c recipient mice, which were then exposed to OVA aerosols. Pulmonary cells were isolated with collagenase and following stimulation with OVA peptide 323-339, supernates were harvested and assayed for IL-5 and IFN-γ by ELISA (n=3, *$p<0.05$).

3.3.3 The proliferation of DO11.10 T cells entering the lung

Previous experiments in which DO11.10 transgenic mice themselves were exposed to aerosolized OVA revealed that the T cells present in the lung tissue enter growth arrest on stimulation with antigen. (Lee SC et al). Associated with this event was a marked reduction in the level of IL-2 production but an increase IFN-γ release. In the course of my experiments I wanted to evaluate whether the attenuation of lung parenchymal T cell proliferative responses was associated with either a Th1 or Th2 mediated inflammation. Th1 or Th2 cells were injected into BALB/c mice, which were then exposed to, aerosolized OVA. After 6 exposures LMC were prepared and restimulated with " plate-bound " anti-CD3 and the proliferation and production of IL-2 determined. Following OVA inhalation the proliferation and production of IL-2 by LMC in response to anti-CD3 was severely attenuated in both Th1 and Th2 recipient mice (Figure 3.10). LMC from control mice that had received either Th1 or Th2 and not inhaled OVA responded normally. We next evaluated whether F4/80+ interstitial macrophages were responsible for limiting the proliferative responses in the lungs of BALB/c mice that had received transgenic T cells. DO11.10 T cells bearing a Th1 phenotype were adoptively transferred into BALB/c mice. As previously, OVA inhalation by animals that had received DO11.10 Th2 cells resulted in a dramatic reduction in lung parencymal proliferative responses and in IL-2 production (Figure 3.11). Importantly, such growth arrest and attenuation of IL-2 production was reversed by the removal of F4/80+ interstitial macrophages (Figure3.11). In contrast, the lung parenchymal T cell responses of control BALB/c mice (which had either received DO11.10 Th2 and not inhaled OVA or had inhaled OVA but not received

Th2 cells) remained unchanged (Figure 3.12). Interestingly, the removal of F4/80+ macrophages had no effect on the level of production of either IL-4 or IL-5 (Figure 3.10).

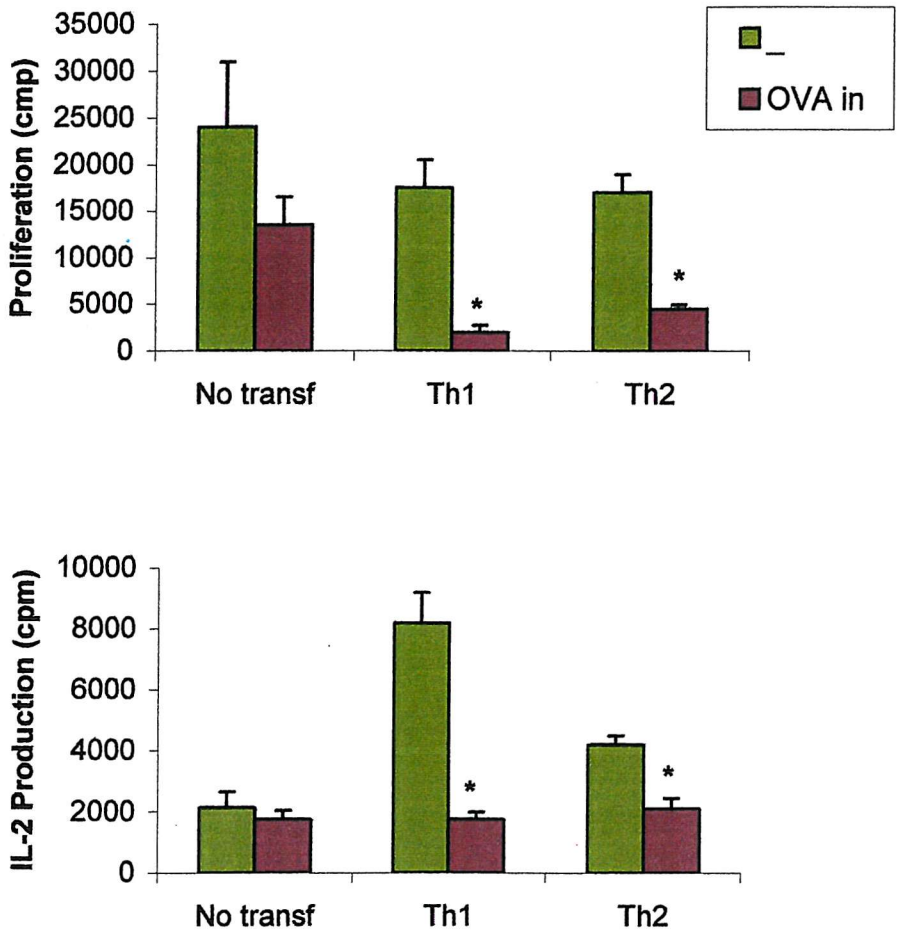


Fig. 3.10
The response of LMC from mice that had received DO11.10 Th1 or Th2 cells and inhaled OVA

DO11.10 Th2 cells (8 days) were transferred into BALB/c mice, which were then exposed to OVA aerosols. LMC from Th1 or Th2 recipients were stimulated with plate-bounb anti-CD3. The proliferation was determined after 3 days using ^{3}H-thymidine incorporation and production of IL-2 determined using the CTLL bioassay (n=3, *$p<0.05$).

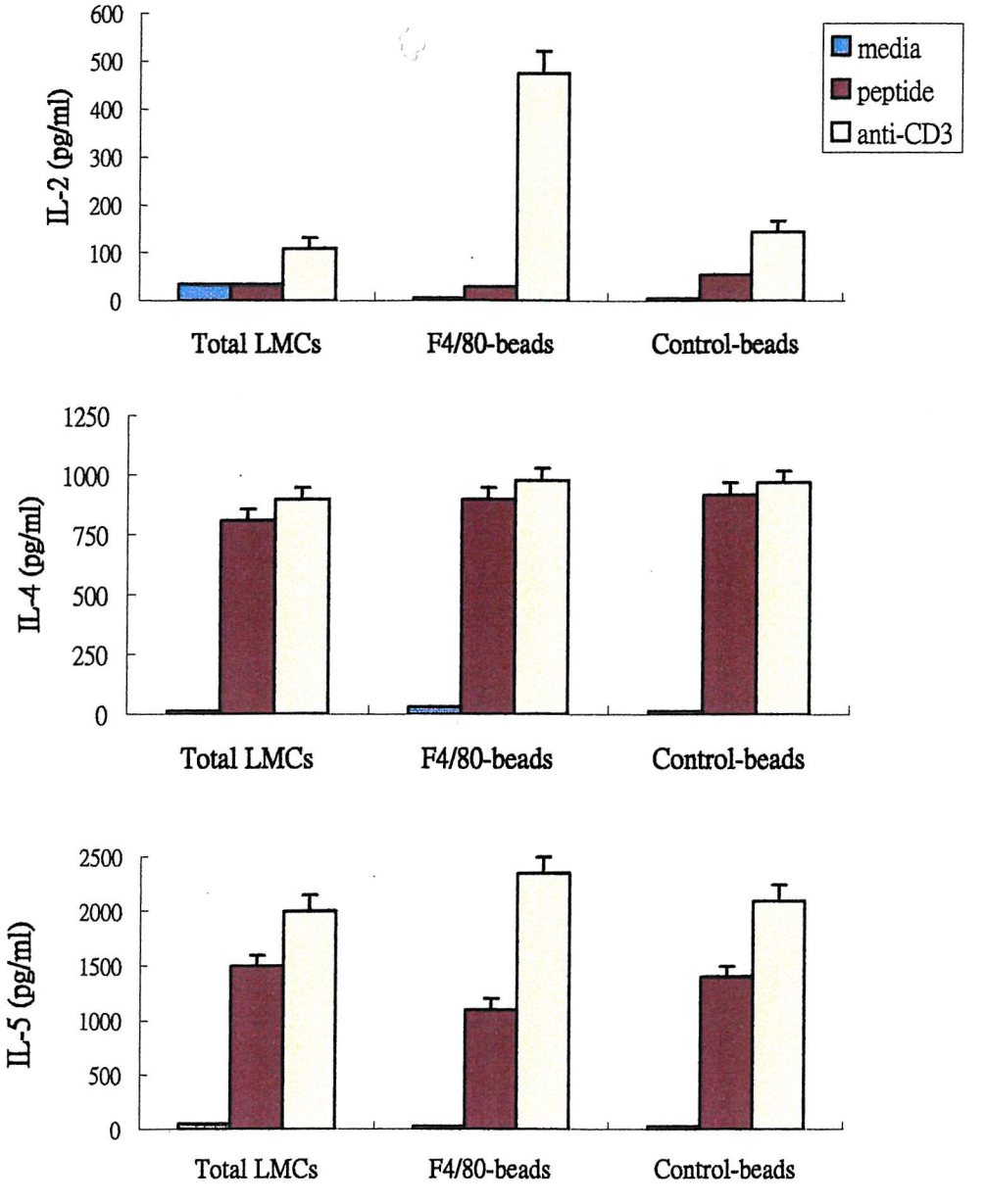


Fig. 3.11

The effect of F4/80+ macrophages on the level of production of cytokines produced by LMC.

Th2 DO11.10 cells (10^7) were injected *i.v.* into naïve BALB/c mice which were then exposed to OVA aerosols for 6 days. Lung tissue was harvested and macrophages depleted using F4/80 Ab and anti-rat Ig-coated Dynabeads, The cytokines produced by LMCs in response to OVA323-339 peptide (1μg/ml) or plate-bound anti-CD3 (10μg/ml) was evaluated after 3 days (n=3).

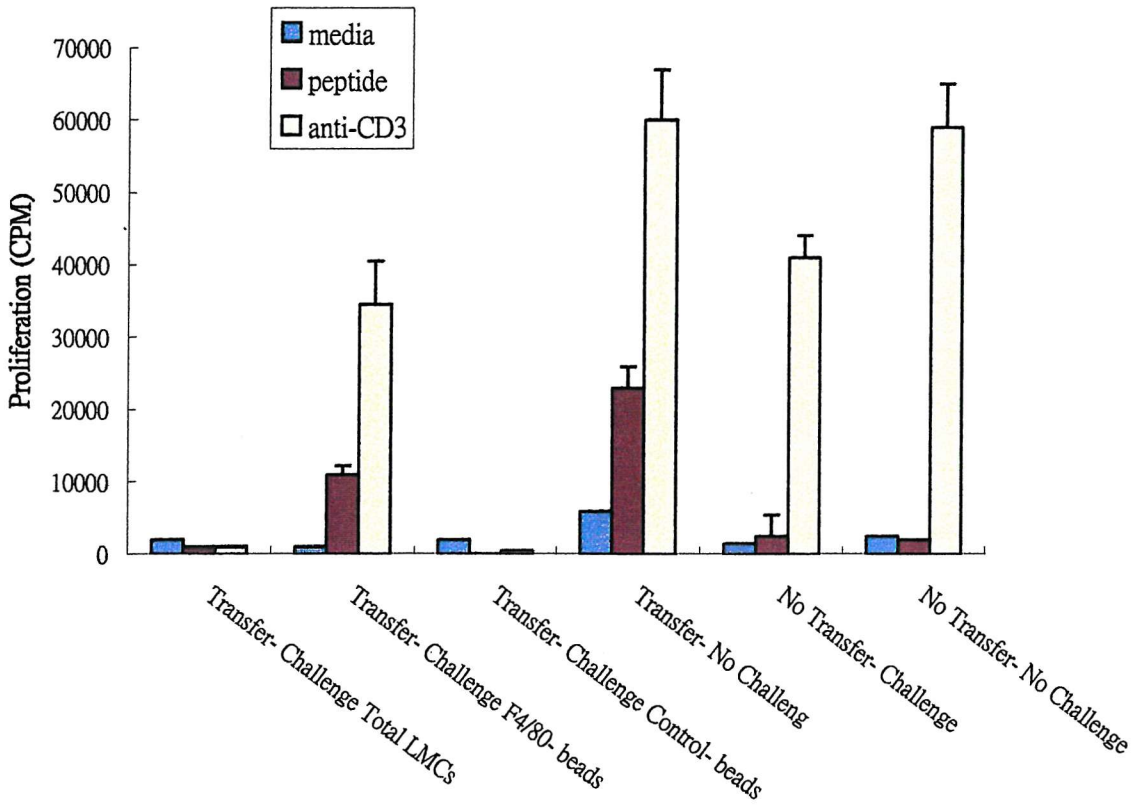


Fig 3.12
The effect of F4/80+ macrophagesion the proliferative responses of LMC
Th2 DO11.10 cells were injected *iv* (10^7) into naïve BALB/c mice which were then exposed to OVA aerosols for 6 days. Controls comprised of mice that had received DO11.10 T cells but had not inhaled OVA or had inhaled OVA but had not been given cells. LMCs prepared from mice that had received DO11.10 T cells and exposed to OVA aerosols were depleted of interstitial macrophages using the F4/80 Ab and anti-rat Ig-coated Dynabeads. The proliferative response of LMC following stimulation with $OVA_{323-339}$ peptide (1 μ g/ml) or plate-bound anti-CD3 was determined by measuring ^{3}H-thymidine incorporation after 72 h.

3.4 The lung inflammatory response that develops following OVA aerosol inhalation

Following intravenous injection of cultured DO11.10 T cells into BALB/c mice; the recipients were either left or exposed to OVA aerosols for 6 consecutive days. Then, mice were lavaged and the BAL cells were collected by cytospin and stained with modified Wrights stain to examine the differentiate count under light microscope. The infiltration of macrophages and neutrophils into the BALF was clearly evident in mice that had received Th1 cells and were subsequently exposed to OVA aerosols (Figure 3.13b). After 3 days of challenge the number of neutrophils present in the BALF had increased and this reached a maximum level after 6 days and had begun to return to normal after 10 days of challenge (Figure 3.13b). The magnitude of the T cell response correlated with the level of recruitment of neutrophils into the lungs since the numbers in the BAL peaked by 6 days of antigen inhalation but were absent by 10 days. In contrast, animals that had received Th2 cells and then inhaled OVA promoted and increased in the number of both macrophages and eosinophils after 6 days. The number of eosinophils remained high even after 10 days of challenge (Figure 3.13a). This was determined by measuring the total levels of the eosinophil peroxidase and by the cytochemical determination of eosinophil numbers (Table 3.1). Animals that had received Th1 cells and inhaled OVA showed no evidence of pulmonary eosinophilia. Since the level of immune-modulaton is directly proportional to the severity of the inflammation, it implies that the resulting inflammation represents a point of equilibrium, which is largely controlled by the immuno-modulatory

events described here. Therefore, to investigate the regulation of pulmonary inflammation following antigen inhalation in the lungs of mice that have received DO11.10 T cells of either Th1 or Th2 phenotype is important.

Experimental Groups	%Lym	%Mac	%Eso	%Neu	%Epi	Total Cell Counts
Th1	7.2	88.0	0	0	9.8	$1.8x10^5$
Th1 OVA *in*	32.1	63.3	0.2	38	0.6	$5.2x10^5$
TH2	6.4	86.5	0	0	7.1	$3.9 x10^5$
Th2 OVA *in*	38.5	40.5	17.0	1.9	1.2	$4.2 x10^5$

Table 3.1
BALF differential cell counts

Th1 or Th2 DO11.10 cells were injected *iv* (10^7) into naïve BABL/c mice which were then exposed to OVA aerosols for 6 consecutive days. On day 7 mice were sacrificed and BALF collected. BAL cells were cytospine onto glass slides using $5x10^5$ cells at 800rpm. Slides were air dried and differentiated by the same examiner blinded to sample identity counting at least 300 cells with a light microscope (n=3).

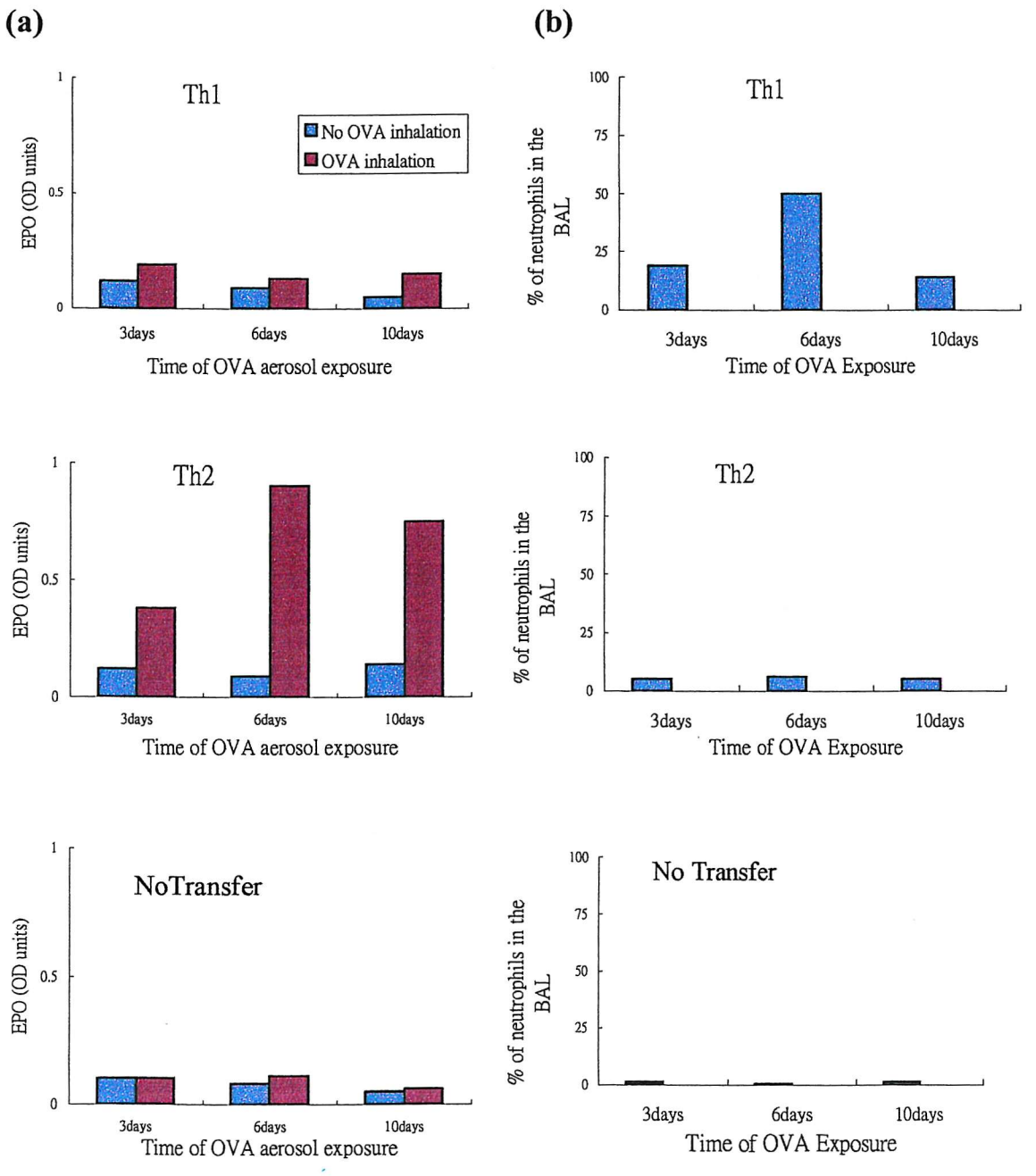


Fig.3.13
Pulmonary inflammation induced following OVA inhalation

DO11.10 T cells bearing either a Th1 or Th2 phenotype were injected into the tail veins of BALB/c mice (10^7/ mouse). Mice were then either left alone or exposed to repeated OVA aerosol challenge. After 3, 6 and 10 days BALF was taken and the levels of eosinophil peroxidase (a) and number of neutrophils (b) present determined.

3.5 Summary

A surprising observation was that during Th1-driven pulmonary inflammation, the proliferative response of the DO11.10 T cells present in the lungs was severely attenuated within 3 days. This immune modulation was associated with a dramatic reduction in the level of IL-2 produced by DO11.10 T cells present in the lung tissue in response to OVA 323-339 peptide. Such kinetics in the modulation of the T cell response was closely associated with the recruitment of neutrophils into the lung, which peaked by 6 days of antigen inhalation above, was completely lost by 10 days. This aerosol-induced unresponsiveness was tissue specific and was similar to the severe attenuation observed in lung T cell response when the DO11.10 mice themselves inhale antigen. A phenomena which we have reported previously. In contrast, when DO11.10 T cells bearing a Th2 phenotype were adoptively transferred into BALB/c hosts, the T cell response taking place in the lung tissue was far more protracted. The proliferative responses and production of IL-2 by DO11.10 T cells in the lungs was not significantly reduced until 6 days of aerosol exposure and then only partially. Interestingly, at this time point the DO11.10 T cells present in the lung tissue begin to produce IL-4 and IL-5.

It is clear that the nature of the T cell response developing in the lung mucosa in response to inhaled antigen not only influences the type of inflammation but also the fate of the T cells in the lungs. Preliminary observation suggest that the attenuation of Th1 and Th2 responses was a consequence of the modulation of T cell function by tissue macrophages but involving epithelial cells. The mechanisms by which these cells mediate these effects

in the site of inflammation remain unknown. Preliminary data demonstrated that T cells that had have been activated are subjected to immune modulation and ultimately killed. Since T cells take primacy in driving the inflammatory response to inhaled antigens, we speculate that the attenuation of the parenchymal T cell response is an effective way to regulate inflammation in the lung mucosa. Moreover, that change in the properties of the inflammatory cells convert them from being effective antigen presenting cells, to a state in which they attenuate the T cell response. Consequently, the level of immune-modulation is site specific and directly proportional to the severity of the inflammation.

In conclusion, DO11.10 T cells do enter the lung tissue following adoptive transfer into the BALB/c recipients and their numbers are increased following exposure of the animals to aerosolized OVA. However, following OVA inhalation, the proliferation and production of IL-2 by LMC in response to anti-CD3 was severely attenuated in both Th1 and Th2 recipient mice. Moreover, removal of F4/80+ macrophage reversed the growth arrest and attenuation restored IL-2 production. The infiltration of neutrophil into the BALB/c was evident in mice that had received Th1 and exposed to OVA aerosol but pulmonary eosinophilia was shown in the mice that had received Th2 cells.The phenotype of DO11.10 Th1 and Th2 cells cultured for 8d was stable in vivo since Th1 DO11.10 T cells found in the lung produced high IFN-γ and IL-2 and the Th2 cell line produced high IL-4, lower IL2 and no detectable INF-γ. Interestingly, no detectable IL-5, IFN-γ or TNF-α was detectable in BALF by ELISA.

Chapter Four

The Role of COX-2 in Modulating Pulmonary Th1 and Th2 Mediated Inflammation

4.1 Introduction

Having developed a model of aerosol induced pulmonary inflammation one aspect that was of interest was the role of cyclooxgenases in modulating the inflammatory process. There were several reasons for this choice. Firstly, aerosol inhalation is associated with entry of the T cells in the lung into a state of growth arrest (Lee SC et al 1999). This is associated with a marked reduction in the levels of IL-2 produced and a failure to progress through cell cycle. The onset of growth arrest is mediated by lung macrophages. Preliminary attempts to deplete macrophages from the lungs of mice using gadolinium chloride or carrageenan did not prove successful. However, some of the immune regulatory effects of lung interstitial macrophages may be mediated by prostaglandins produced by these cells. Certainly, growth arrest and failure to produce IL-2 can be caused by treatment of T cells with prostaglandin E2 (Belz M et al 1991, Snijdwint FG et al 1993). Secondly, prostaglandin E2 has been shown to favour Th2 responses by blocking IL-12 production by dendritic cells (Kalinski P et al 1997). Consequently, treatment of mice with an inhibitor of cyclooxygenase might be expected to ameliorate pulmonary eosinophilic inflammation. Finally, 10-15% of asthma patients is sensitive to aspirin. It has been proposed that in these individuals blocking the cyclooxgenase pathway increase production of cysteinyl leukotrienes by increasing the amount of available arachidonic acid. These mediators subsequently cause bronchocontriction and mucus secretion and may be responsible for the pathogenesis in exacerbation's of asthma following NSAID ing Us of ast aspirin intolerant asthmatics. However, as to whether shunting arachidonic acid into the 5-LO pathway is entirely responsible for aspirin sensitivity remains controversial. In the course of these experiments we attempt to address the question as to whether prostanoids produced in the lung influence the pulmonary T cells and inflammatory response.

4.2 Results

4.2.1 Treatment of mice with NSAID increases the level of pulmonary eosinophils

The first experiments performed were to investigate the effect of a specific inhibitor of COX-2 and the non-specific COX inhibitors indomethacin and lysine-aspirin on pulmonary inflammation. The model of aerosol challenge that had been developed in the previous chapter was used for these studies. Th1 and Th2 DO11.10 T cells were generated by culture with OVA peptide and exogenous cytokines over 8 days. $10x10^6$ Th1 or Th2 cells were transferred to the BALB/c recipients which were then exposed to aerosolised OVA (0.5%) for 6 consecutive days. During the OVA inhalation period, mice were either untreated or treated with the non-steroid anti-inflammatory drugs (NSAIDs) indomethacin, lysine aspirin or NS-398. The NS-398 is a commercially available COX-2 specific inhibitor having minimal effects on COX-1. The experimental groups comprised of animals treated with the COX-2 specific inhibitor, NS-398 (3 *ip* injections a day at 8 hours intervals of 10mg/kg), indomethacin (a single *ip* injection of 5mg/kg) and lysine-aspirin (a single *ip* injection of 100mg/kg). The lung inflammation was monitored by evaluating cell infiltration into the BALF and the functional properties of the T cells was evaluated by dispersing lung tissue in collagenase and measuring cytokine production following stimulation with OVA peptide, or KJ1-26 mAb or anti-CD3 antibody. Mice were then sacrificed on day 7 to perform the bronchoalveolar lavage and prepare lung mononuclear cells. The functional properties of the lung T cells were analysed by measuring cytokine production following stimulation with OVA peptide, KJ1-26 or anti-CD3 mAb. As demonstrated previously BALB/c mice which had received DO11.10 T cells bearing a Th2 phenotype on inhaling aerosolised OVA, developed a pulmonary eosinophilia as determined by the

BALF differential cell count (Table 4.2.1). Over 4 experiments cytochemical staining of BAL cells revealed that in animals treated with indomethacin or the COX-2 specific inhibitor (NS-398), the level of pulmonary eosinophilia was markedly increased in magnitude over that found in animals that had not been treated with NSAID (Table 4.2.1, Fig 4.2.1b). Specifically, following OVA inhalation the number of eosinophils present in the BALF increased from undetectable to 17%. Treatment on mice with indomethacin or NS-398 increased the percentage that were eosinophils to 28.5% and 33.8% respectively (total number was 162450 and 317720 respectively). This was confirmed on measuring the EPO activity of the lysed cells present in the BAL (Figure 4.2.2). The principle advantage of measuring EPO activity is that the measurements are non-subjective. The level of EPO was similarly enhanced in both the indomethacin treated Th2 group and NS-398 treated groups (Fig. 4.2.2). In addition to determining cell associated EPO the levels of soluble EPO in the BALF were also evaluated. This presumably reflects EPO released by activated or dying eosinophils. Low levels of EPO were present in BALF from the indomethacin treated mice but not in the untreated control animals (Fig.4.2.2). The levels of soluble EPO in BALF from NS-398 treated mice were also slightly increased. These data demonstrate that COX-2 specific inhibitors like the other NSAIDs increase the level of pulmonary eosinophilia in Th2 recipients.

Experimental Groups	%Lym	%Mac	%Eso	%Neu	%Epi	Total Cell Counts
Th1	7.2	88.0	0	0	9.8	$1.8x10^5$
Th1 OVA *in*	32.1	63.3	0.2	38	0.6	$5.2x10^5$
Th1 OVA *in*+INDO	20.7	72	0	3.5	3.8	$5.1x10^5$
Th1 OVA *in*+NS398	17.6	77.1	0	2.0	3.3	$5.6x10^5$
Th2	6.4	86.5	0	0	7.1	$3.9 x10^5$
Th2 OVA *in*	38.5	40.5	**17.0**	1.9	1.2	$4.2 x10^5$
Th1 OVA *in*+INDO	31.7	30.7	**28.5**	7.3	1.8	$5.7x10^5$
Th1 OVA *in*+NS398	33.9	27.8	**33.8**	3.3	1.2	$9.4x10^5$

Table 4.2.1

Treatment of mice COX-1 and COX-2 non-selective inhibitorIindomethacin and COX-2 selective inhibitor NS-398 results in increased pulmonary eosinophilic inflammation.
$10x10^6$ DO11.10 Th1 or Th2 effector T cells were injective intravenously into BALS/c mice. Mice were either untreated , exposed to aerosolized 0.5% OVA for 6 consecutive days and treated with Indomethacin (asingle *ip* injection at 5mg/kg) or NS-398 (*ip* injection every 8 h at 10mg/kg). On day 7, mice were sacrificed, and BALF collected and examined by cytochemical staining. The percentage of lymphocytes, macrophages, eosinophils, neutrophils and epithelial cells present are shown.

The total cell number in BALF was increased after OVA aerosols challenge in both Th1 and Th2 recipient mice (Fig.4.2.1). The number of lymphocytes present in BAL was increased following OVA inhalation in the Th2 recipient mice. This was further increased in mice treated with the COX-2 selective inhibitor, NS-398 or indomethacin. In contrast, the macrophage number was slightly

decreased in both NS-398 and indomethacin treated groups in both Th1 and Th2 recipient mice. An airway neutrophilia was observed in Th1 recipient mice following OVA inhalation and this was not changed following treatment with either NS-398 or indomethacin (Fig.4.2.1a).

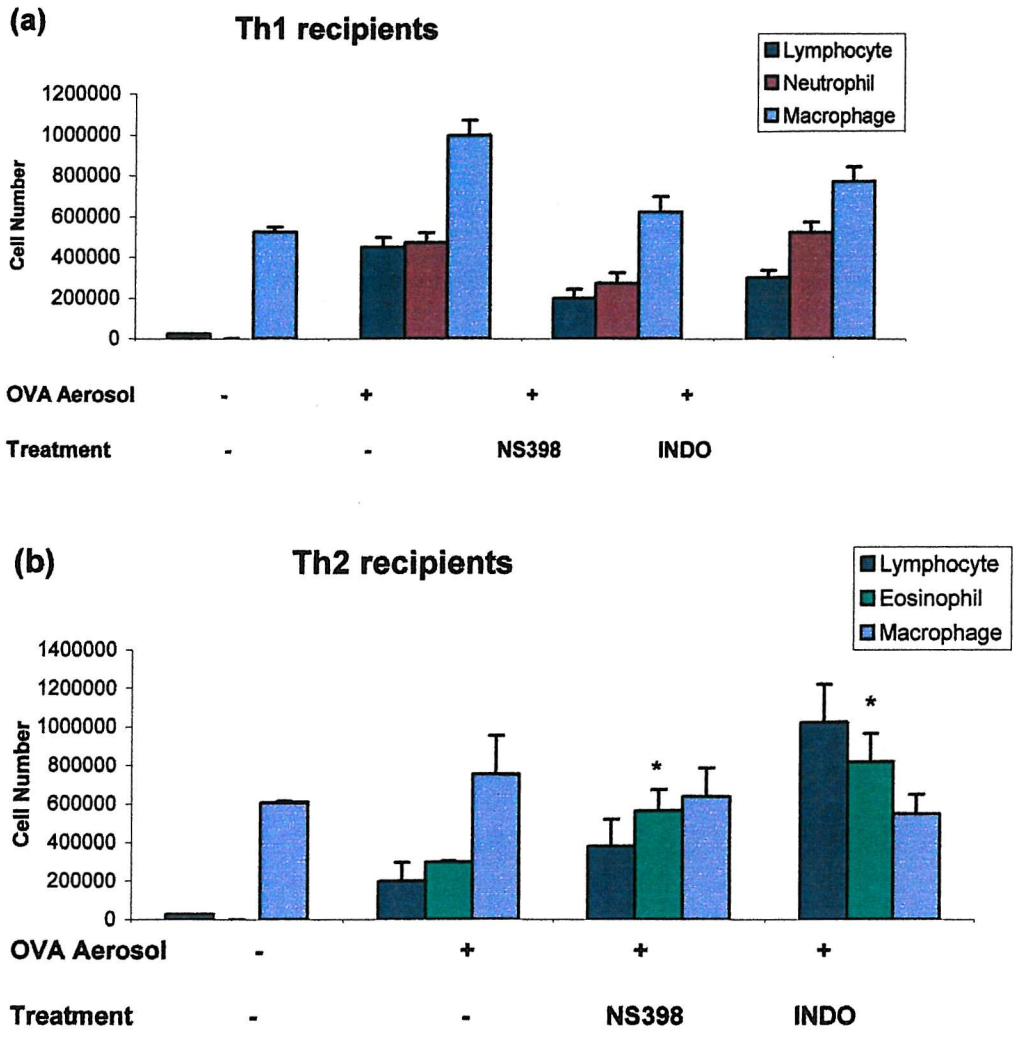


Fig. 4.2.1
COX-2 selective inhibitor NS-398 and non-selective inhibitor INDO increased airway eosinophilic inflammation
DO11.10 Th1 or Th2 effector cells (10^7) were injected *iv* into BALB/c recipients that were then exposed to OVA aerosols for 6 consecutive days. Mice were either untreated or injected *ip* with NS398 (10mg/kg every 8 h) or indomethacin (INDO, 5mg/kg per day). On day 7, BALF was collected and cell differential percentages determined by light microscopic evaluation of cytospin preparations. Data are expressed as absolute cell numbers and are means for 4 separate experiments (*$p<0.05$).

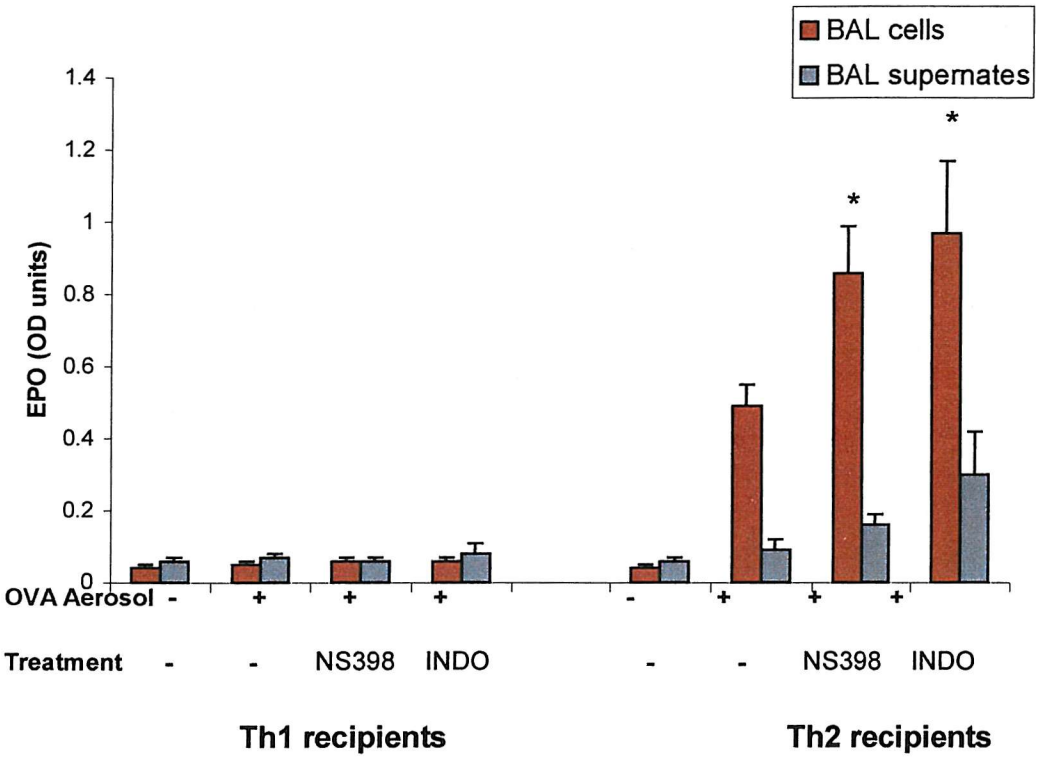


Fig. 4.2.2

Treatment of mice with indomethacin or NS-398 increased cell associated and released EPO in BALF.
DO11.10 Th1 or Th2 cells (10^7) were injected *iv* into BALB/c recipients that were than exposed to OVA aerosols for 6 consecutive days. Mice were either untreated or injected with NS-398 (10mg/kg *ip* every 8h) or indomethacin (INDO, 5mg/kg *ip* per day). On day 7, BAL fluid was collected and eosinophil peroxidase (EPO) levels in BAL cells or BAL supernatants were determined by colorimetric analysis (n=4, *$p<0.05$).

4.2.2 Effect of corticosteroids on differential cell counts and EPO in the BALF of mice

Other models of pulmonary inflammation have demonstrated that the eosoinophilia can be reversed by treating mice with corticosteroids. To examine if this was true of our model, Th1 or Th2 phenotype DO11.10 T cells were adoptively transferred into either untreated or treated with dexamethasone and exposed to OVA inhalation. BALF was harvested and cytospin slide staining examination revealed a marked reduction of absolute eosinopil counts in the Th2 BALB/c recipients treated with dexamethasone. In contrast, Th2 recipients treated with NS-398 alone showed an increase in the eosinophil count significantly. EPO assay of the BALF revealed a decrease in the Th2 recipients following treatment with dexamethasone and conversed Th2 recipient mice treated with indomethacin or NS-398 had high levels of EPO. Therefore, corticosteroids can inhibit the eosinophil production (Table 4.2.2).

(a)

	Lym	Mac	Neu	Eos	Epi	Total cell counts
Th1	$171.4x10^5$	$109x10^5$	$71.8x10^5$	$1.87x10^5$	$21.4x10^5$	$37.5x10^6$
Th2	$128x10^5$	$118.8x10^5$	$3.0x10^5$	$43.8x10^5$	$21.4x10^5$	$30x10^6$
Th2+Dexa	$28.8x10^5$	$207.8x10^5$	$2.5x10^5$	$2.5x10^5$	$8.5x10^5$	$25x10^6$
Th2+NS-398	$72.4x10^5$	$107.1x10^5$	$1.1x10^5$	$62.9x10^5$	$5.1x10^5$	$27x10^6$

(b)

	EPO (OD units)
Th2	0.478
Th2 + Dexamethasone	0.285
Th2 + Indomethacin	0.565
Th2+NS-398	0.480

Table 4.2.2

Effect of corticosteroids on absolute differential cell counts and EPO in BALF of Th2 recipients exposed to OVA challenge

Following adoptive transfer of Th2 effector cells, mice were treated with a dose of 1mg/kg by injection of 100 µl of dexamethasone solution *iv* using the lateral tail vein daily during 6 consecutive days of OVA aerosols challenges. Then, BALF was harvested and EPO assays and cytospin slide staining performed.

4.2.3 Effect of NSAID on prostaglandins present in the BALF of mice

Prostaglandins are the important mediators or modulators in inflammation and thrombosis. It was important to evaluate which members of the prostnoid family were present in the lung environment and susceptible to inhibition following treatment with the COX-2 selective inhibitor NS-398 or the COX-1 and COX-2 non-selective inhibitor indomethacin. Using the experimental method described above, mice were given DO11.10 Th1 or Th2 phenotype T cells by intravenous injection. During the OVA inhalation period, mice were either non-treated or treated with indomethacin, lysine aspirin, or NS-398. Then, BALF was collected and frozen down at –80°C for prostanoid analyses. Analysis of PGD2, PGE2, PGF2α, TXB2, and 6-ketoPG1α levels in BALF by EIA reveiled that:

(1) Some levels of PGD2 were found in the BALF of Th1 and Th2 recipients but the levels were significantly increased after OVA challenge for 6 consecutive days. No change of the PGD2 levels in both Th1 and Th2 recipient groups after treatment of NS-398 but there was more than 50% reduction of the levels in either group treated with indomethacin (Fig.4.2.3).

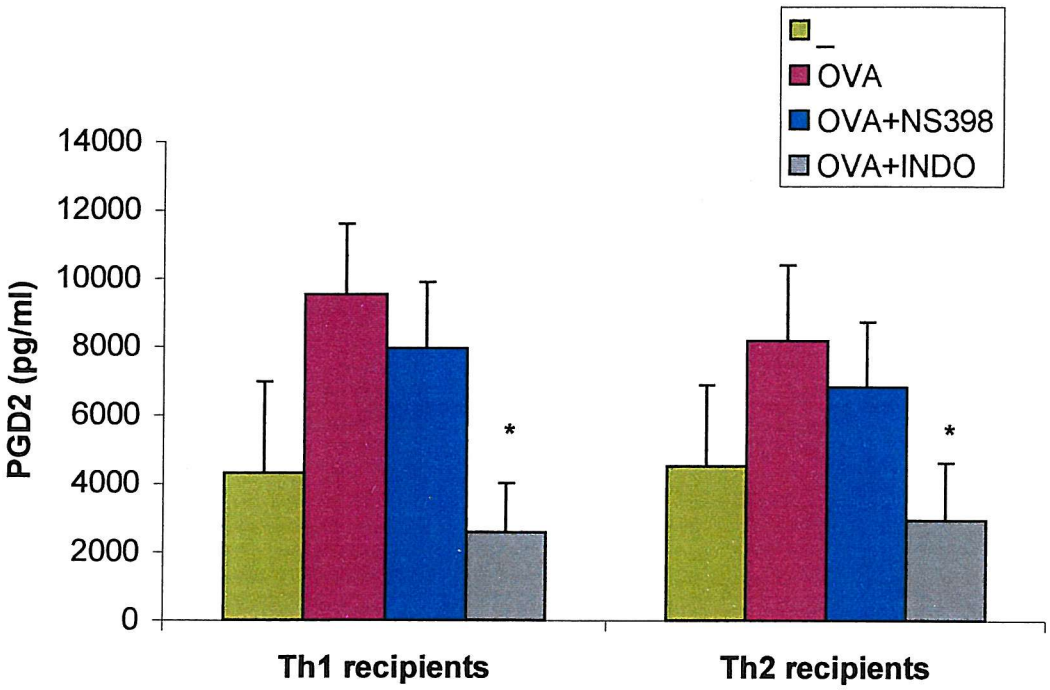


Fig. 4.2.3

PGD_2 levels in BALF are reduced by Indomethacin but not NS-398 treatment.
DO11.10 Th1 or Th2 cells (10^7) were injected *iv* into BALB/c recipients that were then exposed to OVA aerosols for 6 days. Control mice (-) were not exposed to OVA inhalation. Mice were either untreated or injected *ip* with NS-398 (10mg/kg every 8h), or indomethacin (INDO, 5mg/kg oral per day). On day 7, BALF was collected and PGD_2 levels determined by EIA (n=8, *$p<0.05$).

(2) PGE2 levels in BALF, approximately 35000 pg/ml, were measured in Th1 and Th2 recipients mice. These were increased to 75000 pg/ml in Th1 and Th2 recipients after OVA inhalation. There was no effect on the PGE2 levels following the treatment of NS-398 but indomethacin or lysine aspirin significantly reduced the levels (Fig.4.2.4).

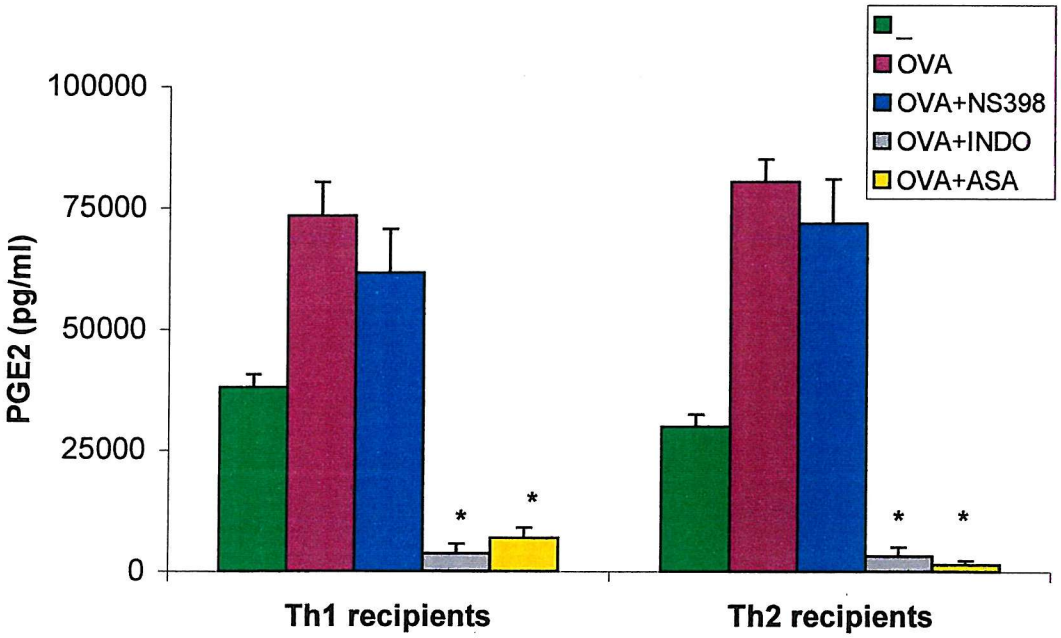


Fig. 4.2.4

PGF_2- levels in BALF are reduced by Indomethacin but not NS398 treatment.
DO11.10 Th1 or Th2 cells (10^7) were injected *iv* into BALB/c recipients that were then exposed to OVA aerosols for 6 days. Control mice (-) were not exposed to OVA inhalation. Mice were either untreated or injected *ip* with NS-398 (10mg/kg every 8h), or indomethacin (INDO, 5mg/kg oral per day). On day 7, BALF was collected and $PGF_{2\alpha}$ levels determined by ELISA (n=8, *$p<0.05$).

(3) The production of PGF2α in BALF was similar to those of PGE2. There was an increase in PGF2α in the Th1 and Th2 recipients, in animals untreated or treated with NS-398 and exposed to OVA aerosols. Also there was significantly reduced the levels in the indomethacin treated Th1 and Th2 BALB/c recipients (Fig.4.2.5).

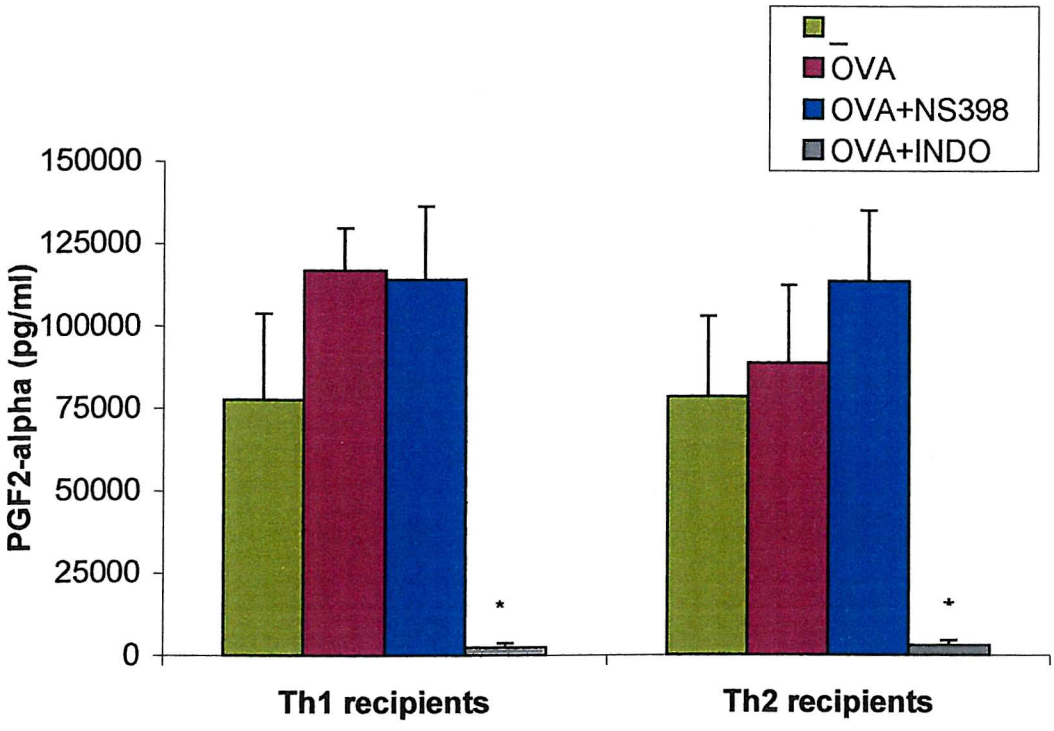


Fig. 4.2.5

$PGF_{2\text{-}\alpha}$ levels in BALF are reduced by Indomethacin but not NS398 treatment.
DO11.10 Th1 or Th2 cells (10^7) were injected *iv* into BALB/c recipients that were then exposed to OVA aerosols for 6 days. Control mice (-) were not exposed to OVA inhalation. Mice were either untreated or injected *ip* with NS-398 (10mg/kg every 8h), or indomethacin (INDO, 5mg/kg oral per day). On day 7, BALF was collected and $PGF_{2\alpha}$ levels determined by ELISA (n=8, *$p<0.05$).

(4) Since TXA2 is rapidly hydrolysed to TXB2 *in vivo*, the later is measured by EIA. Some levels of TXB2 in BALF were measured in both Th1 and Th2 recipients. After OVA challenge, the TXB2 levels were markedly increased but the levels were dramatically reduced following treatment with indomethacin. No change of the TBX2 levels were found in the Th1 and Th2 recipient groups treated with NS-398 (Fig 4.2.6).

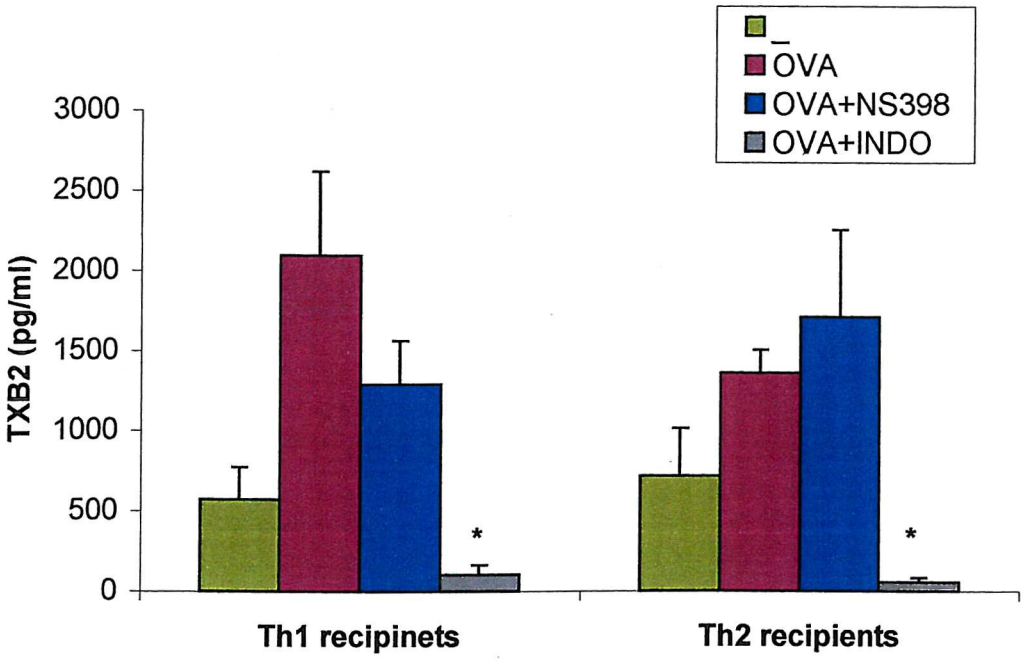


Fig. 4.2.6

TXB_2 levels in BALF are reduced by Indomethacin but not NS-398 treatment.
DO11.10 Th1 or Th2 cells (10^7) were injected *iv* into BALB/c recipients which were then exposed to OVA aerosols for 6 days. Control mice (-) were not exposed to OVA inhalation. Mice were either untreated or injected *ip* with NS-398 (10mg/kg every 8h), or indomethacin (INDO, 5mg/kg per day). On day 7, BALF was collected and TXA_2 levels determined by EIA (n=8, *$p<0.05$).

(5) 6-ketoPG1α is the stable metabolite of PGI2. Indomethacin and aspirin almost completely reduced the 6-ketoPG1α production in BALF in the Th1 and the Th2 recipients when compared to the OVA challenged animals. NS-398 treatment of Th1 and Th2 groups also reduced the 6-ketoPG1α levels in BALF by 50% (Fig.4.2.7).

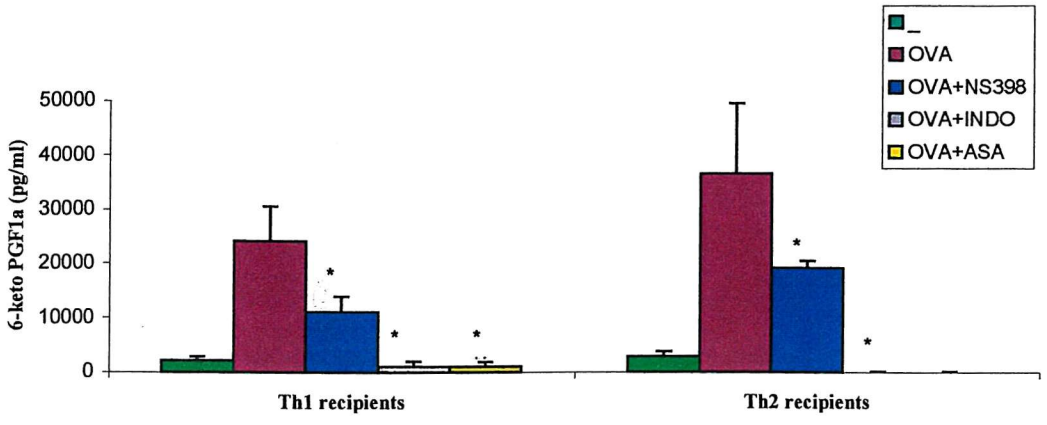


Fig. 4.2.7

Indomethacin, aspirin and NS-398 inhibited 6-ketoPGF1α levels in BALF.
DO11.10 Th1 or Th2 cells (10^7) were injected *iv* into BALB/c recipients that were then exposed to OVA aerosols for 6 days. Control mice (-) were not exposed to OVA inhalation. Mice were either untreated or injected *ip* with NS-398 (10mg/kg every 8h), singly injected *ip* with lysine aspirin (100mg/kg per day) or indomethacin (INDO, 5mg/kg per day). On day 7, BALF was collected and 6-ketoPGF$_{1\alpha}$ levels determined by EIA (n=8, *$p<0.05$).

In conclusion, BALF from mice exposed to aerosolized OVA and treated with NS-398, the PGD2, PGE2, PGF2α, TXB2 levels remained high. This was in marked contrast to mice treated with the COX non-selective inhibitors, indomethacin or lysine aspirin, which dramatically reduced the levels of all five prostanoids. In contrast, the COX-2 selective inhibitor, NS-398 reduced the 6-ketoPGF1α production (Fig.4.2.7) but not PGD2, PGE2, PGF2α, and TXB2.

4.2.4 Effect of NSAID on LTB_4 present in BALF of mice

In addition to measuring prostaglandins present in the BALF, the levels of leukotriene was also evaluated. Some levels of LTB_4 are present in BALF of Th1 recipients, which did not inhale OVA. These levels were reduced following OVA inhalation but were not significantly changed when challenged Th1 recipients were treated with indomethacin. In contrast, unchallenged Th2 recipients had low levels of LTB_4 in the BALF. These were not significantly changed following OVA inhalation or treatment with indomethacin (Fig.4.2.8).

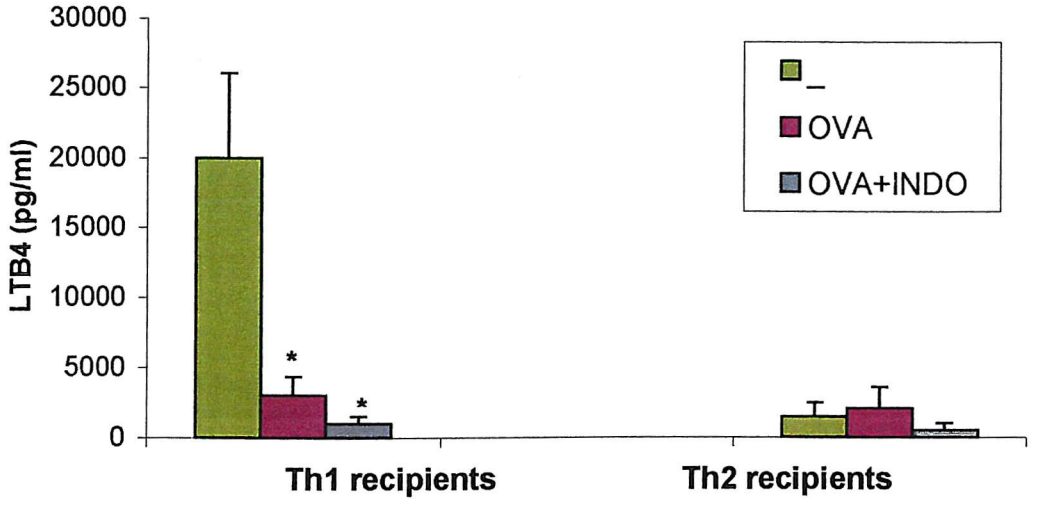


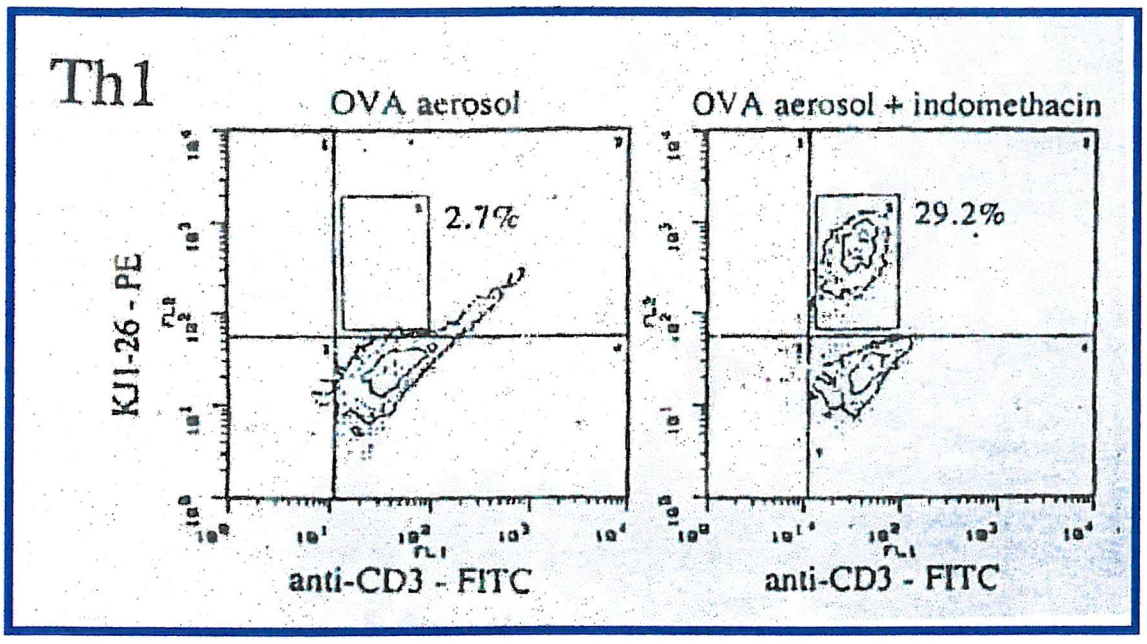
Fig.4.2.8

LTB_4 levels in BALF are not changed by Indomethacin treatment
DO11.10 Th1 or Th2 cells (10^7) were injected iv into BALB/c recipients, which were then exposed to OVA aerosols for 6 consecutive days. Control (-) were not exposed to OVA inhalation. Mice were either untreated or injected ip with indomethacin (INDO, 5mg/kg per day). On day 7, BALF was collected and LTB_4 levels determined by EIA (n=3, *$p<0.05$).

In summary, LTB_4 production does not appear to be involved in the elevated airway eosinophilic inflammation observed following treatment with COX-1 and COX-2 non-selective inhibitors.

4.2.5 Effect of NSAID on lymphocyte infiltration into the BALF

In both Th1 and Th2 recipient mice, OVA inhalation only resulted in the infiltration of small numbers of DO11.10 T cells into the BAL. Treatment of mice with indomethacin increased the number of OVA specific T cells present in the BAL of both Th1 and Th2 recipient mice following exposure to OVA aerosols (Fig. 4.2.9). Specifically, treatment of mice with indomethacin resulted in an increase in the percentage of CD4+ cells that were $CD4^{+}KJ1\text{-}26^{+}$ cells in the airway to 29.2% in the Th1 recipients and 24.8% in the Th2 recipients. However, whether the increase in cell number is a consequence of expansion due to local cell proliferation or alternatively recruitment of cells into the airway is not clear. Interestingly, there was no increase of DO11.10 T cells into the airway in the NS-398 treated group by using FAScan analysis (data not shown).



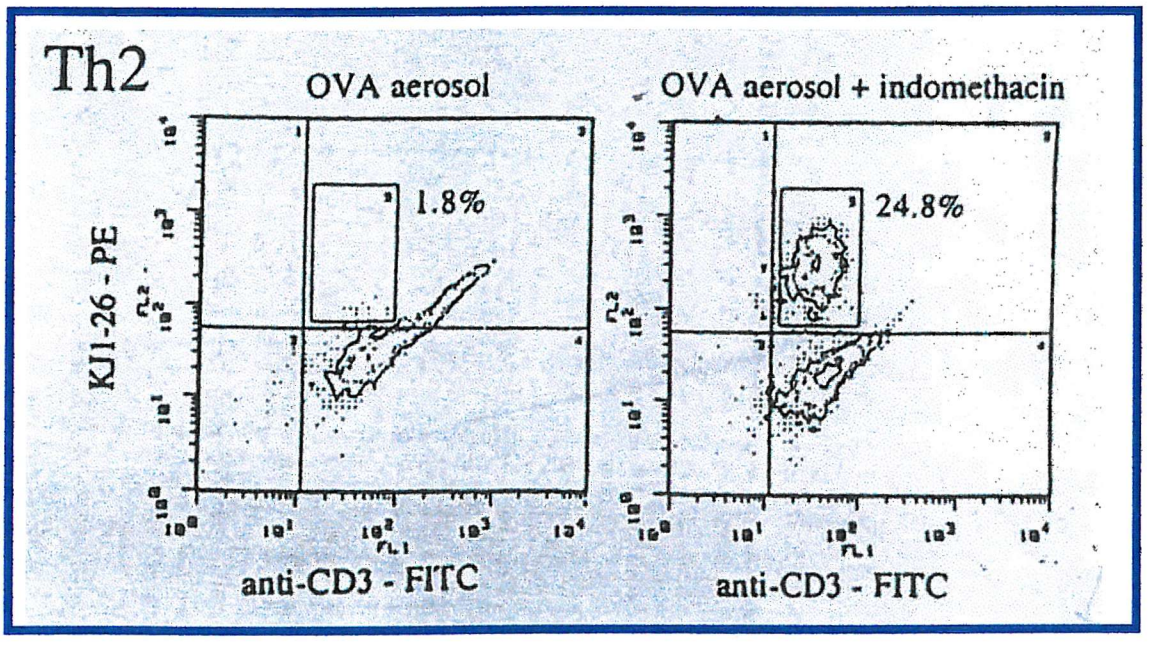


Fig.4.2.9

Indomethacin treatment resulted in an increase in the number of $CD4^+KJ1\text{-}16^+$ cells in the BALF in mice that had received either Th1 or Th2 cells and inhaled OVA.

BALF was collected from Th1 or Th2 BALB/c recipients after 5 days of exposure to OVA aerosols. Mice were either untreated or treated with indomethacin. The BAL cells were double-stained by using the clonotype specific mAb, KJ1-26 PE and anti-CD3-FITC. Immunofluorescence analysis of the BAL cells by using FACScan was then performed.

4.2.6 Effect of NSAID on cytokine production by LMC

The Th1 cytokine IFN-γ has been recognized as the key cytokine that can counter the allergic responses. Hence, it was important to understand whether NS-398 or indomethacin treatment reduced the IFN-γ produced by lung mononuclear cells (LMC).

To determine which cytokines were produced in the lungs of Th1 and Th2 recipient mice, two approaches were adopted. Firstly, lung tissue was dispersed in collagenase and the LMC stimulated with OVA peptide or anti-CD3. The cytokines produced were determined after 48 hours. In addition, the levels of IL-4, IL-5, and IFN-γ in the BALF were determined. LMC from Th1 recipients produced IFN-γ following stimulation with OVA peptide. Treatment of Th1 recipient mice with NS-398 resulted in reduced IFN-γ production by LMC from the Th1 group (Fig.4.2.10). In contrast, no IFN-γ was produced by LMC from Th2 recipients following restimulation with OVA peptide for 48 hours. Stimulation of LMC from Th1 recipients with plate-bound anti-CD3 promoted IFN-γ production from Th1 recipients but not Th2 recipients. For either peptide or anti-CD3 stimulation the production of INF-γ production was markedly increased after OVA inhalation.

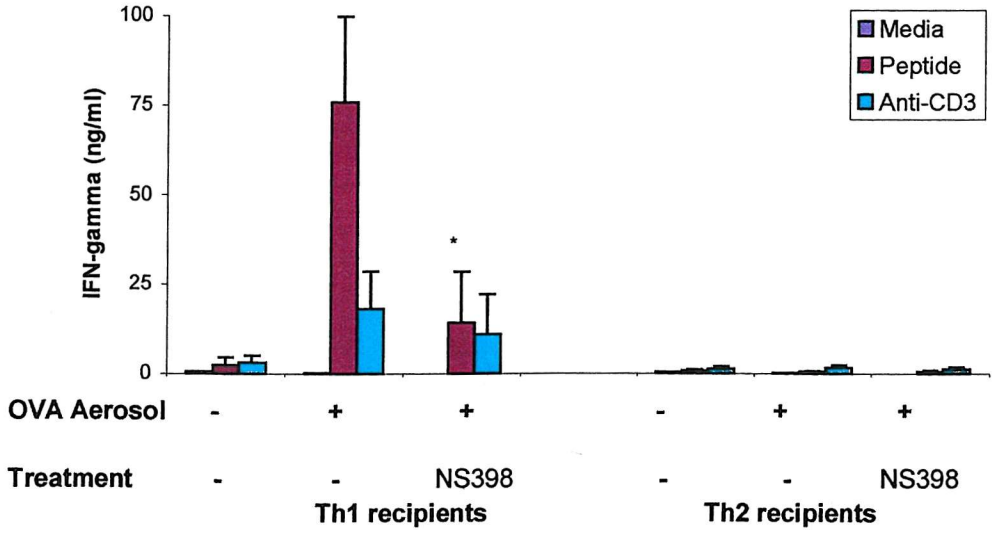


Fig. 4.2.10

Effect of OVA inhalation and NS-398 treatment on IFN-γ produced by LMC.
DO11.10 T cells bearing a Th1 or Th2 phenotype (10^7) were injected into BALB/c recipients that were then exposed to OVA aerosols for 6 consecutive days. Mice were either untreated or injected with NS-398 (10mg/kg *ip* every 8h). On day 7, LMC were isolated and restimulated with OVA peptide or anti-CD3 for 48h. IFN-γ production was determined by ELISA (n=4, *$p<0.05$).

IFN-γ production is induced by the cytokine IL-12 either alone or synergistically with cytokine IL-18. Consequently, we investigated whether the changes in IFN-γ production reflected changes in IL-12 or IL-18. We analyzed the cytokines IL-12 and IL-18 produced by LMC from Th1 or Th2 recipients exposed to aerosolize with OVA. LMC were stimulated with OVA peptide for 48 hours, the supernatant harvested and the IL-12 and IL-18 levels determined using commercial ELISA kits. Very low levels of IL-12 were detected both in Th1 and Th2 BALB/c recipients either treated with NS-398

or untreated and exposed to OVA aerosols (Fig.4.2.11). LMC from either Th1 or Th2 recipients produced IL-18 when simply cultured in media. IL-18 levels were reduced by LMC from mice exposed to aerosolized OVA irrespective of whether stimulated with KJ1-26 or anti-CD3. However, treatment with NS-398 (intraperitoneal injection every 8 hours at 10mg/kg) did not make any significant changes in the IL-18 level (Fig. 4.2.12). Therefore, it seems the decrease of IFN-γ production in mice treated with NSAIDs is not related to the amount of IL-12 and IL-18 produced.

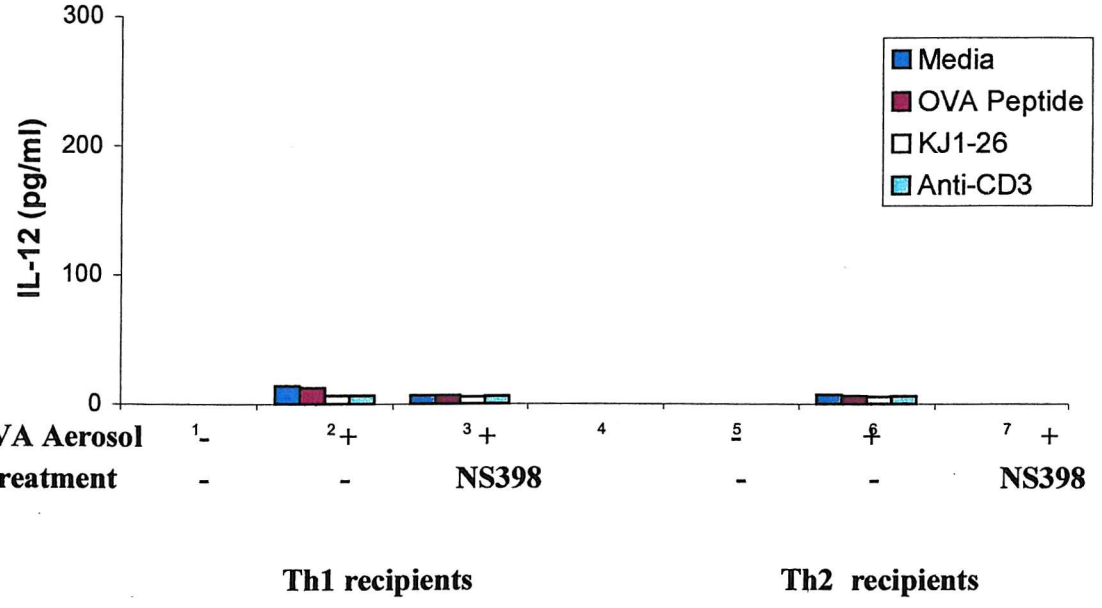


Fig. 4.2.11

Effect of OVA inhalation and NS-398 treatment on IL-12 produced by LMC.
DO11.10 T cells bearing a Th1 or Th2 phenotype (10^7) were injected into BALB/c recipients that were then exposed to OVA aerosols for 6 consecutive days. Mice were either untreated or injected with NS-398 (10mg/kg *ip* every 8h). On day 7, LMC were isolated and restimulated with OVA peptide, KJ1-26 or anti-CD3 for 48h. IL-12 production was determined by ELISA (n=4, *$p<0.05$).

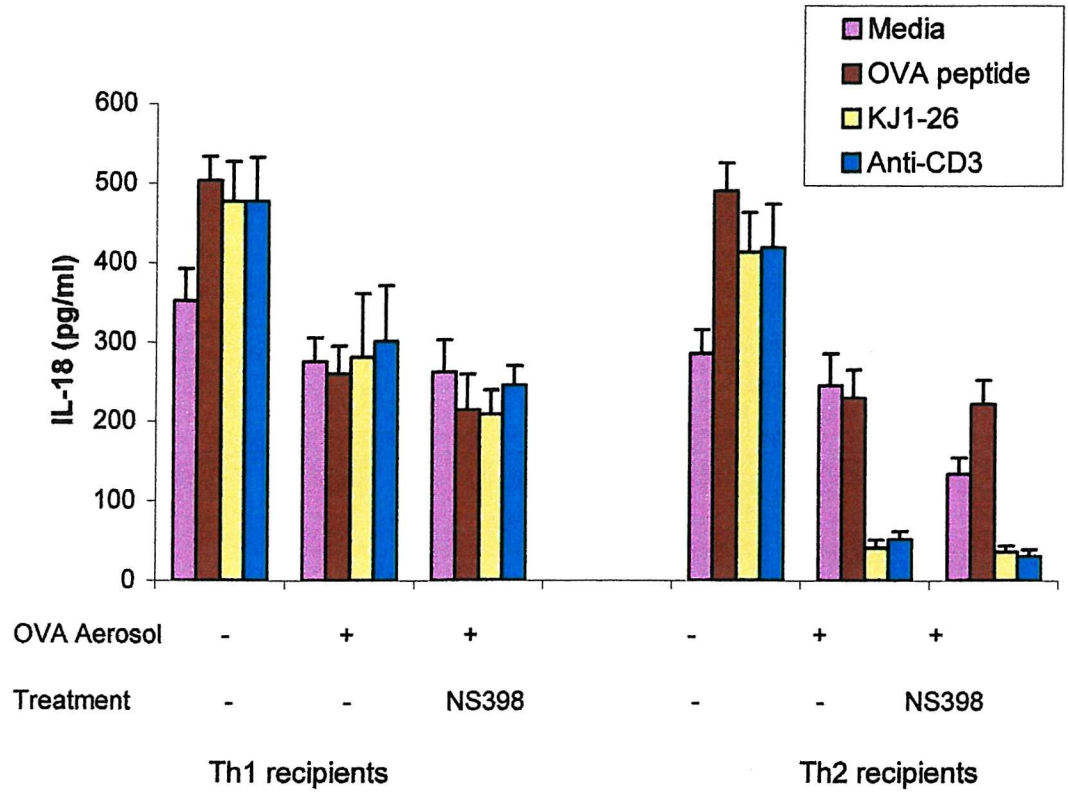


Fig. 4.2.12

Effect of OVA inhalation and NS-398 treatment on IL-18 produced by LMC.
DO11.10 T cells bearing a Th1 or Th2 phenotype (10^7) were injected into BALB/c recipients which were then exposed to OVA aerosols for 6 consecutive days. Mice were either untreated or injected with NS-398 (10mg/kg *ip* every 8h). On day 7, LMC were isolated and restimulated with OVA peptide, KJ1-26 or anti-CD3 for 48h. IL-18 production was determined by ELISA (n=4, *$p<0.05$).

In contrast, when examining the production of IL-5 by LMC, it was found that (1) LMC from Th2 recipient mice produced IL-5 in response to OVA peptide or anti-CD3 but not Th1 recipients (Fig. 4.2.13). (2) There was an increase in the levels of IL-5 in mice receiving Th2 cells and inhaling OVA

in response to anti-CD3 but not peptide. The IL-5 production by LMC from animals treated with NS-398 was similar to the OVA inhalation Th2 group (Fig. 4.2.13).

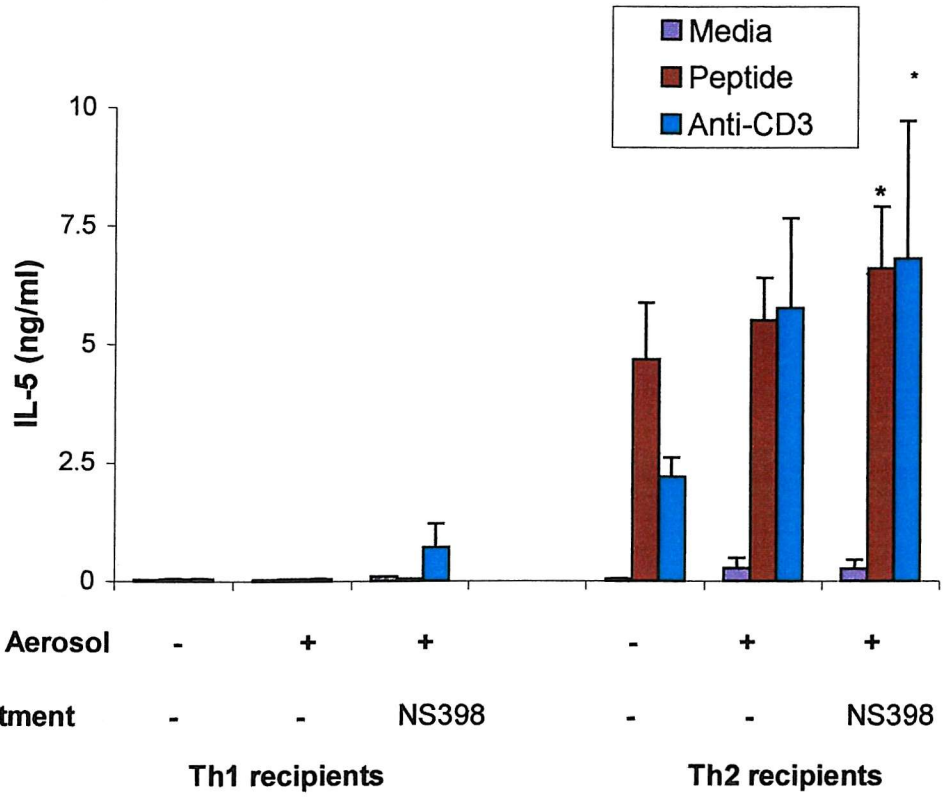


Fig. 4.2.13

Effect of OVA inhalation and NS-398 treatment on IL-5 product by LMC.
DO11.10 T cells bearing a Th1 or Th2 phenotype (10^7) were injected into BALB/c recipients that were then exposed to OVA aerosols for 6 consecutive days. Mice were either untreated or injected with NS-398 (10mg/kg *ip* every 8 h) On day 7, LMCs were isolated and restimulated with OVA peptide or anti-CD3 for 48 h. IL-5 production was determined by ELISA (n=4, *$p<0.05$).

4.2.7 Effect of NSAIDs on IFN-γ, IL-4, IL-5 present in the BALF of mice

Since it has proven difficult to use "real time" indicators of cytokine production by parenchymal LMC, BALF is a good measure of cytokine produced in lung inflammatory responses. Moreover, evaluating cytokines produced by LMC is only an approximation to those produced in an inflammatory response taking place in lungs of animals, BALF can reveal the measure of lung inflammatory response even though cytokine levels are low. It was important to investigate the cytokine levels in the airways of mice and whether they were affected by treatment with COX-2 specific inhibitor, NS-398. Attempts to detect cytokines in BALF using non-commercial ELISA failed to detect IL-4, IL-5 or IFN-γ (sensitivity approximately 100 pg/ml). Consequently, commercial kits were used to assay for these cytokines in BALF (sensitivity ≅ 2 pg/ml). In the mice which had received DO11.10 Th1 or Th2 cells and inhaled OVA, very low levels of IFN-γ were present in BALF of Th1 recipients and not increased in NS-398 treated animal (Fig.4.2.14). No clear difference between Th1 and Th2 recipients could be obtained. In contrast, BALF from Th2 recipients that had inhaled OVA contained 50 pg/ml of IL-4 and 30 pg/ml of IL-5. Furthermore, the IL-4 and IL-5 levels in BALF are increased significantly in NS-398 treated Th2 recipients (90 pg/ml and 70 pg/ml respectively) (Fig.4.2.15, Fig.4.2.16). Very low levels of IL-4, IL-5 were found in BALF of Th1 recipients.

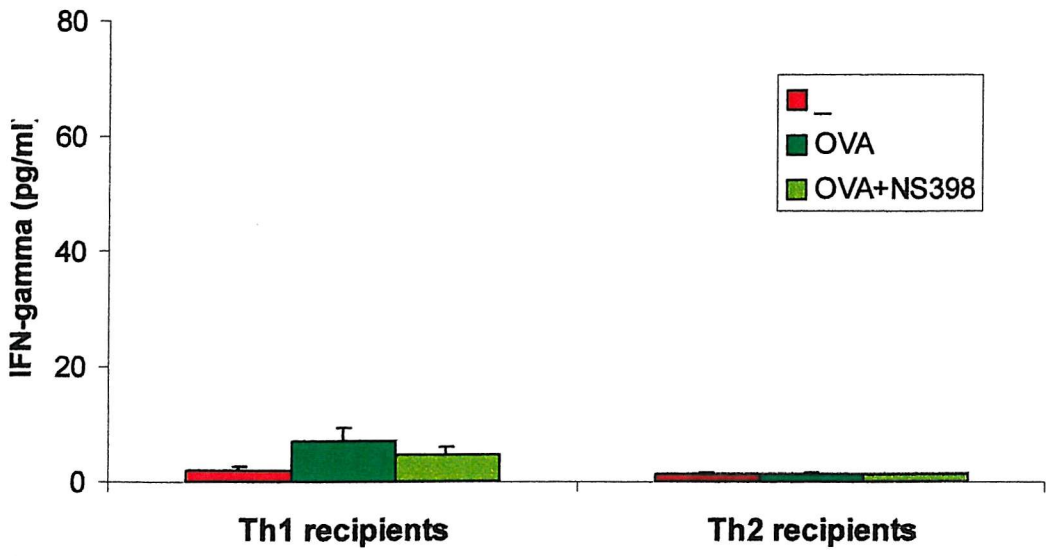


Fig. 4.2.14

Low levels of INF-γ were present in BALF of Th1 recipients and increased in NS-398 treated animal. Following adoptive transfer of Th1 or Th2 cells, recipient mice were either untreated or exposed to OVA aerosols for 6 days. In some control groups, mice were treated with NS-398 (injected *ip* at 10mg/kg every 8h). On day 7, BALF was collected and INF-γ levels determined by ELISA (n=4, *$p<0.05$).

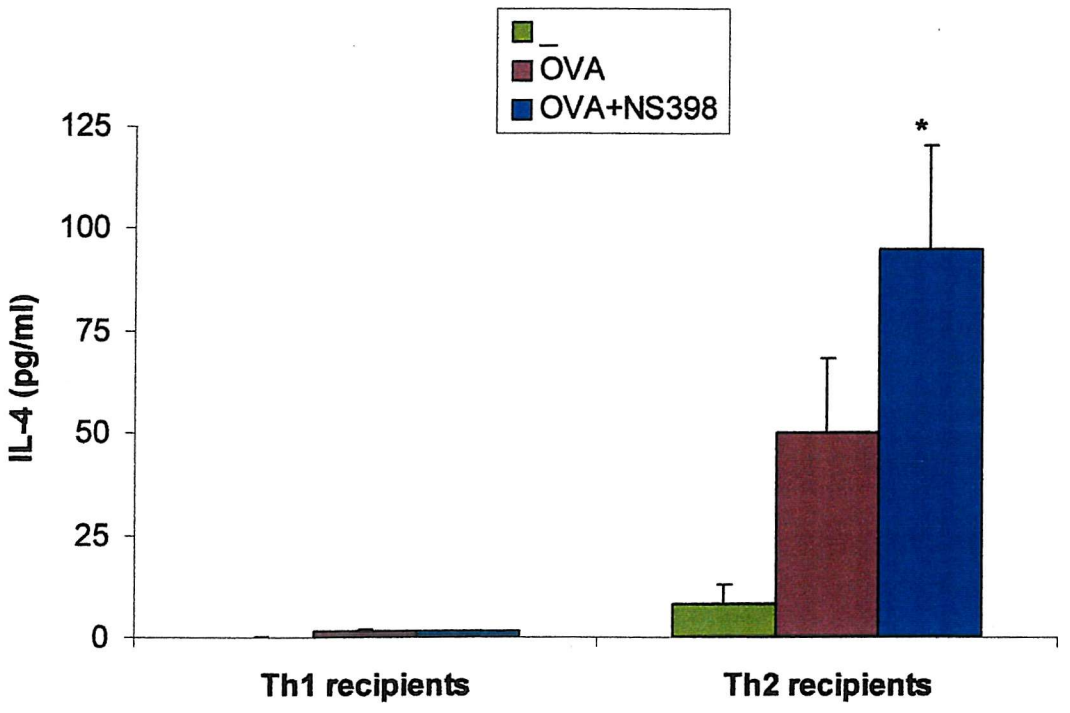


Fig. 4.2.15

IL-4 levels in BALF are increase in NS-398 treated animals.
Following adoptive transfer of Th1 or Th2 cells, recipients mice were either untreated or exposed to OVA aerosols for 6 days. In some control groups, mice were treated with NS398 (injected *ip* at 10mg/kg every 8h). . On day 7, BALF was collected and IL-4 levels determined by ELISA (n=4, *$p<0.05$).

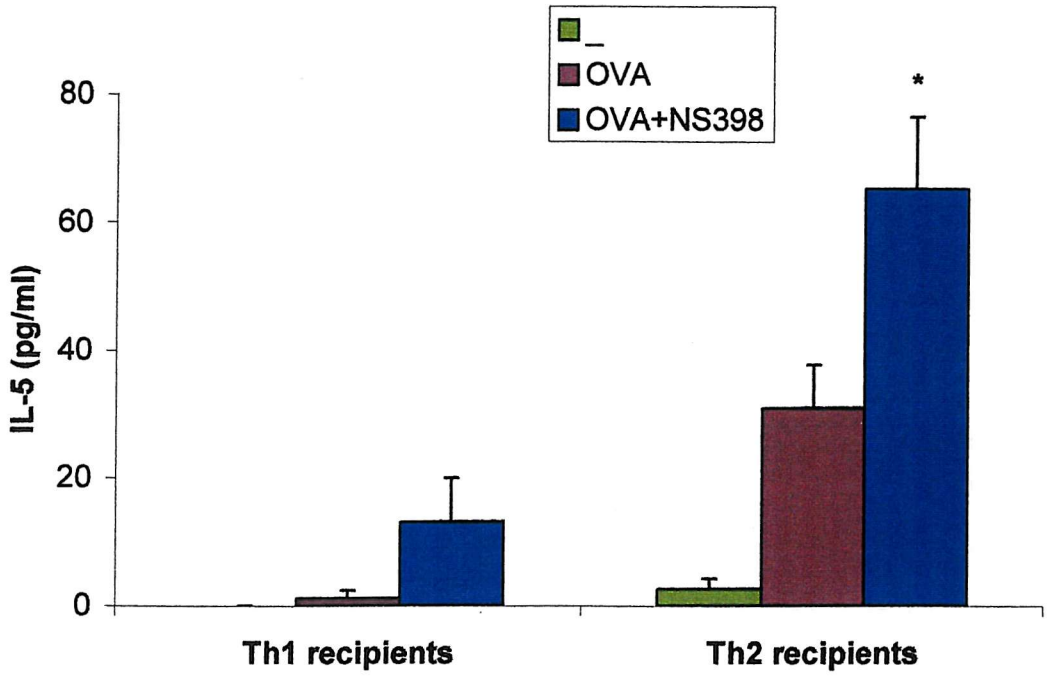


Fig. 4.2.16

IL-5 levels in BALF are increased in NS398 treated animals.
Following adoptive transfer of Th1 or Th2 cells, recipient mice were either untreated, exposed to OVA aerosols for 6 days. In some control groups, mice were treated with NS398 (injected *ip* at 10mg/kg every 8h). On day 7, BALF was collected and IL-5 levels determined by ELISA (n=4, *$p<0.05$).

4.2.8 Role of TNF-α on the level of pulmonary eosinophilic inflammation

TNF-α plays a key role in orchestrating inflammation and immunity. It functions in an autocrine or paracrine manner to amplify the lung inflammatory response through activation of the nuclear factor-kappa B (NF-kappaB) (Lentsch AB et al 1998). TNF-α is a potent inhibitor of IL-12 p40 and p70

secretion from human macrophages induced by LPS or S. aureus. Recent data demonstrate a selective negative regulation on IL-12 by TNF-α, identifying a direct negative feedback mechanism for inflammation-induced suppression of IL-12 gene expression (Ma X et al 2000). TNF-α has the ability to induce neutrophil transendothelial and transepithelial migration and was largely dependent on the generation of IL-8 by the endothelial or pulmonary epithelial cells (Casale TB et al 1999). Abundant evidence supports the importance of TNF-α in the pathogenesis of pulmonary fibrosis. Recent observations also indicate that eosinophils found in fibrotic lung express elevated levels of cytokines known to be important in lung fibrosis. These finding suggest a possible role for TNF-α in eosinophil recruitment and cytokine expression in this disease (Zhang K et al 1997). TNF-α is a primary mediator of acute pulmonary inflammation and contributes to the pathophysiology of chronic lung diseases; consequently it was important to investigate the role of TNF-α playing in our model.

In mice treated with Th2 cells and exposed to OVA aerosols, treatment with COX-2 specific inhibitor, NS-398 or nonspecific COX inhibitors, indomethacin, the production of TNF-α by LMC on restimulation with OVA peptide is significantly increased (Fig. 4.2.17). Moreover, single daily intraperitoneal injection of anti-TNF-α at 10mg/kg together with NS-398 treatment to the Th2 group and exposed to OVA inhalation reduced EPO levels by approximately 50% when compared with the NS-398 treatment only (Fig.4.2.18). These data demonstrate that TNF-α plays a role in promoting pulmonary eosinophilic inflammation.

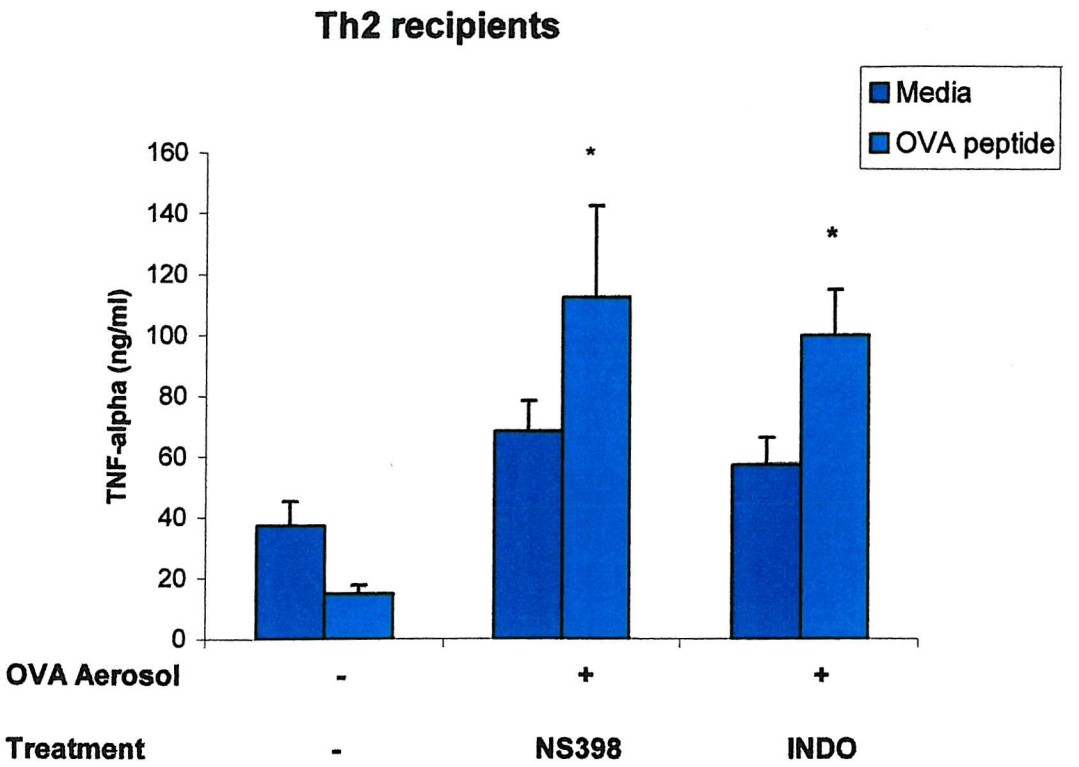


Fig. 4.2.17

TNF-α production by LMCs is increased in aerosol challenged mice treated with NSAID.
Following adoptive transfer of Th2 effector cells, recipient mice were treated with NS398 or indomethacin and exposed to OVA inhalation. Control comprised of mice that had not received DO11.10 cells or inhaled OVA. LMCs were isolated and restimulated with OVA peptide. TNF-α levels after 48h were determined by ELISA. Data were means from 4 separated experiments (*$p<0.05$).

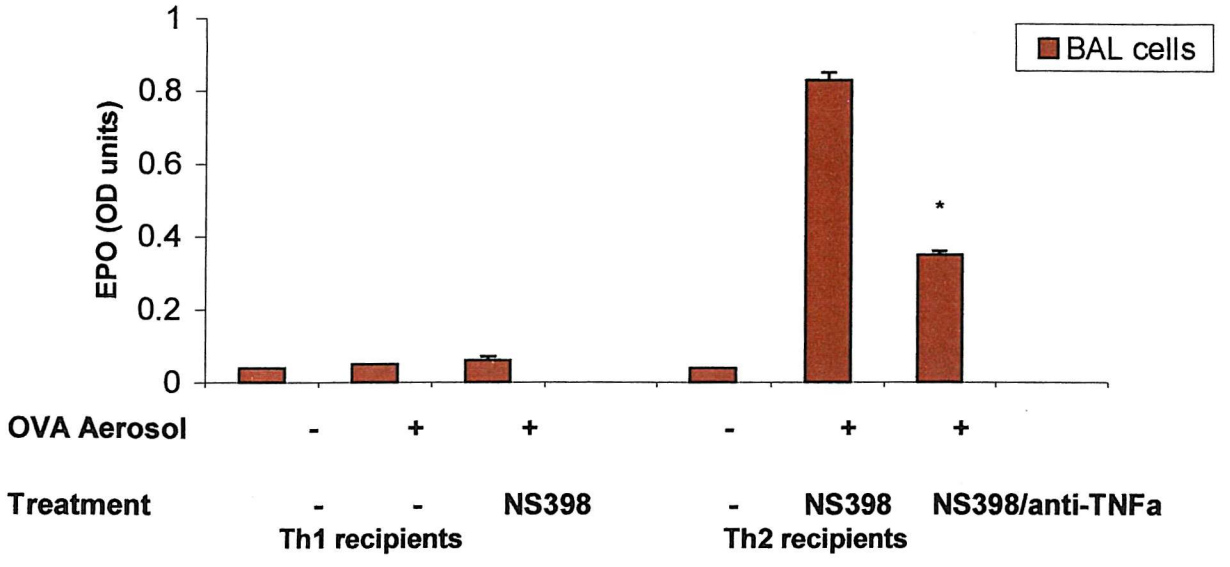


Fig. 4.2.18

Treatment of mice with anti-TNF-α antibody reduces the level of pulmonary eosinophilia in NS-398 treated Th2 recipient mice.
Following adoptive transfer of Th1 or Th2 effector T cells, BALB/c recipients were either treated with NS-398 and alone (*ip* injection every 8h at 10mg/kg) or with NS398 and anti-TNF-α antibody (single daily *ip* injection at 10mg/kg) and exposed to OVA aerosols for 6 days. Control comprised of mice that had received DO11.10 T cells and were either exposed to OVA aerosols or did not inhale OVA. On day 7, EPO activity in BALF was determined. (n=1, *p<0.05).

4.2.9 Effect of NSAID on proliferative responses in the lung

Antigen inhalation is typically associated with the LMC not proliferating in response to antigen. It was important to determine whether NS-398 reversed this process. Following adoptive transfer of Th1 or Th2 T cells, the BALB/c recipient mice were exposed to OVA aerosols. Experimental groups

were treated with NS-398. LMC were purified and restimulated with OVA peptide or 'plate-bound' anti-CD3 for 72 hours. The COX-2 selective inhibitor, NS-398 treatment of mice did not prevent the LMC from entering growth arrest following antigen stimulation (Fig. 4.2.19).

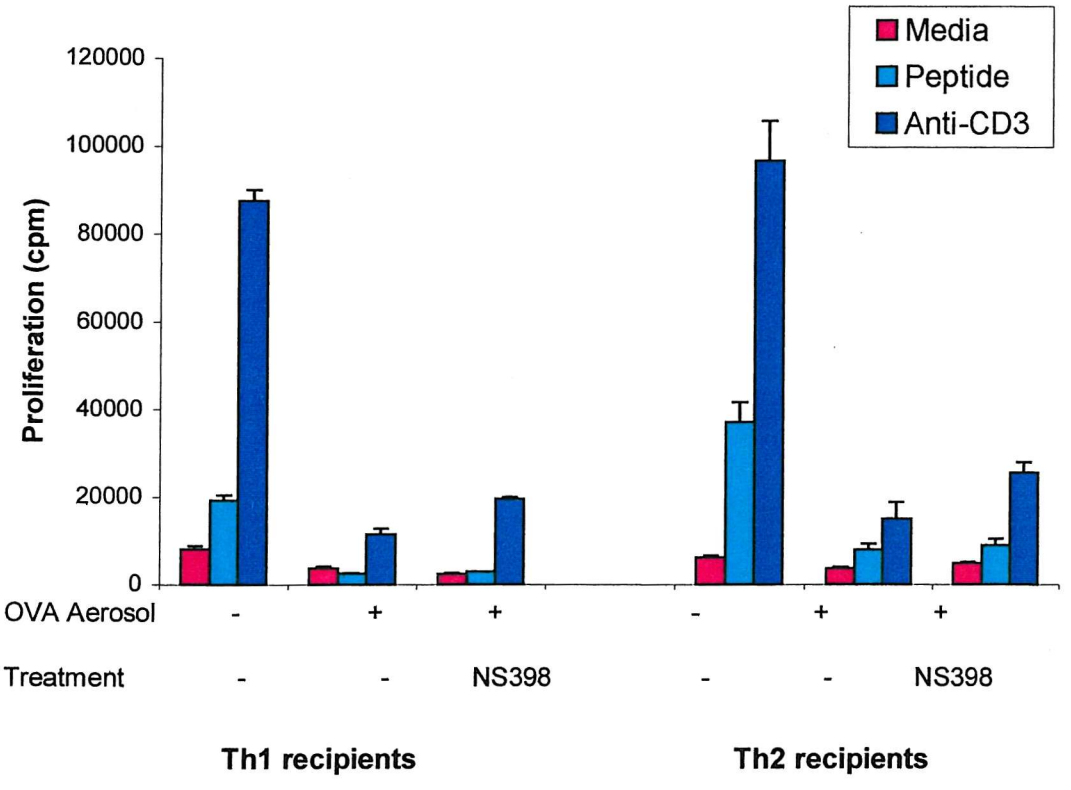


Fig. 4.2.19

Treatments of mice with NS398 did not enhance proliferative responses of LMC.
DO11.10 Th1 or Th2 cells were injected into BALB/c recipients that were then exposed to OVA inhalation for 6 consecutive days, LMC were isolated and restimulated with OVA peptide or 'plate-bound' ant'-CD3 for 72h. Proliferative response was determined using 3H-thymidine incorporation (n=4).

4.2.10 Effect of NSAID on the production of serum IgE antibody

OVA inhalation is typically associated with the production of IgE. To examine if OVA inhalation resulted in IgE production, the serum levels determined. Serum IgE antibodies were increased in the Th2 recipient mice following exposure to OVA aerosols. These levels were the same in animals that had been untreated or treated with COX-2 selective inhibitor, NS-398. In contrast, there was significantly decreased serum IgE levels in the Th2 mice treated with indomethacin or lysin aspirin. The IgE regulation may play an important role in the Th2 mediated pulmonary responses induced by the COX-2 selective inhibitors. However, to date we have measured only total IgE levels and not OVA-specific IgE. The later represent the true IgE responses after OVA challenge. Moreover, when measuring the OVA-specific IgE, can be complicated by cross-reactivity of the reagents with IgG (Fig.4.2.20).

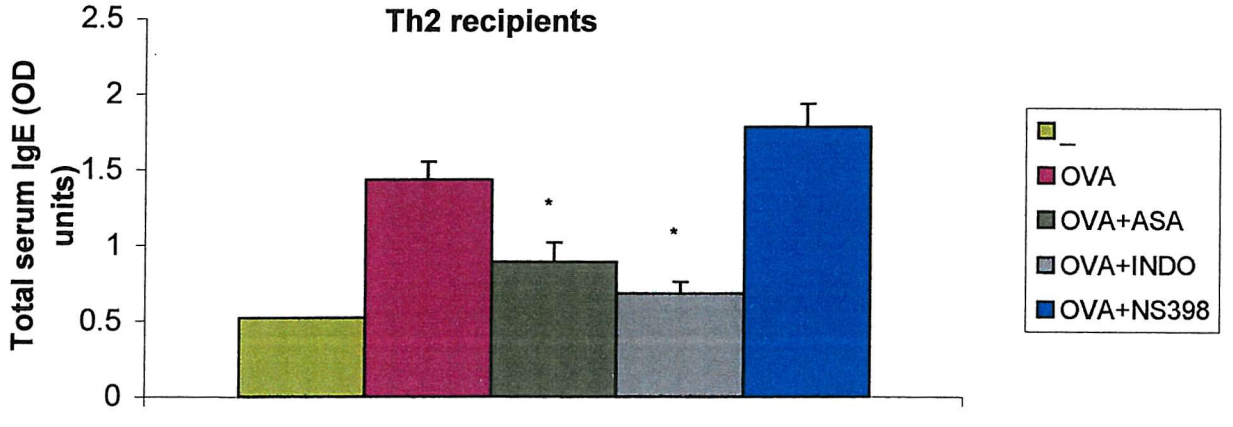


Fig. 4.2.20

Total serum IgE antibody remained high in the Th2 recipients treated with NS-398 and exposed to OVA challenge.
Following adoptive transfer of Th2 effector cells, BALB/c recipients were treated with indomethacine or NS-398 and exposed to OVA aerosols for 6 consecutive days. Controls comprised of mice that had not received DO11.10 T cells with or without OVA inhalation. Serum were collected and total IgE antibodies were determined by ELISA (n=3, *$p<0.05$).

4.3 Summary

Using the DO11.10 model I evaluated the role of COX-1 and COX-2 in modulating pulmonary inflammation. This was achieved by treating mice with indomethacin and lysine-aspirin (which inhibit both COX-1 and COX-2) or the COX-2 specific inhibitor, NS-398. The principle observations were (Table 4.3.1):

1. Treatment of Th2 recipient mice with aspirin, indomethacin or NS-398 resulted in an increase in pulmonary eosinophilia. In contrast, Th1 recipients treated with aspirin, indomethacin, NS-398 there was no increase the level of pulmonary neutrophils.

2. The effect of NS-398 on cytokine production in the lung showed that it downregulated IFN-γ production in Th1 recipients and the decrease of the level was not related to IL-12 or IL-18. In contrast, there was no clear difference in IL-5 production by LMC of Th2 recipients treated with NS-398 and exposed to OVA inhalation. However, IL-4 and IL-5 levels in the BALF were increased significantly in Th2 animals.

3. COX-2 selective inhibitor, NS-398 only reduced the 6-keto-PGF1α prostanoid production in the BALF of Th1 and Th2 recipients. In contrast, COX-1 and COX-2 non-selective inhibitors, indomethacin and aspirin reduced all the levels of PGD2, PGE2, PGF2α, TXB2 and 6-keto-PGF1α.

4. In conclusion, the COX-2 specific inhibitor downregulated PGI2 production that may be responsible for a change in the Th1/Th2 balance observed in LMC from animals that have inhaled OVA.

	Th1 recipients			Th2 recipients		
	NS-398	**Lysin-ASA**	**INDO**	**NS-398**	**Lysin-ASA**	**INDO**
BAL: Eosinophilia	--	--	--	↑ ↑	↑ ↑	↑ ↑
EPO	--	--	--	↑ ↑	ND	↑ ↑
Neutrophilia	--	--	--	--	--	--
IFN-γ	--	ND	ND	–	ND	ND
IL-4 & IL-5	--	ND	ND	↑ ↑	ND	ND
PGI2 (6-keto-PGF1α)	↓	↓ ↓	↓ ↓	↓	↓ ↓	↓ ↓
PGD2, PGE2, PGF2α, TxA2	--	↓ ↓	↓ ↓	--	↓ ↓	↓ ↓
LMC: IFN-γ	↓ ↓	ND	ND	--	ND	ND
IL-12 & IL-18	--	ND	ND	--	ND	ND
IL-5	--	ND	ND	↑	ND	ND
TNF-α	ND	ND	ND	↑	ND	↑

Table 4.3.1. *Summary of the result.*

Chapter Five

Discussion

5.1 The DO11.10 model of pulmonary inflammation

During the course of these experiments the principal objective was to develop a model of Th1- and Th2-mediated pulmonary inflammation. The adoptive transfer of differentiated T cells into naive hosts provides a unique opportunity to exert a high level of control over the nature of the CD4 response elicited by antigen inhalation. This is a marked improvement over models based on immunising mice before exposure to aerosolised antigens, since the immune response that develops following adoptive transfer is independent of antibody generated by the host. Certainly, in immunised animals the antibodies generated are likely to contribute to the inflammation developing to inhaled antigens. Consequently, the contribution of immune complex formation and complement activation to the inflammatory process is obviated by injecting OVA-specific effector T cells. In addition, using the adoptive transfer approach, a high level of control is available as to whether a Th1- or Th2-mediated inflammatory response is studied. Finally, using the DO11.10 mice, antigen-specific T cells can be enumerated by flow cytometric techniques using the KJ1-26 monoclonal antibody.

Flow cytometric analysis revealed that the injected T cells were predominantly KJ1-26+/CD4+ T cells. Moreover, in order for the injected T cells to retain a Th2 phenotype, the cells had to polarise for a minimum of 8 days prior to adoptive transfer into BALB/c mice. If the Th2 cells used had been cultured for shorter periods they began to produce IFN-γ in the lungs of Th2 recipients that had inhaled OVA. Collectively, these data

suggest that the lung environment is strongly Th1 biased. Only very low numbers of DO11.10 T cells penetrated the airways and they were found in the BAL of either Th1- or Th2-recipient mice irrespective of whether they had inhaled OVA or not. OVA inhalation did, however, increase the number of DO11.10 T cells present in the lung tissue, which peaked at day 6 in both Th1 and Th2 recipients.

Irrespective of whether mice had received Th1 or Th2 cells, following antigen inhalation the T cells present in the lung no longer proliferated in response to antigen or anti-CD3 stimulation. Paradoxically, although the T cells were in a state of growth arrest they were capable of producing high levels of cytokines, with LMC from Th1 recipients producing high levels of IFN-γ after inhaling OVA. Conversely, LMC from Th2 recipients produced high levels of IL-4 and IL-5 in response to OVA323-339 peptide. The state of growth arrest was dependent on the presence of F4/80+ interstitial macrophages. This effect has been reported to also occur when the DO11.10 mice themselves inhale OVA (Lee SC et al). These observations extend our analysis of this phenomenon and demonstrate that growth arrest is not only restricted to antigen-specific T cells but to all T cells present in this environment. This would seem to imply that antigen specific T cells do not expand in the lung mucosal environment but in the draining lymph nodes. Implicit in this hypothesis is that any increase in the number of clonotype- positive cells in the lung would be a consequence of increased level of recruitment to this site.

Th1 recipients that had inhaled OVA developed a pulmonary neutrophilia that peaked after 3 days of inhalation but had begun to resolve by 6 days of inhalation. Whether this was a consequence of the neutrophils not surviving in the airways or resolution of the underlying Th1 response was unclear. In this context, it might be expected that the Th1 response in the lung would be of short duration and in an environment in which IL-2 is absent and the T cells enter a state of growth arrest. Indeed, if continually stimulated with antigen, the Th1 cells may apoptose. Adoptive transfer of Th2 cells and exposure of recipient mice to OVA aerosols caused the development of pulmonary eosinophilic inflammation. The eosinophilia peaked after 6 days of challenge and remained high even after 10 days.

It has proven difficult to develop "real time" indicators of cytokine production in the lung in response to antigen inhalation. Consequently, the magnitude of the T cell response taking place in lung tissue was determined by either measuring cytokine production by LMC after stimulation with OVA323-339 peptide, or cytokines present in the BALF.

In the case of Th1-recipient mice, IFN-γ but no IL-4 or IL-5 was produced by LMC in response to restimulation with OVA323-339 peptide. Similarly, in Th2 recipient mice stimulation of LMC with OVA323-339 peptide induced the production of IL-4 and IL-5 but not IFN-γ. These data demonstrate that the transferred DO11.10 T cells cells retain their Th1/Th2 phenotype *in vivo* even after OVA inhalation. Although the cellular provenance of the cytokine was not formally demonstrated it seems highly probable that

DO11.10 T cells were the source of the cytokines since production was elicited by the antigenic peptide OVA323-339 and plate bound KJ1-26. However, this does not completely preclude the possibility that NK cells and macrophages also produce some IFN-γ.

Evaluating cytokine production by LMC following OVA323-339 peptide stimulation does not prove that these cytokines are produced *in vivo* following antigen inhalation. To further determine cytokine production in the lung, the BALF was examined for the presence of IL-4, IL-5 and IFN-γ. Certainly, in Th2 recipient mice that had inhaled OVA the presence of IL-4 and IL-5 was confirmed. However, the quantities of cytokine present were typically low (60pg/ml and 35pg/ml respectively, compared LMC supernates which were >10ng/ml). IFN-γ present in the BALF from the Th1 recipient was low and not changed following OVA inhalation.

5.2 The role of cyclooxygenases in limiting pulmonary inflammation

To address whether cyclooxygenases (COXs) limited the magnitude of the inflammatory responses we used a series of inhibitors of COX activity. Both lysine-aspirin and indomethacin irreversibly inactivate both COX-1 and COX-2. In contrast, NS-398 is a specific COX-2 inhibitor whose actions are reversible.

High levels of PGE2 and PGF2α were present in BALF from mice that had not inhaled OVA. Following OVA inhalation the level of PGE2, PGF2α, PGD2, TxB2 and the stable metabolite of prostacyclin, 6 keto-PGF1α in the BALF increased. Interestingly, treatment of Th1 and Th2 recipient animals with the COX-2-specific inhibitor, NS-398 markedly reduced the levels of 6-keto-PGF1α in the BALF. In contrast, the levels of PGD2, PGF2α, TxB2 and PGE2 were blocked by indomethacin and aspirin but not NS-398. It has been recently been reported in patients treated with the COX-2 specific inhibitor celocoxib, that PGI2 synthesis is inhibited whilst the biosynthesis of other prostanoids was not affected (McAdam BC et al 1999). Treatment of mice with NS-398 had no effect on the concentration of LTB4 present in the BALF. It has been proposed that inhibiting COX activity effectively shunt aracidonic acid metabolism into the 5-lipoxygenase pathway and increased production of leukotrienes. Whether this happens *in vivo* in asthma patients remains controversial (Snyderman CH et al 1995, Watson N et al 1997, Mancini JA et al 1997). In this context it has been recently shown in COX-1 and COX-2 "knockout" mice that the former does show evidence have increased leukotriene production. In contrast, the COX-2 knockout mice do not show any evidence of increased production of leukotrienes (Dinchuh JE et al 1995, Schuligoi R et al 1998). By extrapolation from these findings we would not expect treatment of mice with NS-398 to increase leukotriene levels. This is likely because on examining the level of LTB4 present in the BALF, only low levels were found and they were not elevated in animals treated with NSAID. Collectively these data imply that reduced PGI2 biosynthesis was responsible for the increased level of pulmonary eosinophilia.

It is difficult to know whether this prostanoid inhibits the inflammatory process itself (e.g. by inhibiting eosinophil recruitment to the lung) or the underlying T cell response.

PGI2 may play a role in modulating the Th2 response, since the levels of IL-4 and IL-5 in the BALF are increased significantly and the IFN-γ production by LMC is reduced following treatment with NS-398. The cytokines produced in the lungs of mice after OVA inhalation suggests hypotheses that NS-398 treatment favours Th2 responses. Treatment of Th2 recipient mice with NS-398, aspirin or indomethacin resulted in a marked increase in the level of Th2-mediated pulmonary inflammation. This was detectable at two levels, namely, an increase in the number of eosinophils that entered the airways, and an increase in the levels of IL-4 and Il-5 present in the BALF. However, LMC from NS-398 treated Th2 recipient mice did not produce significantly higher levels of either IL-4 or IL-5. Treatment of Th1 recipient mice with NS-398 resulted in a reduction in the level of IFN-γ produced by LMC on stimulation with OVA323-339 peptide. The mechanism by which this occurs remains speculative but is consistent with the observation that treatment of mice with NS 398 broadly favoured Th2 responses. Blocking the COX-2 isoform, with NS-398, increases the levels of IL-4 and IL-5 production and induces an eosinophilic inflammation, and possibly IgE antibody production. The eosinophilia may be mediated by an increase in TNF-α, IL-5, and associated with eotaxin, RANTES, and CCR-3 to recruit these cells into the airway. Induced TNF-γ is produced by LMC in both Th1 and Th2 recipients following OVA inhalation. Moreover, blocking TNF-α using a neutralising antibody increased the level of pulmonary eosinophilia in Th2 recipients following OVA

inhalation. This demonstrates that in addition to the Th2 cytokines, TNF-α also contributes to the development of the pulmonary eosinophilia.

Pulmonary epithelial cells and inflammatory cells express COX-1 and COX-2. In addition, COX-2 localizes in interstitial macrophages. Consequently, epithelial cells and macrophages are important sources of prostanoid secretion in the lungs. Treatment of mice with indomethacin markedly increases the number of clonotype- positive T cells present in the BALF in both Th1 and Th2 recipients. However, to date treatment with NS 398 has not resulted in similar increases. Since indomethacin blocks the synthesis of all prostaglandins while NS-398 only inhibited PGI2 synthesis. This implies that prostaglandins other than PGI2 limit the recruitment of antigen specific T cells.

Inhalation of OVA has been shown to be associated with an increase in serum IgE levels that typically peak after 10 days of inhalation (Keane-Myere A et al 1997). Continued inhalation is invariably associated with the deviation of such responses to IgG2a and IgG2b. The collapse of the IgE response does not appear to involve γδT cells, CD8+ lymphocytes of IFN-γ (Suzuki M et al 1999). Preliminary experiments to examine whether COX-2 activity influenced the IgE response proved to be inconclusive.

It has been proposed that the COX-2 specific inhibitors provide an opportunity to inhibit the inflammatory process without dramatically affecting the housekeeping function of COX-1 and associated undesirable side effects. The experiments presented here imply that

inhibition of COX-2 in cases were Th2-mediated inflammatory processes are taking place in the lung may exacerbate the inflammation simply because of the immune regulatory effects of PGI2.

That NSAID influence the magnitude of Th2-mediated pulmonary inflammation has implications in the treatment of asthma. In asthma, 10-15% patients are highly sensitive to aspirin. Ingestion of aspirin by sensitive patients elicits a severe exacerbation of the symptoms most evident of which is loss of lung function. This contributes in a significant way to the number of asthma related deaths that have been reported.

5.3 Mechanism by which NS-398 treatment influence lung mucosal T cell responses.

These studies demonstrate that following OVA inhalation large amounts of PG-I2 are produced in the lung. The production of PGI2 is highly dependent on COX-2 and its cellular origin is currently unclear but likely sources include macrophages and epithelial cells. Treatment of animals with the COX-2 inhibitor NS-398 augments pulmonary Th2 responses and reduces the severity of pulmonary Th1 responses. It is likely that these effects are mediated by PGI2 itself, although whether this is by influencing the Th1/Th2 balance or recruitment of inflammatory cells to the lung is unclear. Preliminary

experiments demonstrate that PGI2 and its analog carbaprostacyclin have no effect on the production of IFN-γ by Th1 effector cells or IL-4 and IL-5 by Th2 effector cells. However, both PGI2 and its analog prevent Th2 polarization of naive T cells into Th2 effector cells. In addition PGI2 does not inhibit the development of Th1 effectors. The observation that PG-I2 in vitro limits Th2 responses is further evidence that the principle effect of inhibiting COX-2 is by reducing PGI2 levels (Fig.5.1). Since macrophages are the principle source of PGI2, this may serve as a way in which activated macrophages prevent the development of Th2 responses and favour Th1 polarization (Fig.5.1).

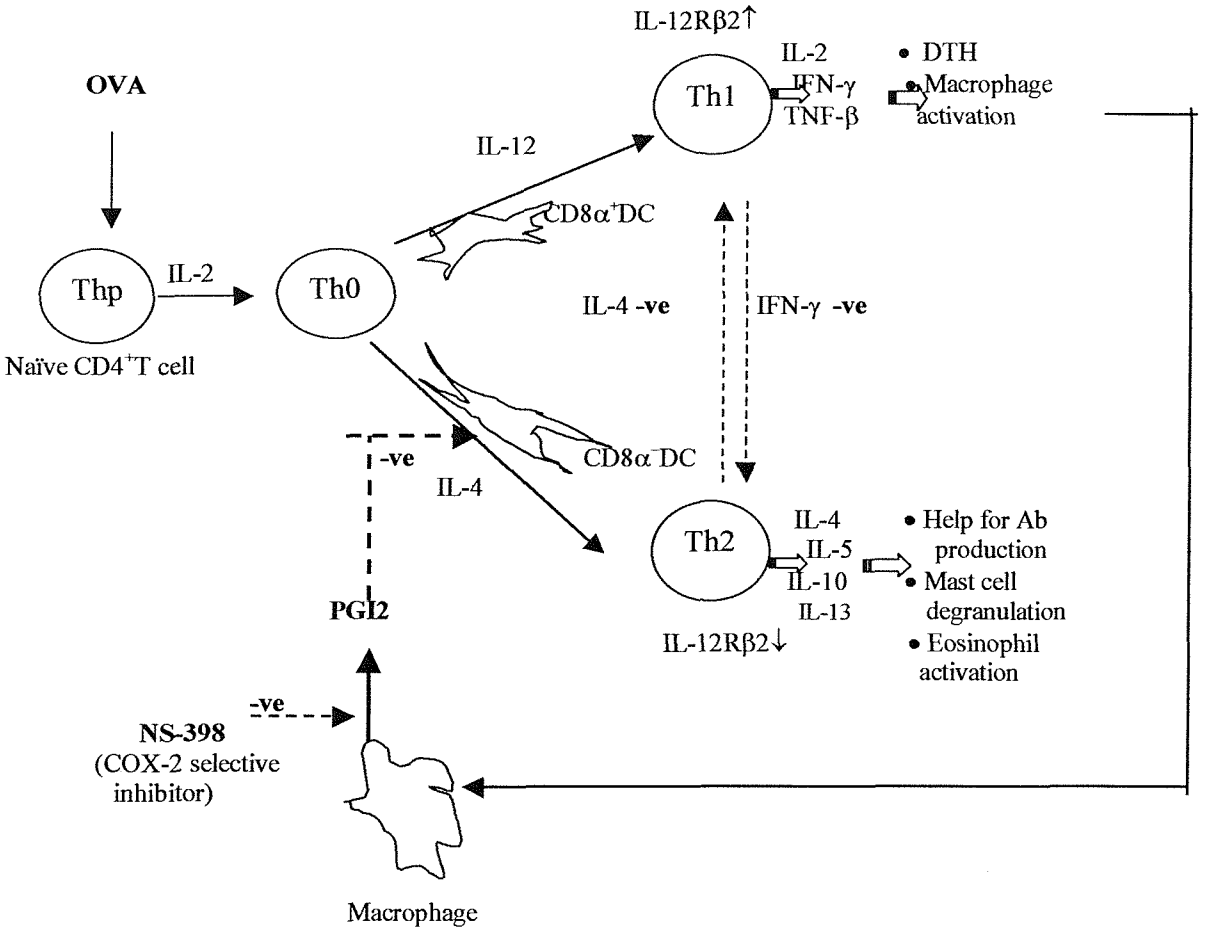


Fig.5.1

Overview of the effect of PGI2 on Th cell differentiation

Step 1. Naïve CD4+ T cell is activated via the TCR when it encounters antigen presented by an antigen-presenting cell.

Step 2. Once activated, the Thp cell starts to proliferate and secrete IL-2 and expresses the IL-12 receptor.

Step 3. When the Th0 cell encounters IL-12 secreted by macrophages and or on contact with $CD8\alpha^{+}$DCs, a Th1 differentiation programme is initiated. The IL-12Rβ2 chain is upregulated leads to the activation of Stat4 and T-bet and initiates to Th1 differentiation. IL-4 produced intrinsically by several cell types that contact with $CD8^{-}$DCs induces differentiation into the Th2 subset by activated the Stat6 and GATA3 transcription, and downregulates the IL-12Rβ2 expression.

Step 4. The Th1 and Th2 cells produce their specific cytokines and mediate delayed type hypersensitivity, macrophage activation (Th1 responses), helping antibody production, mast cell degranulation and eosinophil activation (Th2 responses).

Step 5. The activated macrophages produce PGI2, which inhibits the Th0 cell differentiation into Th2 effector cells.

Step 6. COX-2 selective inhibitors downregulate the PGI2 production.

5.4 Aspects of the project that need clarification

As mentioned in the previous chapter, cyclooxygenase (COX) exists as two isoforms, COX-1, (a constitutive isoform), and COX-2 (which is inducible by cytokines or inflammatory stimuli and may participate in airway inflammation). However, the cellular localization of COX-1 and COX-2 in the lungs of the mice is not known. In rat lungs, the expression of both COX-1 and COX-2 was readily detectable (Ermert L et al 1998) by immunohistochemical analysis. COX-1 localized predominantly to bronchial epithelial cells, smooth muscle cells of large hilum veins and with lower expression in alveolar macrophages and pulmonary artery endothelial cells. The most intense COX-2 staining was noted in macrophage- and mast cell-like cells detected in bronchial epithelium. Strong COX-2 expression was found in smooth muscle cells of pulmonary muscular vessels and large vein of the hilum. This suggests the important homeostatic role of COX-2 in the regulation of human airway contractility, inflammation and immune responses (Walkins DN et al 1999). LPS-induced COX-2 expression varied in freshly isolated polymorphonuclear leukocytes (PMN), monocytes and macrophages indicating that COX-2 expression differentially regulated myeloid cells of different lineages and degrees of maturation (Maloney CG et al 1998). No data has been published on the effect of PGI2 on the Th1/Th2 response.

(a) The expression of COX-1, COX-2 and prostacyclin synthase in the lung

Prostacyclin synthetase (PGIS) is the final committed enzyme in the metabolic pathway leading to prostacyclin (PGI2) production. COX-2 is a major source of systemic prostacyclin biosynthesis in healthy humans (McAdam BF et al 1999).

Using RT-PCR, further work is required to determine the levels of mRNA for prostacyclin synthetase in the lungs of mice pre- and post-exposure to OVA aerosols. Using RT-PCR and specific primers for COX-1, COX-2, and prostacyclin synthase further work is required to determine mRNA levels for both isoforms in the lungs of mice pre- and post-exposed to OVA aerosols. COX activity will be determined by making cell free lysates from lung tissue and following the conversion of arachidonic acid to PGE2. To resolve which cell types in the lung tissue produce COX-1, COX-2 and prostacyclin synthetase, LMC and BAL cells will be sorted using magnetic bead prior to analysis by RT PCR.

(b) The effect of PGI2 on the T cell response

Th1 and Th2 cells will be cultured in the presence of PGI2 (or the stable PGI2 analogue, 6-keto-prostaglandin I1). The effect of this prostanoid on their proliferative responses and cytokine production will be examined. It is important to resolve whether PGI2 inhibits T cell differentiation. T cells from DO11.10 mice will be stimulated with OVA peptide in the presence of either IL-12 or IL-4 (to drive Th1 and Th2 phenotypes respectively). The effect of adding PGI2 to Th1 and Th2 cultures on the spectrum of cytokines produced will be examined. The distribution of the PGI2 receptor (IP-receptor) will be examined using RT-PCR and binding of ^{3}H-Iloprost.

Reference

1. Abbas AK, Murphy KM, and Sher A 1996. Functional diversity of helper T lymphocytes. Nature 383: 787-793.

2. Akbar AN, Terry L, Janossy G, et la 1988. Loss of CD45R and gain of UCHL1 reactivity is a feature of primed T cells. J Immunol 140: 2171-2178.

3. Aman MJ, Tayebi N, Obiri NI, et al 1996. cDNA cloning and characterization of the human IL-13 receptor alpha chain. J Biol Chem 271: 29265-70.

4. Amiri P, Locksley RM, McKerrow JH, et al 1992. TNF-α restores granulomas and induces parasite egg laying in schistosome-infected SCID mice. Nature 356: 604-610.

5. Anderson GD, Hauser SD, McGarity KL, et al 1996. Selective inhibition of COX-2 reverses inflammation and expression of COX-2 and IL-6 in rat adjuvant arthritis. J Clin Invest 97: 2672-2679.

6 Aoki Y, Qui D, Uyei A, et al 1997. Human airway epithelial cells express IL-2 in intro Am J Physiol 272: L276-86.

6. Arase H, Arase N, and Onoe K, et al 1993. NK1.1+CD4+CD8- thymocytes with specific lymphocyte secretion. Eur J Immunol 23, 307-310.

7. Aversa G, Punnonen J, Cocks BG, et al 1993. An IL-4 mutant protein inhibits both IL-4 or IL-13-induced human IgG4 and IgE synthesis and B cell proliferation: support for a common component shared by IL-4 and IL-13 receptors. J Exp Med 178: 2213-8.

8. Banchereau J and Steinman RM 1996. Dentritic cells and the fcontrol of immunity. Nature 392: 245-252.

9. Bascom R, Wachs M, Lichtenstein LM, et al 1988. Basophil influx occurs after nasal antigen challenge: Effects of topical corticosteroid pretreatment. J Allergy Clin Immunol 81: 580-589.

10. Beasley R, Varley J, Robinson C, Holgate ST 1987. Cholinergic-mediated bronchoconstriction induced by PGD2, its initial metabolite 9a,11B-PGF2α, in asthma. Am Rev Respir Dis 136: 1140-1144.

11. Bentley AM, Hamid Q, Robinson DS, et al 1996. Prednisolone treatment in asthma. Reduction in the numbers of eosinophils,T cells, tryptase-only positive mast cells, and modulation of IL-4, IL-5, and IFN-γ cytokine gene expression within the bronchial mucosa. Am J Respir Crit Care Med 153: 551-6.

12. Bendelac A, Matzinger P, Seder A, Paul WE, and Schwartz RH 1992. Activation events during thymic selection. J Exp Med 174, 731-742.

13. Benz M, Fox BS 1991. PGE2 inhibits production of Th1 lymphokines but not of Th2 lymphokines. J Immunol 146(1): 108-13.

14. Bergh K, Olsen PO, Iversen OJ, et al 1991. Complement activation and pulmonary dysfunctioin in experimental Escherichia coli septisemia. Acta Anaestheiol Scand 35: 267-274.

15. Berkman N, John M, Roesems G, et al 1995. Inhabition of macrophage inflammatory protein-1a by IL-10: differential sensitivities in human blood monocytes and alveolar macrophages. J Immunol 155: 4412-8.

16. Berkman N, Roesems G, Jose PJ, et al 1995. IL-13 inhibits expression of macrophage-inflammatory protein-1α from human blood monocytes and alveolar macrophages. Am J Respir Cell Mol Biol 15: 382-9.

17. Betz M and Fox BS 1991. Prostaglandin E_2 inhibits production of Th1 lymphokines but not of Th2 lymphokines. J Immunol 146: 108.

18. Bienen stock J, Befus AD, McDermott M 1980. Mucosal immunity. Monogr Allergy 16:1-18.

19. Bilyk N and Holt PG 1993. Inhibition of dthe immuosuppressive activity of resident pulmonary alveolar macrophages by GM-CSF. J Exp Med 177: 1773-1777.

20. Blotta MH, Marshall JD, DeKruyff RH, et al 1996. Cross-linking of the CD40 ligand on human CD4+ T lymphocytes generates a costimulatory signal that up-regulates IL-4 synthesis. J Immuno 156: 3133-40.

21. Blotta MH, Dekruyff RH, Umetsu DT 1997. Corticosteroids inhibit IL-12 production in human monocytes and enhance their capacity to induce IL-4 synthesis in CD4+ lymphocytes. J Immunol 158: 5589-95.

22. Bochner BS, Luscinskas FW, Gimbrone MA Jr, et al 1991. Adhesion of human basophils, eosinophils, and neutrophils to IL-1-activated human vascular endothelial cells: contributions of endothelial cell adhesion molecules. J Exp Med 173:1553-7.

23. Boguniewicz M, Mafrtin RJ, Martin D, et al 1995. The effects of nebulized recombinant INF-γ in asthmatic airways. J Allergy Clin Immunol 95: 133-5.

24. Bone RC, 1992. Inhibitors of complement and neutrophils: A critical evaluation of their role in the treatment of sepsis. Crit Care Med 20: 891-898.

25. Bradding P, Roberts JA, Britten KM, et al 1994. IL-4, -5 and –6 and tumor necrosis factor α in normal and asthmatic airways: evidence for the human mast cell as a source of these cytokines. Am J Respir Cell Mol Biol 10: 471-80.

26. Brain JD, 1988. Lung macrophages. How many kinds are there? What do they do? Am Rev Respird Dis 137: 507-509.

27. Brain W, Seymour P, Coffman RL, et al 1998. Aerosol-induced IgE unresponsiveness to ovalbumin does not require CD8+ or T cell receptor-γδ+ T cells or IFN-γ in murine model of allergy sensitization. J Exp Med 187: 721-731.

28. Breeze RG, Wheeldon EB 1977. The cells of the pulmonary airways. State of the art. Am Respir Dis 116: 705-777.

29. Brinkman GL 1968. The mast cell in normal bronchus and lung. J Ulstrastruct Res 23:115-123.

30. Broide D, Schwarze J, Raz E, et al 1998. Immunostimulatory DNA sequences inhibit IL-5, eosinophilic inflammation, and airwayhyperresponsiveness in mice. J Immunol 161: 7054-7062.

31. Brunner T, Heusser CH, and Dahinden CA 1993. Human peripheral blood basophils primed with IL-3 produce IL-4 in response to IgE receptor stimulation. J Exp Med 177: 605-612.

32. Brusselle G, Kips J, Tavernier J, van der Heyden J, Cuvelier C, Pauwels R, and Bluethmann H 1994. Attenuation of allergic airwayin flammation in IL-4 deficient mice. Clin Exp Allergy 24, 73-80.

33. Brusselle G, Kips J, Pauwels R, et al 1995. Allergen-induced airway inflammation and bronchial responsivenes in wild-type and IL-4 deficient mice. Am J Respir Cell Mol Biol 12: 254-259.

34. Brusselle G, Kips J, Peleman RA, et al 1997. Role of IFN-γ in the inhibition of the allergic airway inflammation caused by IL-12. Am J Respir Cell Mol Biol 17: 767-71.

35. Carey PD, Jenkens JK, Fowler AA, et al 1992. The neutrophil respiratory burst and tissue injury in septic acute lung injury: The effect of cyclooxygenase inhibition in swine. Surgery 112: 45-55.

36. Carlos T, Kovach N, Harlan J, et al 1991. Human monocytes bind to two cytokine-induced adhesive ligands on cultured human endothelial cells: Endothelial-leucocyte adhesion molecule-1 and vascular cell adhesion molecule-1. Blood 77: 2266-2271.

37. Casale TB and Carolan EJ 1999. Combination of IL-8 plus TNF-α induces additive neutrophil migration. Allergy Asthma Proc 20(6): 361-3.

38. Caughey GE, Pouliot M, James MJ et al 1997. Regulation of tumor necrosis factor-alpha and IL-1 beta synthesis by thromboxane A2 in nonadherent human monocytes. J Immonol 158(1): 351-358.

39. Cembrzynska-Nowak M, Szklarz E, Inglot AD, and Teodorczyk-Injeyan JA 1993. Elevated release of tumor necrosis factor-alpha and interferon-gamma by bronchoalveolar leukocytes from patients with bronchial asthma. AM Rev Respir Dis 147:291-295.

40. Chakkalath HR, and Titus RG 1994. Leishmania major-parasitized macrophages augment Th2-type T cell activation. J Immunol 153: 4378-4387.

41. Chapuis F, Rosenzwajg M, Gluckman JC, et al 1997. Differentiation of human dendritic cells from monocytes *in vitro*. Eur J Immunol 27: 431-441.

42. Chensue SW, Otterness IG, Kunkel SL, et al 1992. Monokine production by hypersensitivity (Schistosoma mansoni egg) and foreign body (Sephadex bead)-type granuloma macrophages: Evidence for sequential production of IL-1 and TNF. J Immunol 142: 1281-1287.

43. Cher DJ, Mosmann TR 1987. Two types of murine helper T cell clone. II: delayed-type hypersensitivity is mediated by Th1 clones. J Immunol 138: 3688-3694.

44. Cherayil BJ, Weiner SJ, Pillai S 1989. The Mac-2 antigen is a galactose-specific lectin that binds IgE. J Exp Med 170: 1959-1972.

45. Christman BW and Christman JW 1990. PGE_2 decrease leukotriene B_4 production by A23187-stimulated rat alveolar macrophages: analysis by gas chromatography/mass spectrometry. Am Rev Resperi Dis 141: A393.

46. Coffman RL, and Carty J 1986. A T cell activity that enhances polyclonal IgE production and its inhibition by IFN-γ. J Immunol 136: 949-954.

47. Coffman RL, et al 1988. The role of helper T cell products in mounse B cell differentiation and isotype regulation. Immunol Rev 102: 5-28.

48. Coffman RL, Seymour BWP, and Rennick D, et al 1989. Antibody to IL-5 inhibits helminth-induced eosinophilia in mice. Science 245, 308-310.

49. Cohn L, Homer RJ, Niu N, and Bottomly K 1999. Th1 cells and IFN-γ regulate allergic airqay inflammation and mucus production. J Exp Med 190(9): 1309-1317.

50. Cohen P, Noveral JP, Grunstein MM, et al 1995. LTD4 facilitates airway smooth muscle cell proliferation via modulation of the IGF axis. Am J Physiol 269: L151-L157.

51. Coleman RA, Eglen RM, Jones RL, et al 1995. Prostanoid and leukotriene receptors: a progress report from the IUPHAR working parties on classification and nomenclature. Adv Prostaglandin Thromboxane Leukot Res 23: 283-5.

52. Coles SJ, Neill KH, Reid LM, et al 1983. Effects of LTC4 and D4 on glycoprotein and lysozyme secretion by human bronchial mucosa. Prostaglandins 25: 155-70.

53. Collins PD, Griffiths-Johnson DA, Jose PJ, et al 1995. Cooperation between IL-5 and the chemokine, eoxtaxin, to induce eosinophil accumulation in vivo. J Exp Med 182: 1169-74.

54. Constan SL et al 1997. Induction of the Th1 and Th2 CD4+ T cell responses: The alternative approaches. Annu Rev Immunol 15: 297-322.

55. Corry DB, Folkesson HG, and Young IG, et al 1996. IL-4, but IL-5 or eosinophils, is required in a murine model of acute airway hyperreactivity. J Exp Med 183:109.

56. Corrigan CJ, Hartnell A, Kay AB 1988. T lymphocyte activation in acute severe asthma. Lacet 1: 1129.

57. Corrigan CJ, Kay AB 1990. CD4 T-lymphocyte activation in acute severe asthma. Am Rev Respir Dis 141: 970.

58. Corrigan CJ, Haczku A, Gemon-Engesaeth V, et al 1993. CD4+ T lymphocyes activation in asthma is accoimpanied by increased serum concentration of IL-5: effect of glucocorticoid therapy. Am Rev Respir Dis 147: 540-7.

59. Costa JJ, Matossian K, Resnick MB, et al 1993. Human eosinophils can experess the cytokines tumor necrosis factor-α and macrophage inflammatory protein-1α. J Clin Invest 91: 2673-84.

60. Crowley M, Inaba K, Steinman RM, et al 1989. The cell surface of mouse dendritic cells: FACS analyses of dendritic cells from different tissues including thymus. Cellular Immunol. 118: 108-125.

61. Curry AJ, and Kaye PM, 1992. Recombinant IL-1α augments granuloma formation and cytokine production but not parasite clearence in mice infected with Leishmania donavani. Infect Immun 60: 4422-4426.

62. Cutz E, 1982. Neuroendocrine cells of the lung. An overview of morphologic characteristics and development. Exp Lung Res 3: 185-208.

63. Dahlen SE, Hedqvist P, Samuelsson B, et al 1980. Leukotrienes are potent constrictors of human bronchi. Nature 288: 484-6.

64. D' #Andrea AO A-Amezaga M, Trinchieri G, et al 1993. IL-10 inhibits lymphocyte IFN-γ -production by suppressing NKCSF/IL-12 synthesis in accessory cells. J Exp Med 178: 1041-1048.

65. deKruyff RH, Gieni RS, Umetsu DT 1997. Antigen-driven but not LPS-driven IL-12 production in macrophages requires triggering of CD40. J Immunol 158: 359-66.

66. Del Prete G, de Carli M, Romagnani S, et al 1993. Human IL-10 is produced both by Th1 and Th2 cell clones and inhibits their antigen-specific proliferation and cytokine production. J Immunol 150: 353-360.

67. Dent LA, Strath M, Mellor AL, et al 1990. Eosinophilia in transgenic mice expressing IL-5. J Exp Med 172:1425-31.

68. Derek W, Gilroy PR, Willoughby DA, et al 1999. Inducible cyclooxygenase may have anti-inflammatory properties. Nature Medicice 5(6): 698-701.

69. Desreumauzx P, Janin A, Capron M, et al 1992. IL-5 messenger RNA expression by eosinophils in the intestinal mucosa of patients with celiac disease. J Exp Med 175: 293-296.

70. de Waal Malefyt R, Haanen J, and de Vries JE, et al 1991. IL-10 and v-IL-10 strongly reduce antigen specific human T cell responses by diminishing the antigen presenting capacity of monocytes via down-regulation of class I MHC expression. J Exp Med 174, 915-924.

71. de Waal Malefyt R, Yssel H, and de Vries JE, 1993. Direct effects of IL-10 on subsets of human CD4+ T cell clones and resting T cells. Specific inhibition of IL-2 production and proliferation. J Immunol 150, 4754-4765.

72. De Waal Malefyt R, Figdor CG, Huijbens R, et al 1993. Effects of IL-13 on phenotype, cytokine production, and cytotoxic function of human monocytes. J Immunol 151: 6370-81.

73. Diaz P, Gonzalez MC, Gallequillos FR, et al 1989. Leukoctyes and mediators in bronchoalveolar lavage during allergen induced late-phase asthmatic reactions. Am Rev Respir Dis 139: 1383.

74. Dinarello CA 1999. IL-18: A Th1-inducing, proinflammatory cytokine and new member of IL-1 family. J Allergy Clin Immunol 103(1 Pt 1):11-24.

75. Dinchuh JE, Car BD, Czaniah PM, et al 1995. Renal abnormalities and an altered inflammatory response in mice lacking COX-2. Nature 378(6555): 406-9.

76. Drazen JM, Lewis RA, Austen KE, et al 1979. Differential effects of a partially purified preparation of SRSA on guinea pig tracheal spirals and parenchymal strips. J Clin Invest 63: 1-5.

77. Drazen JM, Austen KF, Lewis RA, et al 1980. Comparative airway and vascular activities of LTC1 and D *in vivo* and *in vitro*. Proc Natl Acad Sci USA 77: 4354-8.

78. Dusting GJ and MacDonald PS 1990. Pharmcol Ther 48:323-344.

79. Dworski R and Sheller JR 1997. Prostaglandins 53: 237-251.

80. Eder J 1997. TNF-α and IL-1 signalling: do MAPKK kinases connect it all? Trends Pharmacol Sci 18: 319-22.

81. Eichacker PQ, Hoffman WD, Natanson C, et al 1991. TNF but not IL-1 in dogs causes lethal lung injury and multiple organ dysfunction similar to human sepsis. J Appl Physiol 71: 1979-1989.

82. Elias JA, Gustilo K, Freundlich B et al 1987. Synergistic stimulation of fibroblast prostaglandin production by recombinant interleukin 1 and tumor necrosis factor. J Immunol 138: 3812.

83. Enk AH, Katz SI, 1992. Identification and induction of keratinocyte-derived IL-10. J Immunol 149: 92-5.

84. Ermert L, Ermert M, Seeger W, et al 1998. Cytooxygenase isoenzyme localization and mRNA expression in lungs. Am J Respir Cell Mol Biol 18(4): 479-88.

85. Ermert L, Ermert M, Seeger W et al 2000. In situ localization and regulation of thromboxane A2 synthase in normal and LPS-primed lungs. Am J Physiol Lung Cell Mol Physiol 278(4): L744-53.

86. Evett GE, Xie W, Simmons DL , et al 1993. Arch Biochem Biophys 306:169-177.

87. Fantuzzi G, Puren AJ, Dinarello CA, et al 1998. IL-18 regulation of IFN-γ productioin and cell proliferation as revealed in IL-1β converting enzyme-deficient mice. Blood 91: 2118-25.

88. Fantuzzi G, Reed DA, Dinarello CA 1999. IL-12-induced IFN-γ is dependent on caspase-1 processing of the IL-18 precursor. J Clin Invest 104(6): 761-767.

89. Fantoe JC, Kunkel SL, Ward PA 1982. Chemotactic mediators in neutrophil-dependent lung injury. Ann Rev Physiol 44: 283.

90. Fedyk ER and Phipps RP 1996. Prostaglandin E2 receptors of the EP2 and EP4 subtypes regulate activation and differentiation of mouse B lymphocytes to IgE-secreting cells. Proc Natl Acad Sci USA 93: 10978-10983.

91. Ferreri NR, Howland WC, Spiegelberg HL 1986. Release of leukotrienes C4 and B4 and PGE2 from human monocytes stimulated with aggregated IgG, IgA, and IgE. J Immunol 136: 4188.

92. Finkelman FD, Katona IM, Ohara J, et al 1988. IL-4 is required to generate and sustain in vivo IgE responses. J Immunol 141: 2335-2341.

93. Fiorentino DF, Bond MW, and Mosmann TR 1989. Two types of mouse T helper cell. IV. Th2 clones secrete a factor that inhibits cytokine productioin by Th1 clones. J Exp Med 170, 2081-2095.

94. Fiorentino DF, Zlotnik A, O'Garra A, et al 1991. IL-10 acts on the antigen-presenting cell to inhibit cytokine production by Th1 cells J Immunol 146: 3444-3451.

95. Fiorentino DF, Zlotnik A, Mossmann TR, et al 1991. IL-10 inhibits cytokine production by activated macrophages. J Immunol 147: 3815-22.

96. Firestein GS, Roeder WD, Laxer JA, et al 1989. A new murine CD4-T cell subset with an unrestricted cytokine profile. J Immunol 143: 518-525.

97. Fletcher BS, Kubuju DA, Herschman HR, et al 1992. Structure of the mitogen-inducible TIS10 gene and demonstration that the TIS10-encoded protein is a functional prostaglandin G/H synthase. J Biol Chem 267: 4338-44.

98. Flint KC, Leung KBP, Johnson NM, et al 1985. Human mast cells recovered by BAL: their morphology, histamine release and the effects sodium cromoglycate. Clin Sci 68: 427-432.

99. Flory CM, Jones ML, and Warren JS 1993. Pulmonary granuloma formation in the rat is partially dependent on monocyte chemoattractant protein 1. Lab Invest 69: 450-462.

101.Ford WL et al 1975. Lymphocyte migration and immune responses. Pro Allergy 19:1.

102.Foster PS, Hogan SP, Ramsay AJ, et al 1996. IL-5 deficiency abolishes eosinophilia, airways hyperreactivity, and lung damage in a mouse asthma model. J Exp Med 183: 195-201.

103.Franke-Ullmann G, Pfortner C, Kobzik L, et al 1996. Characterization of murine lung interstitial macrophages in comparison with alveolar macrophages *in vitro*. J Immunol 157: 3097-3104.

104.Fu YH, and Blankehorn EP 1992. Nitrc oxide induced anti-mitogenic effects in high and low responder rat strain.J Immunol 148: 2217-2222.

105.Fujimura M, Sasaki F, Nakatsumi Y, et al 1986. Effects of a thromboxane synthetase inhibitor (OKY-046) and lipoxygenase inhibitor (AA-861) on bronchial responsiveness to acetylcholine in asthmatic subjects. Thorax 41: 955-959.

106.Funk CD, Funk LB, Kennedy ME, et al 1991. Human platelet/erythroleukemia cell prostaglandin G/H synthase: cDNA cloning, expression and gene chromosomal assignment. FASEB J 5: 2304-2312.

107.Gadek JE, Fells GA, Zimmerman RL, et al 1984. Role of connective tissue proteases in the pathogenesis of chronic inflammatory lung disease. Environ Health Perspect 55: 297.

108.Galve de Rochemonteix B, Nicod LP, Dayer JM, 1996. TNF solube receptor 75: the principal receptor form released by human alveolar macrophages and moncytes in the presence of IFN-γ. Am J Respir Cell Mol Biol 14: 279-87.

109.Gardiner PJ 1989. Eicosanoids and airway smooth muscle. Pharm Ther 44: 1-62.

110.Gauvreau GM, Watson RM, O'Byrne PM 1999. Protectiv $Byrne PM199. Protec t 2 on allergen-induced airway responses and airway inflammation. Am J Respir Crit Care Med 159(1): 31-6.

111.Gavett SH, Chen X, Wills-Karp M, et al 1994. Depletion of murine CD4+ T lymphocytes prevents antigen-induced airway hyperreactivity and pulmonary eosinophilia. Am J Respir Cell Mol Biol 10: 587-593.

112.Gavett SH, O'Hearn DJ, L *Hearn DJ, Li X e-12 inhibits antigen-induced airway hyperresponsiveness, inflammation, and Th2 cytokine expression in mice. J Exp Med 182:1527-36.

113.Gearing AJ, Beckett P, Christodoulou M, et al 1994. Processing of TNF-α procursor by metalloproteinases. Nature 370: 555-7.

114.Geraci MW, Gao B, Voelkel NF, et al 1999. Pulmonary prostacyclin synthase overexpression in transgenic mice prltects against development of hypoxic pulmonary hypertension. J Clin Invest 103(11):1509-15.

115.Gerosa F, Paganin C, Peritt D, Paiola F, Scupoli MT, Aste-Amegaza M, et al 1996. IL-12 primes human CD4 and CD8 T cell clones for high production of both IFN-γ and IL-10. J Exp Med 183: 2559-69.

116.Ghayur T, Banerjee S, Cater A, et al 1997. Capase-1 processes IFN-γ-inducing factor and regulates LPS-induced IFN-γ production. Nature 386: 619-23.

117.Ghosh S, Latimer RD, Oduro A, et al 1993. Endotoxin-induced organ injury. Crit Care Med 21:S19-S28.

118.Giembycz MA, Kroegel C, Barnes PJ 1990. PGE_2 inhibits platelet activating factor-induced eosinophil activation. Am Rev Respir Dis 141(part 2):A393.

119.Glanville AR, Tazelaar HD, Robin ED, et al 1989. The distribution of MHC class I and II antigens on bronchial epithelium. Am Rev Respir Dis 139: 330-334.

120.Glassberg MK, Ergul !, Puett D, et al 1994. Endothelin-1 promotes mitogenesis in airway smooth muscle cells. Am J Resp Cell Mol Biol 10:316-321.

121.Gleich GH 1986. The eosinophilic leukocyte: Structure and function. Adv Immunol 39: 177-253.

122.Gold WM, Meyers GL, Bourne HR, et al 1977. Changes in airway mast cells and histamine caused by antigen aerosol in allergic dogs. J Appl Physiol 43: 271-175.

123.Godding V, Stark JM, Sedgwick JB, et al 1995. Adhesion of activated eosinophils to respiratory epithelial cells is enhanced by TNF-α and IL-1β. Am J Respir Cell Mol Biol 13: 555-62.

124.Goodwin JS, and Ceuppens J 1983. Regulation of the immune response by prostaglandins. J Vlin Immunol 3: 295.

125.Goodwin JS, Bankhurst AD and Messner RP 1977. Suppression of human T-cell mitogenesis by prostaglandin: existence of a prostaglandin-producing suppressor cell. J Exp Med 146:1719.

126.Gordon JR, Burd PR, Galli SJ 1990. Mast cells as a source of multifunctional cytokines. Immunol Today 11: 458-464.

127.Gounni AS. Lamkhioued SB, Tanaka E, et al 1994. High-affinity IgE receptor on eosinophils is involved in defense against parasites. Nature 367: 183-186.

128.Greenbaum LA, Horowitz JB, Woods A, et al 1988. Autocrine growth of CD4+ T cells. Differential effects of IL-1 on helper and inflammatory T cells. J Immunol 140: 1555-60.

129.Gribando G et al 1993. IFN inhibit onset of murine CMV immediated-early gene transctiption. Virology 197(1): 303-11.

130.Grunig G, Warnock M, Wakil AE, et al 1998. Requirement for IL-13 independently of IL-4 in experimental asthma. Science 282: 2261-3.

131.Grzych JM, Pearce E, Sher A, et al 1991. Egg deposition is the major stimulus for the production of Th2 cytokines in murine schistosomiasis mansoni. J Immunol146: 1322-1329.

132.Gundel RH, Wegner CD, and Letts LG 1992. Antigen-induced acute and late-phase responses in primates. Am Rev Respir Dis 146: 369-373.

133.Gurlo T, Huang WW, von Grafenstein H 1998. PGE2 inhibits IL-2 and IL-4-dependent proliferation of CTLL-2 and HT2 cells. Cytokine 10(4): 265-74.

134.Guvett SH, Chen XL, Will-Karp M, et al 1994. Depletion of murine CD4+ T lymphocytes prevents antigen-induced airway hyperractivity and pulmonary eosinophilia. Am J Respir Cell Mol Biol 10:587-593.

135.Hakkert BC, Kuijpers TW, Roos D, et al 1991. Neutrophil and monocyte adherence to and migration across monolayers of cytokine-activated endothelial cell: The contribution of CD18, ELAM-1, and VAL-4. Bolld 78: 2721-2726.

136.Hamid QA, Azzawi M, Sun Ying, et al 1991. Expression of mRNA for IL-5 inmucosal bronchial biopsies from asthma. J Clin Invest 87: 1541-9.

137.Hamid QA, Schotman E, Jacobson MR, et al 1997. Increases in allergen-induced late skin responses after successful grass pollen immunotherapy. J Allergy Clin Immunol 99: 254-60.

138.Hamzaoui A, Hamzaoui K, Chabbou A, et al 1999. Lymphocytes apoptosis in patients with acute exacerbation of asthma. Mediators Inflamm 8:4-5 237-43.

139.Hansel TT, Braunstein JB, Virchow C Sr, et al 1991. Sputum eosinophils from asthmatics express ICAM-1 and HLA-DR. Clin Exp Immunol 86: 271-277.

140.Hansen G, Berry G, DeKruyff RH and Umetsu DT 1999. Allergen-specific Th1 cells fail to conterbalance Th2 cell-induced airway hyperreactivity but cause severe airway inflammation.

141.Hartnell A, Robinson DS, Wardlaw AJ, et al 1993. CD69 is expressed by human eosinophils activated *in vivo* in asthma and *in vitro* by cytokines. Immunology 80: 281-286.

142.Haye-Legrand I, Cerrina J, Brink C, et al 1986. Histamine concentrations of isolated human airway smooth muscle preparartions: role of prostaglandins. J Pharmacol Exp Ther 239: 536-41.

143.He X, Stuart JM 1999. PGE2 selectively inhibits human CD4+ T cells secreting low amounts of both IL-2 and IL-4. J Immunol 163(11): 6173-9.

144.Heinen E, Cormann N, Simar LJ et al 1986. Isolation of follicular dendritic cells from himan tonsils and adenoids. VI. Analysis of prostaglandin secretion. Ann Inst Pasteur Immunol 137D: 369.

145.Herbert CA, Edwards D, Robinson C, et al 1991. In vitro modulation of the esoinophil-dependent enhancement of the permeability of the bronchial epithelium. Br J pharmacol 104: 391-398.

146.Heppleston AG, Young AE, 1973. Uptake of inert particulate matter by alveolar cells: an ultrastructural study. J Pathol 111: 159-164.

147.Higgs GA, Moncada S, Seager K, et al 1983. The source of thromboxane and prostaglandins in experimental inflammation. Br J Pharmacol 79: 863-868.

148.Hilkens CM, Vermeulen H, Kapsenber ML, et al 1995. Differential modulation of Th1 and Th2 cytokine secretion by PGE2 critically depends on IL-2. Eur J Immunol 25(1): 59-63.

148.Hirata M, Kakizuka A, Narumiya S, et al 1994. Proc Natl Acad Sci USA 91: 11192.

149.Hlat T and Neilson K 1992. Proc Natl Acad Sci USA 89:7384-7388.

150.Holt PG 1979. Alveolar macrophages. II. Inhibition of lymphocyte proliferation by purified macrophages from rat lung. Immunology. 37: 429-436.

151.Holt PG, Oliver J, Thepen T, et al 1993. Downregulation of the antigen presentating cell functions of pulmonary dendritic cells *in vivo* by resident alveolar mactophages. J Exp Med 177: 397-407.

152.Holtzman MJ 1991. Arachidonic acid metqbolism: implications of biological chemistry for lung function and disease. Am Rev Respir Dis 143: 188-203.

153.Horsfield K, Cumming G 1968. Morphology of bronchial tree in man. J Appl Physiol 24: 373-383.

154.Hosfstra CL, Van Ark I, and Van Oosterhout AJ 1998. Prevention of Th2-like cell responses by coadministration of IL-12 and IL-18 is associated with inhibition of antigen-induced airway hyperresponsiveness, eosinophilia, and serum IgE levels. J Immunol 161(9):5054-60.

155.Hoshino T, Wiltrout RH, and Young HA 1999. IL-18 is a potent coinducer of IL-13 in NK and T cells: A new potential role for IL-18 in modulating the immune response. J Immunol 162: 5070-5077.

156.Hsieh CS, Heimberger AB, and Murphy KM, et al 1992. Differential regulation of T helper phenotype development by IL-4 and 10 in an alpha beta T-cell-receptor transgenic system. Proc Natl Acad Sci USA 89, 6065-6069.

157.Hsieh CS, Macatonia SE, Tripp CS, et al 1993. Development of Th1 CD4+ T cells through IL-12 produced by Listeria-induced macrophages. Science 260: 547-9.

158.Hui KP, Lotvall J, Barnes PJ, 1991. Attenuation of inhaled allergen-induced airway microvascular leakage and airflow obstruction in guiea pigs by a 5-lipoexyenase inhibitor (A-63162). Am Rev Respir Dis 143: 1015-9.

159.Humbert M, Durham SR, Kimmitt P, et al 1997. Elevated expression of RNA encoding IL-13 in the bronchial mucosa of atopic and nonatopic subjects with asthma. J Allergy Clin Immunol 99: 657-65.

160.Huninghake GW, Gadek JE, Fales HM, et al 1980. Human alveolar macrophage-derived chemotactic factors for neutrophils. Stimuli and partial characterization. J Clin Invest 66: 473.

161.Huygen K, Abramowicz D, Goldman M, et al 1992. Spleen cell cytokine secretion in Mycobacterium bovis SCG-infecter mice. Infect Immun 60: 2880-2886.

162.Iwama T, Nagai H, Suda H, et al 1992. Effect of murine recombinant IL-5 on the cell population in guinea-pig airways. Br J Pharmacol 105: 19-22.

163.Iwamoto I, Nakajima H, Endo H, et al 1993. IFN-γ regulates antigen-induced eosinophil recruitment into the mouse airways by inhibiting the infiltration of CD4+ T cells. J Exp Med 177: 573-6.

164.Janoff A 1985. Elastases and emphysema. Current assessment of the protease-antiprotease hypothesis. Am Rev Respir Dis 132: 417.

165.Jeannin P, Delneste Y, Seveso M, Life P, Bonnefoy JY 1996. IL-12 synergizes with IL-2 and other stimuli in inducing IL-10 production by human T cells. J Immunol 156: 3159-65.

166.Jianfei Yang, Teresa L, Murphy KM et al 1999. Induction of IFN-γ production in Th1 CD4+ T cells: evidence for two distinct pathways for promoter acgtivation.

167.Johnson KJ, Chapman WE, and Ward PA 1979. Immunopathology of the lung: A review. Am J Pathol 95: 795-844.

168.Johson KJ, and Ward PA 1981. Role of oxygen metabolites in immune complex injury of the lung. J Immunol 126: 2365-2369.

169.Johnson SL, Freezer NJ, Howarth PH, et al 1995. Prostaglandin D2-induced bronchoconstriction is mediated only in part by the tromboxane poostanoid receptor. Eur Respir J 8: 411-415.

170.Jones DA, Carlton DP, Prescott SM, et al 1993. J Biol Chem 268:9049-9054.

171.Jones ML, and Warren JS 1992. Monocyte chemoattractant protein 1 in a rat model of pulmonary granulomatosis. Lab Invest 66: 498-503.

172.Joose GF, Pkauwels R, van der Straeten ME 1989. The effect of nedocromil sodium on the bronchoconstrictor effect of neurokinin A in subjects with asthma. J Allergy Clin Immunol 83: 663-668.

173.Jorgensen JL, et al 1992. Moecular components of T cell recognition. Annu Rev Immunol 10: 835.

174.Kaplan DR, et al 1984. Influenza virus-specific human cytotoxic T cell clone: Heterogeneity in antigenic stimulation. Cell Immunol 88: 193.

175.Kawakami Y, Uchiyama K, Murao M, et al 1973. Evaluation of aerosol of PGE1 and E2 as bronchodilators. Eur J Clin Pharmacol 6: 127-32.

176.Kay AB 1988. The eosinophil. In "Pulmonary Diseases and Disorders", 2nd ed, pp.599-606. McGraw-Hill, New York.

177.Kay AB, Ying S, Varney V 1991. Messenger RNA expression of cytokine gene cluster. IL-3, IL-5 and GM-CSF, in allergen-induced late-phase cutaneous reactions in atopkic subjects. J Exp Med 173: 775.

178.Kay AB, and Corrigan CJ 1992. Asthma, esoinophils, and neutrophils. Br Med Bull 48: 51-64.

179.Kazuya T, Kazuyuki O, Kinya N et al 2000. Differential production of prostaglandin D2 by human helper T cell subsets. J Immunol 164: 2277-2280.

180.Keane-Myers A, Game WC, Wills-Karp M, et al 1997. B7-CD28/CTLA-4 costimulatory pathways are required for the development of Th2 cell-mediated allergic responses to inhaled antigens. J Immunol 158(5): 2042-9.

181.Keatings VM, O'Connor BJ, Wright LG, et al 1997. Late response to allergen is associated with increased concentrations of TNF-α and IL-5 in induced sputum. J Allergy Clin Immunol 99: 693-8.

182.Keegan AD, Nelms K, Paul WE, et al 1994. IL-4 receptor: Signaling mechanisms. Immunol Today 15(9):423-432.

183.Kennedy MK, Picha KS, Fanslow WC, Grabstein KH, Alderson MR, Clifford KN, et al 1996. CD40/CD40 ligand interactions are required for T cell-dependent production of IL-12 by mouse macrophages. Eur J Immunol 26: 370-8.

184.Kern JS, Lamb RJ, Reed JC, et al 1988. Dexamethasone inhibition of IL-1β production by human monocytes: posttranscriptional mechanisms. J Clin Invest 81: 237-44.

185.Keshav S, Chung LP, Gordon S 1990. Macrophage products in inflammation. Diagn Microbiol Infect Dis 13: 439.

186.Kim YM, Talaian RV, Li J and Billiar TR 1998. Nitric oxide prevents IL-1β and IFN-inducing factor (IL-18) release from mcrophages by inhibiting caspase-1 (ICE). J Immunol 161: 122-128.

187.Kindler V, Sappino AP, Vassalli P, et al 1989. The inducing role of TNF in the development of bactericidal granulomas during BCG infection. Cell 56: 731-740.

188.Kips JC, Tavernier J, Pauwels RA, 1992. TNF causes bronchial hyperresponsiveness in rats. Am Rev Respir Dis 145: 332-6.

189.Kips JC, Brusselle GJ, Joos GF, et al 1996. IL-12 inhibits antigen-induced airway hyperresponsiveness in mice. Am J Respir Crit Care Med 153:535-9.

190.Kita H, Ohnishi T, Gleich GJ, et al 1991. GM-CSF and IL-3 release from human peripheral blood eosinophils and neutrophils. J Exp Med 174: 745-748.

191.Knudsen PJ, Dinarello CA, Strom TB, 1986. Prostaglandins posttranscriptionally inhibit monocyte expression of IL-1 activity by increasing intracellular cyclic adenosine monophosphate. J Immunol 137: 3189-94.

192.Kobayashi M, Fitz L, Trinchieri G, et al 1989. Identification and purification of natural killer cell stimulatory factor (NKSF), a cytokine with multiple biologic effects on human lymphocytes. J Exp Med 170: 827-846.

193.Koning H, Neijens HJ, Baert MR, et al 1997. T cell subsets and cytokines in allergic and non-allergic children. I. Analysis of IL-4, IFN-γ and IL-13 mRNA expression and protein production. Cytokine 9: 416-26.

194.Kopf M, Le Cros G, and Kohler G, et al 1993. Disruption of the murine IL-4 gene blocks Th2 cytokine responses. Nature 362, 245-248.

195.Koto H, Mak JC, Haddad EB, et al 1996. Mechanisms of impaired beta-adrenoceptor-induced airway relaxation by IL-1β in vivo in the rat. J Clin Invest 98: 1780-7.

196.Koyama S, Rennard SI, Robins RA, et al 1989. Bronchial epithelial cells release chemoattractant activity for monocytes. Am J physiol 257: L130-L136.

197.Kradin RL, McCarthy, Schmeeberger EE, et al 1991. The poor accessory cell function of macrophages in the rat may reflect their inability to form clusters with T cells. Clin Immunol Immunopathol 44: 348-363.

198.Kraemer SA, Meade SA, DeWitt DL 1992. Prostaglandin endoperoxide synthase gene structure: Identification of thetranscriptional start site and 5'-flanking regulatory sequences. Arch Biochem Biophys 293: 391-400.

199.Kubin M, Kamoun M, and Trinchieri G, et al 1994. IL-12 dynergizes with B7/CD28 interaction in inducing efficient proliferation and cytokine production of human T cells. J Exp Med 180, 211-222.

200.Kunkel SL, Strieter RM, Chensue SW, et al 1994. Molecular aspects of granulomatous inflammation. J Immunol Immunopharmacol 14: 71-77.

201.Kurland JI and Bockman R 1978. Prostaglandin E production by human blood monocytes and mouse peritoneal macrophages. J Exp Med 147: 952.

202.Kuroda E, Sugiura T, Yamashita U, et al 2000. Sensitivity diffenence to the suppressive effect of PGE2 among mouse strains: a possible mechanism to polarize Th2 response in BALB/c mice. J Immunol 164(5): 2386-2395.

203.Lack G, Bradley KL, Hamelmann E, et al 1996. Nebulized IFN-γ inhibits the development of secondary allergic responses in mice. J Immunol 157: 1432-9.

204.Laitinen LA, Haahtela T, Lee TH, et al 1993. LTE4 and granulocytic infiltration into asthmatic airway. Lancet 341: 989-90.

205.Laneuville O, Breuer DK, DeWitt DL, et al 1994. Differential inhibition of human prostaglandin endoperoxide H synthase-1 and –2 by NSADs. J Pharmacol Exp Ther 271: 927-934.

206.Langman MJS, Weil J, Wainwright P, et al 1994. Risks of bleeding peptic ulcer associated with individual NSAIDs. Lancet 343: 1075-1078.

207.Lee SC, Jaffar ZH, Wan KS, Holgate ST, and Roberts K 1999. Regulation of pulmonary T cell responses to inhaled antigen: Role in Th1- and Th2-mediated inflammation. J of Immunol 162: 6867-6879.

208.Le Gros G, Ben-Sasson SZ, Seder RA, et al 1990. Generation of IL-4-producting cells in vivo and in vitro: IL-2 and IL-4 are require4d for in vitro generation of IL-4-producting cells J Exp Med 172:921-9.

209.Leikauf GD, Veki IF, Nadel JA 1985. Bradykinin stimulates chloride secretion and PGE_2 release by anine tracheal epithelium. Am J Physiol 248: F48.

210.Lentsch AB, Czermak BJ, Ward PA, et al 1998. NF-kappaB activation during IgG immune comples-induced lung injury: requirements for TNF-α and IL-1β but not complement. Am J Pathol 152(5): 1327-36.

211.Levi Schaffer F, Barkans J, Newman TM, et al 1996. Identification of IL-2 in human peripheral blood eosinophils. Immunology 87: 155-61.

212.Lipscomb MF, Lyons CR, Toews GB, et al 1986. Human alveolar macrophages HLA-DR-positive macrophages that are poor stimulators of a primary mixed leukocyte reaction. J Immunol 136: 497-504.

213.Lopez AF, Sanderson CJ, Gamble JR, et al 1988. Recombinant human IL-5 is a selective activator of human eosinophil function. J Exp Med 167: 219-24.

214.Lukacs NW, Strieter RM, Kunkel SL, et al 1993. Cytokines in acute inflammation. Curr Opin Hematol. 1993: 26-31.

215.Lukacs NW, Strieter R, Chensue S, and Kunkel S 1994. Interleukin-4-dependent pulmonary eosinophil infiltration in a murine model of asthma. Am J Respir Cell Mol Biol 10, 526-532.

216.Lukacs NW, Strieter RM, Kunkel SL, et al 1995. TNF a mediates recruitment of neutrophils and eosinophils during airway inflammation. J Immunol 154: 5411-5417.

217.Lukacs NW, Strieter RM, Kunlel SL, et al 1955. MIP-1α influences eosinophil recruitment in antigen-specific airway inflammation. Eur J Immunol 25: 245-251.

218.Lundberg JM, Saria A, Folkers RA, et al 1983. A substance P antagonist inhibits vagally-induced increase in vascular permeability and bronchial smooth muscle contraction in the guinea pig. Proc Natl Acad Sci 80: 1120-1124.

219.Luscinskas FW, Cybuisky MI, Gimbrone MA, et al 1991. Cytokine-activated human endothelial monolayers support enhanced neutrophil transmigration via a mechanism involving both endothelial-leukocyte adhesion molecule-1 and intracellular adhesion molecule 1. J Immunol 146:1617.

220.Luttmann W, Knoechel B, Foerster M, et al 1996. Activation of human eosinophils by IL-13. Induction of CD69 surface antigen, its relationship to messenger RNA expression, and promotion of cellular viability. J Immunol 157: 1678-83.

221.Ma X, Sun J, Montaner LJ, et al 2000. Inhibition of IL-12 production in human monocyte-derived macrophages by TNF. J Immunol 164(4): 1722-9.

222.Macatonia SE, Hosken NA, Litton M, et al 1995. Dentritic cells produce IL-12 and direct the development of Th1 cells from naïve CD4+ T cells. J Immunol 154: 5071-9.

223.MacDermot J, Kelsey CR, Blair IA, et al 1984. Synthesis of LTB4 and prostanoids by human alveolar macrophages: alalysis by gas cromotography/mass spectrometry. Prostaglandins 27: 163-79.

224.Maggie LB Jr, Sadeghi H, Heitmeier MR et al 2000. Anti-inflammatory actions of 15-deoxy-delta 12,14-prostaglandin and troglitazone. Diabetes 49(3): 349-55.

225.Malone CG, Kutchera WA, Zimmerman GA, et al 1998. Inflammatory agonists induce cyclooxygenase type expression in human neutrophils. J Immunol 160(3): 1402-10.

226.Malstalerz L, Nizankowska E, and Schzeceklik A 1994. Protective effects of PGE_2 on airway sbstruction induced by aspirin in aspirn-intolerant asthmatics. Eur Respir J 7: 434S.

227.Manetti R, Parronchi P, Giudizi MG, et al 1993. Natural killer cell stimulatory factor (IL-12) induces Th1-specific immune responses and inhibits the development of IL-4-producing Th cells. J Exp Med 177:1199-204.

228.Manetti R, Gerosa F Giudizi MG, et al 1994. IL-12 induces stable priming for IFN-γ production during differentiation of human Th cells and transient IFN-γ production in established Th2 cell clones. J Exp Med 179: 1273-83.

229.Marom Z, Schelhamer JH, Kaliner M, et al 1982. Slow reacting substances, leukotrienes C4 and D4 increase the release of mucus from human airways *in vitro*. Am Rev Respir Dis 126: 449-451.

230.Martin WJ II 1984. Neutrophils kill pulmonary endothelial cells by a H_2O_2 dependent pathway: an *in vitro* model of neutrophil mediated lung injury. Am Rev Respir Dis 130: 209.

231.Martling C-R, Theordorsson-Norheim E, Lundberg JM 1987. Occurrencde and effects of multiple tachykinins: substance P, neurokinin A. neuropeptide k in human lower airways. Life Sci 40: 1633-1643.

232.Masferrer JL, Zweifel BS, Needleman P, et al 1990. J Clin Invest 86:1375-1379.

233.Masferrer JL, et al 1994. Selective inhibition of inducible cyclooxygenase 2 in vivo is antiinflammatory and nonulcerogenic. Proc Natl Acad Sci USA 91: 3228-3232.

234.Mathe AA, Hedqvist P 1975. Effect of PGF2α and E2 on airway conductance in healthy subjects and asthmatic patients. Am Rev Respir Dis 111: 313-20.

235.Maucini JA, Vickers PJ, Riendeau D, et al 1997. Altered sensitivity of aspirin-acetylated prostaglandin G/H synthase-2 to inhibition by NSAID. Mo/pharmacol 51(1):52-60.

236.Mauser PJ, Pitman AM, Fernandez X, et al 1995. Effects of an antibody to IL-5 in a monkey model of asthma. Am J Respir Crit Care Med 152:467-72.

237.McAdam BC, Catella-Lawson F, Fitzgerald GA, et al 1999. Systemic biosythesis of prostacyclin by COX-2: The human pharmacology of a selective inhibitor of COX-2. Proc Natl Acad sci USA 96: 272-277.

238.McCoombs CC, Michalske JP, Light RW, et al 1982. Human alveolar macrophages suppress the proliferagtive response of peripheral blood lymphocytes. Chest 82: 266-271.

239.McCormack DG, Salonen RO, Barnes PJ 1989. Effect of sensory neuropeptides on canine bronchial and pulmonary vessels *in vitro*. Life sci 45: 2405-2412.

240.McDonald DM, 1988. Respiratory tract infections increase susceptibility to neurogenic inflammatio in the rat treachea. Am Rev Respir Dis 137: 1432-1440.

241.McKenzie ANJ, Culpepper JA, de WaalMalefyt R, et al 1993. IL-13, a T cell-derived cytokine that regulates human monocyte and B cell function. Proc Natl Acad Sci USA 90:3735-9.

242.McMenamin C, Mckersey M, Holt PG, et al. γδ T cells downregulate primary IgE responses in reats to inhaled soluble protein antigens. J Immunol 154: 4390-4394.

243.McWilliam AS, Napoli S, Holt PG, et al 1996. Dendritic cells are recruited into the airway epithelium during the inflammatory response to broad spectrum of stimuli. J Exp Med 184: 2429-21432.

244.Meade EA, Smith WL, DeWitt DL 1993. Differential inhibition of prostaglandin endoperoxide synthase (cyclooexygenase) isozymes by aspirin and other non-steriodal anti-inflammatory drugs. J Biol Chem 268: 6610-6614.

245.Melillo E, Woolley KL, O'Byrne PM et al 1994. Effect of inhaled PCE_2 on exercise -induced bronchoconstriction in asthmatic subjects. Am J Respir Crit Care Med 149: 1138-1141.

246.Mertz PM, DeWitt DL, Wahl LM, et al 1994. J Biol Chem 269: 21322-21329.

247.Meyrick B, Reid L, 1970. Ultrastructure of cells in in the human bronchial submucosal glands. J Anat 107: 281-299.

248.Mitchell JA, Akarasereenont P, Thiemermann C, et al 1994. Selectivity of NSADs as inhibitors of constitutive and inducible cyclooxygenase. Proc Natl Acad Sci 90: 11693-11697.

249.Minty A, Chalon P, Derocq J-M, et al 1993. IL-13 is a new human lymphokine regulating inflammatory and immune responses. Nature 362: 248-50.

250.Moller GM, Overbeek SE, Hoogsteden HC, et al 1996. Increased numbers of dendritic cells in the bronchial mucosa of atopic asthmatic patients : downregulatioin by inhaled corticosteroids. Clin Exp Allergy 26: 517-524.

251.Monick M, Glazier J, Hunninghake GW 1987. Human alveolar macrophages suppressIL-1 activity via the secretion of PGE2. Am Rev Respir Dis 135: 72-77.

252.Moqbel R, Hamid Q, Kay AB, et al 1991. Expression of mRNA and immunoreactivity for the GM-CSF in activated human eosinophils. J Exp Med 174: 749-752.

253.Morgan DA, Ruscetti FW, Gallo R 1976. Selective in vitro growth of T lymphocytes from normal human bone marrows. Science 193: 1007-8.

254.Morham SG, Langenbach R, Loftin CD, et al 1995. Prostaglandin synthase 2 gene disruption causes severe renal pathology in the mouse. Cell 83: 473-482.

255.Mosmann TR, Cherwinski H, Coffman RL, et al 1986. Two types of murine helper T cell clone. I. Definition according to profiles of lymphokine activities and secreted proteins. J Immunol. 136: 2348-2357.

256.Mosmann TR, and Coffman RL 1989. Heterogeneity of cytokine secretion patterns and functions of helper T cells. Adv Immunol 46: 111-147.

257.Mostov KE, Blobel G 1982. A transmembraqne precursor of secretory component. The receptor for transcellular transport of polymeric immunoglobulins. J Biol Chem 257: 118.

258.Mould AW, Matthaei KI, Young IG, et al 1997. Relationship between IL-5 and eotaxin in regulating blood and tissue eosinophilia in mice, J Clin Invest 99: 1064-71.

259.Mulligan MS, Hevel JM, Ward PA, et al 1991. Tissue injury caused by deposition of immune complexes is L-arginine dependent. Proc Natl Acad Sci USA 88: 6338-6342.

260.Mulligan MS, Warren JS, Ward PA, et al 1992. Immune complex-induced lung and dermal vasculare injury. Differing requirements for TNF-α and IL-1. J Immunol 149: 331-339.

261.Mulligan MS, Paulson JC, Ward PA, et al 1993. Protective effects of oligosaccharides in P-selectin-dependent lung injury. Nature 364: 149-151.

262.Munder M, Mallo M, Eichmann K, and Modolell M 1998. Murine macrophaes secrete IFN-γ upon combined stimultion with IL-12 and IL-18: a novel pathway of autocrine macrophage activation. J Exp Med 187:2103-2108.

263.Munoz NM, Leff AR 1995. Blockade of eosinophil migration by 5-lipoxygenase and cyclooxygenase inhibition in explanted guinea pig trachealis. Am J Physiol 268: L446-L454.

264.Munoz E, Zubiaga AM, Huber BY et al 1990. Cholera toxin discriminates between T helper 1 and 2 cells in T cell receptor-mediated activation: role of cAMP in T cell proliferation. J Exp Med 172: 95.

265.Murakami M, Matsumoto R, Austen KF 1994. Prostaglandin endoperoxide synthase-1 and –2 couple to different transmembrane stimuli to generate PGD2 in mouse bone marrow-derived mast cells. J Biol Chem 269: 22269-75.

266.Murphy EE, Terres G, and O'Gara A, et al 1994. B7 and IL-12 cooperate for proliferation and IFN-γ production by mouse Th1 clones that are unresponsive to B7 co-stimulation. J Exp Med 180, 223-231.

267.Murphy E, Shibuya K, O'Garra A et al 1996. Reversibility of Th1 and 2 populations is lost after long-term stimulation. J Exp Med 183: 901-913.

268.Murphy KM, Heimberger AB, Loh DY 1990. Induction by antigen of intrathymic apoptosis of CD4+CD8+ TCR1o thymocytes *in vivo*. Science 250: 1720-1722.

269.Murray JJ, Tonnel AB, Brash AR, et al 1986. Release of PGD2 into human airways during acute antigen challenge. N Engl J Med 315: 800-804.

270.Nadel JA, David B, Phipps RG 1979. Control of mucus secretion and ion transport in airway. Annu Rev Physiol 41:369-381.

271.Nagai H, Yamaguchi S, Takatsu K, et al 1993. Effect of anti-IL-5 monoclonal antibody on allergic eosinophilia and airway hyperresponsiveness in mice. Life Sci 53: PL243-PL247.

272.Nakajima H, Iwamoto I, Yoshida S 1993. Aerosolized recombinant IFN-γ prevents antigen-induced eosinophil recruitment in mouse trachea. Am Rev Respir Dis 148: 1102-4.

273.Nakajima H, Sano H, Iwamoto I, et al 1994. Role of vascular cell adhesion molecule1/VLA-4 and intracellular adhesion molecule/lymphocyte function-associated antigen 1 interaction in antigen-induced eosinophil and T cell recruitment into tissue. J Exp Med 179: 1145-1154.

274.Naseer T, Mnshall EM, Leucg DY, et al 1997. Expression of IL-12 and IL-13 mRNA in asthma and their modulation in response to steroid therapy. Am J Respir Crit Care Med 155: 845-51

275.Needleman P, Isakson PC 1997. The discovery and function of COX-2. J Rheumatol. 24(supple 49): 6-8.

276.Niiro H, Otsuka T, and Niho Y, et al 1994. IL-10 inhibits prostaglandin E2 production by lipopolysaccharide-stimulated monocytes. Int Immunol 6, 661-664.

287.Niiro H, Ostsuka T, Nakashima H, et al 1997. Blood 89: 1621-1628.

288.Nophar Y, Kemper O, Brakebusch C, et al 1990. Soluble forms of TNF-Rs. The cDNA for the type 1 TNF-R, cloned using amino acid sequence datqa of its soluble form, encodes both the cell surface and a soluble form of the receptor. EMBO J 9: 3269-78.

289.Novak TJ and Rothenberg EV 1990. cAMP inhibits induction of IL-2 but not of IL-4 in T cells. Proc Ntl Acad Sci USA 87: 9353.

290.Oates JA, FitzGerald GA, Branch RA, et al 1988. Clinical implications of prostaglandin and thromboxane A2 formation. N Engl J Med 319: 689-698, 761-767.

291.Ohlsson K, Bjork P, Thompson RC, et al 1990. IL-1 receptor antagonist reduces mortality from endotoxin shock. Nature 348: 550-552.

292.Ohno I, Lea RG, jordana M, et al 1992. Eosinophils in chronically inflamed human upper airway tissues express transforming growth factor β1 gene. J Clin Invest 89: 1662-1668.

293.Onoe Y, Miyaura C, Kaminakayashiki T, et al 1996. IL-13 and IL-4 inhibit bone resorption by suppressing COX-2-dependent prostaglandin synthesis in osteoblasts. J Immunol 156:758-64.

294.Oppenheim JJ, Zachariae COC, Matsushima K, et al 1991. Properties of the novel proinflammatory intercrine cytokin family. Annu Rev Immunol 9:617

295.O'Sullivan MG, Huggins EMJ, McCall CE, et al 1992. LPS induces prostaglandin H synthase-2 in alveolar macrophages. Biochem Rev Comm 187:1123-7

296.OSullivan MG, Chilton FH, McCall CE, et al 1992. LPS priming of alveolar macrophages for enhanced synthesis of prostanoids involves induction of a novel prostaglandin H synthase. J Bio Chem 267: 14547-50.

297.Qureshi MH, Zhang T, and Kawakami K 1999. Combeined effects of IL-12 and IL-18 on the clinical course and local cytokine production in murine pulmonary infection with Cryptococcus neoformans. Eur J Immunol 29:643-649.

298.Owen Jr WF, Rothenberg ME, Soberman RJ, et al 1987. Regulation of human eosinophil viability, density and function by GM-CSF in the presence of 3T3 fibroblasts. J Exp Med 166: 129-141.

299.Pabst R 1992. Compartmentalization and kinetics of lymphoid cells in the lung. Reg Immunol 3: 62-71.

300.Pang L, Holland E, Knox AJ, 1998. Role of cyclooxygenase-2 inductioin in IL-1β induced attenuation of cultured human airway smooth muscles cells cyclic-AMP generation in response to isoprenaline. Br. J Pharmacol 125: 1320-8.

301.Panina-Bordignon P, Mazzeo D, Lucia PD, et al 1997. Beta$_2$-agonists prevent Th1 development by selective inhibition of IL-12. J Clin Invest 100: 1513-9

302.Pavord ID, Wong CS, Tattersfield AE, et al 1993. Effect of inhaled PGE2 on allergen-induced asthma. Am Rev Respir Dis 148(1): 87-90.

303.Penglis PS, Cleland LG, James MJ et al 2000. Differential regulation of prostaglandin E2 and thromboxane A2 production in human monocytes: implications for the use of cyclooxygenase inhibitors. J Immunol 165(3): 1605-11.

304.Peters JH, Gieseler B, Steinbach F, et al 1996. Dendritic cells: from ontogenetic orphans to myelomonocytic descendants. Immunol Today 17: 273-277.

305.Phipps RP, Stein SH, and RL Roper 1991. A new view of prostaqglandin E regulation of the immune response. Immunol today 12(10): 349-352.

306.Piacentini GL, Kakiner MA 1991. The Potential roles of leukotrienes in bronchial asthma. Am Rev Respir dis 143: S96-S99.

307.Piper PJ 1983. Pharmacology of leukotrienes. Br Med Bull 39: 255-9.

308.Pizarro TT, Michie MH, Cominelli F, et al 1999. IL-18, a novel immunoregulatory cytokine, is up-regulated in Crohn's disease: Expression and localization in intestinal mucosal cells. J Immunol 162: 6829-6835.

309.Pober JS, Gimbrone MA, Jr., Lapierre LA, et al 1986. Overlapping patterns of activation of human endothelial cells by IL-1, TNF, and immune interferon. J Immunol 137: 1893-6.

310.Porreca E, Di Febbo C, Di Dciullo A, et al 1996. Cysteinyl leukotriene D4 induced vascular smooth muscle cell proliferation: a oossible role in myointimal hyperplasia. Thromb Haemost 76: 99-104.

311.Pretolani M, Lapa C, Vargaftig BB, et al 1994. Antibody to very late activation 4 prevents antigen-induced bronchial hyperreactivity and cellular infiltration in the guinea pig airway. J Exp Med 180: 795-805.

312.Pryhuber GS, Huyck HL, OReilly MA, et al 2000. Tumor necrosis factor-alpha-induced lung cell expression of antiapoptotic genes TRAF1 and cIAP2. Am J Respir Cell Mol Biol 22(2): 150-6.

313.Rainsford KD, Velo GP, eds 1992. Side-effects of Anti-infalmmatory Drugs-4. Dordrecht: Kluwer Academic Publishers.

314.Rashad S, Rainsford K, Walker FS, et al 1989. Effect of NSADs on the course of osteoarthritis. Lancet 2: 519-522.

315.Randolph DA, Carruthers CJL, Szabo SJ, Murphy KM, and Chaplin DD 1999. Modulation of Airway Inflammation by Passive Transfer of Allergen-Specific Th1 and Th2 Cells in a Mouse Model of Asthma. J Immunol.162:2375-2383.

316.Raskovic S, Bogic M, Spiric V, et al 1998. The role of prostaglandins in allergic inflammation. Arp Arh Celok Lek 126(9-10): 388-93.

317.Raskin JB 1999. Gastrointestinl Effects of NSAIDs. Am J Med 106(5B): 5B-3S.

318.Raud J, Dahlen SE, Hedqvist P, et al 1988. Enhancement of acute allergic inflammation by indomethacin is reversed by PGE2: apparent correlation with *in vivo* modulation of mediator release. Proc Natl Acad Sci USA 85: 2315-9.

319.Ray CA, Black RA, Kronheim SR, et al 1992. Viral inhibition o inflammation: cowpox virus encodes an inhibitor of the IL-1β converting enzyme. Cell 69: 597-604.

320.Reddy ST, Herschman HR 1994. Ligand-induced prostaglandin synthesis requires expression of the TIS10/PGS-2 prostaglandin synthase gene murine fibroblasts and macrophages. J Bio Chem 269: 15473-80.

321.Resnick MB, and Weller PF 1993. Mechanisms of eosinophil recruitment. Am J Respir Cell Mol Biol 8: 349-355.

322.Ristimaki A, Garfinkel S, Hla T, et al 1994. Induction of COX-2 by IL-1β. Evidence for posttranscriptional regulation. J Biol Chem 269: 11769-75.

323.Robbins RA, Shoji S, Rennard SI, et al 1989. Bronchial epithelial cells release chemotactic activity for lymphocytes. Am J Physiol 257: L109-L115.

324.Robinson DS, Hamid Q, Kay AB, et al 1992. Predominant Th2-like bronchoalveolar T-lymphocyte population in atopic asthma. N Engl J Med 326:298-304.

325.Robinson DS, Hamid Q, Ying S, et al 1993. Prednisolone treatment in asthma is associated with modulation of bronchoalveolar lavage cell IL-4, IL-5, and IFN-γ cytokine gene expression. Am Rev Respir Dis 148: 401-6.

317.Rodney DN, William FS, and Robin GL 1999. Cyclooxygenase-2-depentarachidonic acid metabolites are essential modulators of the intenstinal immune response to dietary antigen. Nature medicine 5(8):900-905.

318.Rogers DF, Awvdkij B, Barnes PJ 1989. Effects of tachykinins on mucus secretion in human bronchi *in vitro*. Eur J pharmacol 174: 283-286.

319.Rogy MA, Auffenberg T, Moldawer LL, et al 1995. Human TNF receptor (p55) and IL-10 gene transfer in the mouse reduces mortality to lethal endotoxemia and also attenuates local inflammatory responses. J Exp Med 181: 2289-2293

320.Romagnani S et al 1999. Th1 and Th2 cells. Inflamm Bowel Dis 5(4): 285-95.

321.Roth MD, Golub SH 1993. Human pulmonary macrophages utilize prostaglandin and TGF beta 1 to suppress lymphocytes activation. J Leuk Biol 53: 366-371.

322. Rossman CM, Lee R, Forrest JB, et al 1984. Nasal ciliary ultrastructure and function in patients with primary ciliary dyskinesia compared wirh that in normal subjects and in subjects with various respiratory diseases. Am Rev Respir Dis 129: 161.

323.Rothenberg ME, Owen Jr WF, Stavens RL, et al 1988. Human eosinophils have prolonged survival, enhanced functional properties, and become hypodense when exposed to human IL-3. J Clin Invest 81: 1986-1992.

324.Rothenberg ME, Petersin J, Stevens RL, et al 1989. IL-5 dependent conversion of normodense human eosinophils to the hypodense phenotype uses 3T3 fibroblasts for enhanced viability: accelerated hypodensity and sustained antibody-dependent cytotoxicity. J Immunol 143: 2311.

325.Rothenberg ME, Ownbey R, Mehlhop PD, et al 1996. Eotaxin triggers eosinophil-selective chemotaxis and calcium flux via a distinct receptor and induces pulmonary eosinophilia in the presence of IL-5 in mice. Mol Med 2: 334-48.

326.Rothenberg ME, MacLean JA, Pearlman E, et al 1997. Targeted disruption of the chemokine eotaxin partially reduces antigen-induced tissue eosinophilia. J Exp Med 185: 785-90.

327.Saltini C, Kirby M, Crystal RG, et al 1990. Biased accumulation of T lymphocytes with "memory" – type CD45 leukocyte common antigen gene expression on the epithelial surface of the human lung. J Exp Med 171: 1123-1140.

328.Sano H, Hla T, Maier JAM, et al 1992. In vivo cycloozygenase expression in synovial tissues of patients with rhematoid arthritis and osteoarthritis and rats with adjuvant and streptococcal cell wall arthritis. J Clin Invest 89: 97-108.

329.Schafer AI 1999. Effects of Nonsteroid anti-inflammatory Therapy on Platelets. Am J Med 106: 5B-25S.

330.Schelhamer JH, Marom Z, Kaliner M, et al 1982. The effects of arachnoids and leukotrienes on the release of mucus from human airways. Chest 81-36S-37S.

331.Schon-Hegrad MA, Oliver J, Holt PG, et al 1991. Studies on the density, distribution, and surface phenotype of intraepithelial class II major histocompatability complex antigen (Ia)-bearing dendritic cells in the conducting airways. J Exp Med 173: 1345-1356.

332.Schuligoi R, Amann R, Reshar BA, et al 1998. Effects of COX-2 inhibitor, NS-398 on thromboxane and leukotriene synthesis in rat peritoneal cells. Inflamm Res 47(5): 227-30.

333.Schweizer RC, Welmers BA, and Koenderman L, et al 1994. RANTES- and IL-8-induced responses in normal human eosinophils: Effects of priming with IL-5. Blood 83, 3697-3704.

334.Secor WE, Stwart SJ, Coley DG 1990. Eosinophils and immune mechanisms. VI. The synergistic combination of GM-CSF and IL-5 accounts for eosinophil-stimulation promoter activity in Schistosoma mansoni-infected mice. J Immunol 144: 1484.

335.Seder RA, Paul WE, David MM, and Fazekas de St. Groth B 1992. The presence of IL-4 during in vitro priming determines the lymphocyte producing potential of CD4+ T cells from T cell receptor transgenic mice. J Exp Med 176: 1091-1098.

336.Seder RA, Gazzinelli R, Paul WE 1993. IL-12 acts directly on CD4+ T cells to enhance priming for IFN-γ production and diminishes IL-4 inhibition of such priming. Proc Natl Acad Sci USA 90: 10188-10192.

337.Seder RA, and Paul WE 1994. Acquisition of lymphokine-producing phenotype by CD4+ T cells. Annu Rev Immunol. 12, 635-673.

338.Sehmi R, Wood LJ, Watson R, et al 1997. Allergen-induced increases in IL-5 receptor alpha-subunit expression on bone marrow-derived CD34+ cells from asthmatic subjects. A novel marker of progenitor cell commitment towards eosinophilc differentiation. J Clin Invest 100: 2466-75.

339.Seibert K, Masferrer JL, Needleman P, et al 1991. The biochemical and hparmacologic manipulation of cellular cyclooxygenase (COX) activity. Adv Prostaglandin Thromboxane Leukot Res 21: 45-51.

340.Serhan CN, Haeggstrom JZ, Leslie CC 1996. Lipid mediator networks in cell signaling: update and impact of cytokines. FASEB J 10: 1147-58.

341.Shah A, Church MK, Holgate ST, 1995. TNF-α: a potential mediator of asthma. Clin Exp Allergy 23: 247-50.

342.Shanley TP, Schmal H, Ward PA, et al 1991. Role of MIP-1α in acute lung injury in rats. J Immunol 154(9): 4793-4802.

343.Shasby DM, Schwarz MI, Kassan SS, et al 1982. Pulmonary immune complex deposition in Wegner's granulomatous. Ch Us gr aul o-340.

344.Shibata Y, Foster LA, Metzger WJ, et al 1998. Immunoregulatory roles of IL-10 in innate immunity: IL-10 inhibits macrophage production of IFN-γiinducing factors but enhances NK cell production of IFN-γ. J Immunol 161: 4283-4288.

345.Shoji S, Ertl R, Rennard SI 1987. Cigarette smoke stimulates release of neutriphil chemotactic activity from cultured bronchial epithelial cells. Clin Res 35: 539A.

346.Smith KA 1988. Interleukin-2: inception, impact, and implications. Science 240: 1169-76.

347.Smith PG, Janiga KE, Bruce MG 1994. Strain increases airway smothmuscle cell proliferation. Am J Respir Cell Mol Biol 10:85-90.

348.Smith RT, Norcross M, Maino V, and Konaka Y 1980. Helper mechanisms in T cell activation. Immunol Rev 51: 193-214.

349.Smith WL, DeWitt DL 1995. Biochemistry of prostaglandin endoperoxide H synthase-1 and synthase-2 and their differential susceptibility to NSAIDs. Semin Nephrol 15: 179-94.

350.Smith WL, Garavito RM, and Dewitt DL 1996. Prostaglandin endoperoxide H synthases (cyclooxygenases)-1 and –2. J Biol Chem 271: 33157-33160.

351.Snijdewint FG, Kalinski P, Kapsenberg ML, et al 1993. PGE2 differentially modulaes cytokine secretion profiles of human T helper lymphocytes. J Immunol 150(12): 5321-9.

352.Snyderman CH, Abbas MM, DAmico F, et al 1995. Inhibition of growth of a murine squamous cell carcinoma by a COX inhibitor increase leukotriene B4 production. Arch Otolaryngol Head Neck Surg 121(9): 1017-20.

353.Sousa AR, Lane Sj, Nakhosteen JA, et al 1996. Expression of IL-1β and interleukin-1 receptor antagonist (IL-1ra) expression in asthmatic bronchial epithelium. Am J Respir Crit Care Med 154: 1061-6.

354.Soutar CA, 1976. Distribution of plasma cells and other cells containing immunoglobulin in the respiratory tract of normal man and class of immunoglobulin contained therein. Thorax 31:158-166.

355.Spahn JD, Szefler SJ, Surs W, et al 1996. A novel action of IL-13: induction of diminished monocyte glucocorticoid receptor-binding affinity. J Immunol 157: 2654-9.

356.Spits H, de Waal Malefyt R, 1992. Functional characterization of human IL-10. Int Arch Allergy Appl Immunol 99: 9-15.

357.Standiford TJ, Strieter RM, Kunkel SL, et al 1995. Neutralization of IL-10 increases lethality in endotoxemia: Cooperative effects of macrophage inflammatory protein-2 and TNF. J Immunol 155: 2222-2229.

358.Stein Sh, Phipps RP 1992. Anti-class II antibodies potentiate IgG2a production by lipopolysaccharide-stimulated B lymphocytes treated with PGE2 and IFN-γ. J Immunol 148(12): 3943-9.

359.Steinman RM 1991. The dendritic cell system and its role in immunogenicity. Annu Rev Immunol 9: 271-296.

360.Stephen HG, Sharon LM, Darryl CZ, et al 1999. Allergic lung responses are increased in prostaglandin H synthase-defieient mice. J Clin Invest 104(6): 721-732.

361.Stolina M, Sharma S, Dubinett SM, et al 2000. Specific inhibition of cyclooxygenase 2 restores antitumor response by altering the balance of IL-10 and IL-12 synthesis. J Immunol 164(1): 361-70.

362.Strieter RM, Kunkel SL and Bone RC 1993. Role of TNF-α in disease states and inflammation. Crit Care Med. 21(10 Suppl): S447-S463.

363.Sugawara I, Yamada H, and Akira S 1999. Role of IL-18 in mycobacterial infection in IL-18-gene-disrupted mice. Infect Immunol 67(5):2585-9.

364.Sun J, Elwood W, Chung KF, et al 1994. Contribution of intercellular-adhesion molecule-1in allergen-induced airway hyperresponsiveness and inflammation in sensitised brown-Norway rats. Int Arch Allergy Immunol 104(3): 291-295.

365.Suzuki M, Taka R, Mariin JG, et al 1999. CD8+ T cell modulate late allergic airway response in Brown Norway rats. J Immunol 163(10): 5574-81.

366.Swain SL, Weinber AD, English M, et al 1990. IL-4 directs the development of Th2-like helper effectors. J Immunol 145: 3796-806.

367.Swain SL, Bradley LM, and Huston G, et al 1991. Helper T cell-subsets: Phenotype, function and the role of lymphocytes in regulating their development. Immunol Rev 123, 115-155.

368.Swain SL, 1993. IL-4 dictates T cell differentiation. Res Immunol 144: 567-651.Takagi M, Koike K, Nakahata T 1990. Antiproliferative effect of IFN-γ on proliferation of mouse connective tissue-type mast cells. J Immunol 145: 1880.

369.Takahiko M, Fumitaka U, Shuh N, et al 1997. Altered pain perception and inflammatory response in mice lacking prostacyclin receptor. Nature 388: 678-682.

370.Tanaka T, Hu-Li J, and Paul WE, et al 1993. IL-4 suppresses IL-2 and IFN-γ production by naive T cells stimulated by accessory cells-dependent engagement. Proc Natl Acad Sci USA 90, 5914-5918.

371.Tauber AL, Kaliner M, Austen KF, et al 1973. Immunological release of histamine and SRSA from human lung. J Immunol 111: 27-32.

372.Tavernier J, Devos R, Cornelis S, et al 1991. A human high affinity IL-5 receptor is composed of an IL-5-specific α chain and β chain with the receptor for GM-CSF. Cell 66: 1175-84.

373.Thepen T, van Rooijen N, Kraal G 1989. Alveolar macrophage elimination in vivo is associated with an increase in pulmonary immune response in mice. J Exp Med 170:499-509.

374.Thepen T, McMenamin CE, Holt PG, et al 1991. Regulation of immune response to inhaled antigen by alveolar macrophages: differential effects of in vivo alveolar macrophage elimination on the induction of tolerance vs. immunity. Eur J Immunol 21: 2845-2850.

375.Thornhill MH, Wellicome SM, Mahiouz DL, et al 1991. TNF combines with IL-4 or IFN-γ to selectively enhance endothelial cell adhesiveness for T cells. The contribution of vascular adhesion molecule-1-dependent and independent binding mechanisms. J Immunol 146: 592-8.

376.Till S, Li B, Durham S, et al 1995. Secretion of the eosinophil-active cytokines IL-5, granulocyte/macrophage colony-stimulating factor and IL-3 by bronchoalveolar lavage CD4+ and CD8+ T cell lines in atopic asthmatics, and atopic and non-atopic controls. Eur J Immunol 25: 2727-31.

377.Tomassini M, Tsicopoulos A, Cpapron M, et al 1991. Release of granule proteins by eosinophils from allergic and non-allergic patientswith eosinophilia on immunoglobulin-dependent activation. J Allergy Clin Immunol 88: 365-375.

378.Tomura M, Maruo S, and Fujiwara H, et al 1998. Differential capacities of CD4+, CD8+, and CD4-CD8- T cell subsets to express IL-18 receptor and produce IFN-γ in response to IL-18. J Immunol 160(8): 3759-65.

379.Torigoe K, Ushio S, Okura T, et al 1997. Purification and characterization of the human IL-18 receptor. J Biol Chem 272: 25737-42.

380.Todt J, Sonstein J, Curtis JL, et al 2000. Repeated intratracheal challenge with particulate antigen modulates murine lung cytokines. J Immunol 164(8): 4037-4047.

381.Trautinger F, Hammerle AF, Micksche M, et al 1991. Respiratory burst capability of PMN and TNF-α serum levels in relationship to the development of septic syndrome incritically ill patients. J Leukocyte Biol 49: 449-454.

382.Trinchieri G 1995. IL-12: a proinflammatory cytokine with immunoregulatory functions that bridge innate resistance and antigen-specific adaptive immunity. Annu Rev Immunol 13: 251-6.

383.Tumang MC, Keogh C, Murray HW, et al 1994. Role and effect of TNF-α in experimental visceral leishmaniasis. J Immunol 153: 768-775.

384.Ushikubi f, Hirata M, Hirata T, et al 1995. Prostaglandin receptors. Gendai Iryo 27: 1873-86 (in Japanese).

385.van der Pouw Kraan TC, Boeije LC, de Groot ER, et al 1997. Reduced production of IL-12 and IL-12-dependent IFN-γ release in patients with allergic asthma. J Immunol 158: 5560-5.

386.van der Pouw Kraan TC, Boeije LC, Smeenk RJ, et al 1995. PGE2 is a potent inhibitor of human IL-12 production. J Exp Med 181:775-9.

387.Vane JR, Flower RJ, Botting RM 1990. History of aspirin and its mechanism of action. Stroke 21: Suppl: IV-12-IV23.

388.Van Oosterhout AJ, Ladenius AR, Nijkamp FP, et al 1993. Effect of anti-IL-5 and IL-5 in airway hyperreactivity and esoinophils in guinea pigs. Am Rev Respir Dis 147: 548-52.

389.Van Oud Alblas AB, Van Der Linden-Schrever B, Van Furth R 1983. Origin and kinetics of pulmonary macrophages durijg an inflammatory reaction induced by intralveolar administration of aerosolized heat-killed BCG. Am Rev Respir Dis 128: 276-281.

390.Venge J, Lampinen M, Haansson L, et al 1996. Identification of IL-5 and RANTES as the majoreosinophil chemoattractants in the asthmatic lung. J Allergy Clin Immunol 97: 1110-5.

391.Vremec D and Shortman K 1997. Dentritic cell subtypes in mouse lymphoid organs. J Immunol 159: 565-573.

392.Walker C, Vicrhow JC, Blaser K, et al 1991. T cells and asthma. II. Regulation of eosinophilia of asthma by T cell cytokines. In Arch Allergy Appl Immunol 94: 248-250.

393.Walters EH, OByrne PM, Nadel JA et al 1984. Control of neurotransmission by prostaglandins in canine trachealis smooth muscle. J Appl Physiol 57: 129-134.

394.Wardlaw AJ, Moqbel R, Kay AB 1995. Eosinophils: Biology and role in disease. Adv In Immunol 60: 151-262.

395.Ward PA, Warren JS, and Johnson K 1988. Oxygen radicals, inflammation, and tissue injury. Free Rad Biol Med 5(5-6): 403-408.

396.Watkins DN, Peroni DJ, et al 1999. Expression and localization of COX-2 in human airways and cultured airway epithelial cells. Eur Respir J 13(5): 999-1007.

397.Watson N, Magnussen H, Robe KF 1997. Inherent tone of human bronchus: role of eicosanoids and the epithelium. Br J Pharmacol 121(6): 1099-104.

398.Weirenga EA, Snoek M, DeGroot C 1990. Evidence for compartmentalization of subsets of CD4+ T lymphocytes in atonic patients. J Immunol 144: 4651.

399.Wenzel SE 1997. Arachidonic acid metabolites: mediators of inflammation in asthma Pharmacotherapy 17: 3S-12S.

400.Willoughby DA, Moore AR, Colville-Nash PR 2000. Cyclopentenone prostaglandins-new allies in the war on inflammation. Nature Medicine 6(2):137-138.

401.Wills-Karp M, Luyimbazi J, Xu X, et al 1998. IL-13: central mediator of allergic asthma. Science 282: 2258-61.

402.Witthaut R, Farhood A, Jaeschke H, et al 1994. Complement and TNF-α contribute to Mac-1 upregulation and systemic neutrophil activation during endotoxemai in vivo. J Leukocyte Biol 55: 105-111.

403.Wizemann TM, and Laskin DL 1994. Enhanced phagocytosis, chemotaxis, and production of oxygen intermediates by interstitial lung macrophages following acute endotoxemia. Am J Respir Cell Mol Biol 11: 358-365.

404.Wolf SF, Temple PA, Perussia B, et al 1991. Cloning of cDNA for natural killer cell stimulatory factor, a hegterodimeric cytokine with multiple biologic effects on T and Natural killer cells. J Immunol 146: 3074-3081.

405.Wong DTW, Elovic A, Weller PF, et al 1991. Eosinophils from patients with blood eosinophilia express transforming growth factor β1. Blood 78: 2702-2707.

406.Xu D, Chan WL, Leung BP, et al 1998. Selective expression and functions of IL-18 receptor on Th1 type but not Th2 cells. J Exp Med 188:1485-92.

407.Yang JF, Murphy TL, Ouyang WJ, and Murphy KM 1999. Induction of interferon-γ production in Th1 CD4+ T cells: evidence for two distinct pathways for promoter activation. Eur J Immunol 29: 548-555.

408.Yamaguchi Y, Hayashi Y, Sugama Y, et al 1988. Highly purified murine IL-5 stimulates eosinophil function and prolongs in vitro survival. IL-5 as an eosinophil factor. J Exp Med 167: 1737-40.

409.Yen SS, Maathe AA, Dugan JJ 1976. Rease of prostaglandins from healthy and sensitized guinea-pig lung and trachea by histamine. Prostaglandins 11: 227-239.

410.Yokomizo T, Izumi T, Shimizu T, et al 1997. A G- Protein-coupled receptor for LTB4 that mediates chemotaxis. Nature 387: 620-4.

411.Zhang K, Gharaee-Kermani M, Phan SH, et al 1997. TNF-α-mediated lung cytokine networking and eosinophil recruitment in pulmonary fibrosis. J Immunol 158(2): 954-9.

412.Zurawski G, and deVries JE 1994. IL-13, an IL-4-like cytokine that acts on monocytes and B cell, but not on T cells. Immunol Today 15(1): 19-26.