Manning the barricades: lung fibroblasts and CD4+ T cells as the last line of defence against bacterial invasion?

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Abstract

Fibroblasts are a major structural cell in the human lung, being responsible for the production of extracellular matrix components that provide the intricate structure necessary for correct lung function. Generally located in the submucosa, fibroblasts do not usually directly interact with the commensal microbes we now know are resident in the airways. However, during situations where alveolar macrophages and epithelial cells are impaired, for example during severe viral infections leading to pneumonia, bacteria can invade the lung mesenchyme. In these circumstances, fibroblasts may represent another immunological barrier to bacterial invasion, not just as innate immune effectors but also by interacting with migrating and tissue-resident adaptive immune cell populations, such as CD4+ T cells. The cytokines produced by CD4+ T helper cells are integral in directing appropriate innate and adaptive immune responses against bacteria but the nature of fibroblast-CD4+ cell interaction, unlike the CD8+ T cell interaction, is not clearly established. Here, we review the responses of lung fibroblasts to bacteria and discuss emerging data indicating a key role for these cells in directly presenting bacterial antigens to CD4+ T cells.

Key words: Human, Lung, fibroblast, antigen presentation, T cells, bacteria, NTHi

Abbreviations

APC	Antigen presenting cell
CCL	C-C chemokine ligand
CCR	C-C chemokine receptor
CD	Cluster of differentiation
CF	Cystic Fibrosis
COPD	Chronic obstructive pulmonary disease
CXCL	C-X-C motif chemokine ligand
DAMP	Danger associated molecular pattern
ECM	Extracellular matrix
HLA	Human leukocyte antigen
ICAM-1	Intercellular adhesion molecule-1
ICOS	Inducible T cell co-stimulator
IFNγ	Interferon gamma
IL	Interleukin
IPF	Idiopathic Pulmonary Fibrosis
MHC	Major histocompatibility complex
NTHi	Nontypeable *Haemophilus influenzae*
PAMP	Pathogen associated molecular pattern

PRR	Pattern recognition receptor
RSV	Respiratory syncytial virus
Spn	*Streptococcus pneumoniae*
TCR	T cell receptor
TGFβ	Transforming growth factor-beta
Th1, 2,17	T helper cell subtype 1,2,17
TLO	Tertiary Lymphoid Organs
TLR	Toll-like receptor
TNF-α	Tumour necrosis factor- alpha
Treg	Regulatory T cell

Introduction

The human lung is one of the most intricate organs in the body whose delicate structure allows the gas exchange processes vital to life. To achieve this structure, the lung is made up of an array of cells including the epithelia, endothelia, and stromal cell populations such as fibroblasts and smooth muscle cells arranged on a complex network of extracellular matrix proteins such as collagen and elastin. To protect the lung architecture against damage and maintain gas exchange, the lung has a number of mechanical, chemical and cellular means of clearing particulates, bacteria and viruses.

In the specific case of bacteria, as a result of new culture-independent means of detection, it is becoming clear that even in health the lungs are not a sterile environment and that the lung has its own microbiota.[1-4] However, many of these healthy lung commensals, such as non-typeable *Haemophilus influenzae* (NTHi), can also switch to being pathogenic and cause pneumonias, compromising gas exchange.[5,6] Therefore, the lung must permit a switch from an immunosuppressive/tolerant steady state to one that allows the development of a proinflammatory immune response against specific threats. This response requires a complex interplay between different cell types of both the innate (e.g. macrophages) and the adaptive immune system and in particular tissue-resident, antigen-experienced T cells, a large number of which reside in the submucosa in close proximity to fibroblasts.[7,8] This review focuses on the interaction between lung-resident T cells and fibroblasts and specifically the role of these interactions in response to bacterial infection.

Bacteria in the human lung

As a mucosal surface, the lung is regularly exposed to pathogenic and potentially pathogenic factors that must be dealt with by the immune system. These include both transient viral infections as well as bacterial populations that colonize the lungs in both healthy individuals and those with underlying lung pathologies. Whereas in the past the healthy lung was considered a largely sterile environment beyond the nasopharyngeal area, it is now known that large numbers of bacteria inhabit the upper and lower respiratory tracts of the human lung.[1] These colonizing bacteria are collectively referred to as the lung microbiota, a term originally coined to describe the vast numbers of bacteria resident on other mucosal surfaces, such as the gut.[9]

Utilizing modern 16s rRNA analysis techniques, it has been shown that an average of $2.2x10^3$ bacteria are present per square cm of lung airway.[2] The most common genera present in health are Actinobacteria, Bacteroidetes, Firmicutes, Proteobacteria and Fusobacterium.[2] However, within these genera potentially-pathogenic microorganisms (PPM), were also detected. For example, *Streptococcus* spp were identified in the Firmicutes genera and *Haemophilus* spp in the Proteobacteria genera. Furthermore, in asthma, chronic obstructive pulmonary disease (COPD) and idiopathic pulmonary fibrosis (IPF), there were increases in the proportion of Proteobacteria species and NTHi in particular compared to samples derived from healthy controls.[2,6,10]

NTHi is a Gram-ve non-encapsulated bacterium that is associated with respiratory infections throughout life. In the very young, this bacterium is associated with Otitis Media,[11] whereas in the elderly, as discussed above, it is commonly associated with COPD and disease exacerbations in particular.[12] It is also increasingly being detected in pneumonia cases.[5,6] Most significantly NTHi is emerging as a distinct cause of invasive bacterial disease, possibly as a

result of it overtaking the niche left by the encapsulated members of this species since the introduction of the *H. influenzae* serotype b (Hib) vaccine programme.[13]

As these bacteria are present in healthy individuals, it is thought there is extensive cross-talk between the immune system and the microbiota ensuring bacterial numbers are kept in check while limiting inflammation to prevent immunopathology. This tolerance could arise from interactions between pathogen-associated molecular patterns (PAMPs) and their associated Pattern-Recognition Receptors (PRRs) or via signalling from microbial metabolites, such as short chain fatty acids.[6,14,15] However the tolerance arises, what is not clear are the signals that promote the shift away from commensalism to pathogenesis. What is evident is a role for viral infections, and for influenza in particular, in disrupting the microbiota and leading to secondary bacterial pneumonias.

Influenza infection leads to bacterial invasion of parenchymal tissue

Respiratory viruses such as influenza, respiratory syncytial virus (RSV) and rhinovirus are common lung pathogens that typically invade the epithelial cells of the respiratory tract. RSV invades primarily the upper respiratory tree and is a cause of morbidity and mortality in young children.[16] Rhinovirus again invades epithelial cells primarily in the upper respiratory tract in healthy individuals, although infection has been shown to spread to the lower tract.[17] Both RSV and rhinovirus infections are associated with a transient, mild illness and are usually effectively cleared in immunocompetent adults. In contrast, influenza infection can cause much more serious disease. Influenza can be broadly split into 2 main categories: seasonal and pandemic strains. Seasonal strains are typically dealt with more robustly by the immune system due to similarities between the current strain and the previously encountered seasonal strains.[18] Pandemic strains are much less well controlled due to their radically different antigenic profile

(usually due to reassortment of immunodominant antigens between two different influenza strains).[19,20] Through infection of epithelial cells and macrophages,[21,22] influenza has considerable effects upon the host immune system, and this virus is therefore considered a major lung pathogen. However, secondary bacterial pneumonia following viral infection appears to be the major cause of mortality in the context of influenza infections largely as a result of significant infiltration of neutrophils into the airways and alveolar spaces and marked damage to the lung epithelium.[23,24] *Streptococcus pneumoniae* (Spn) is the bacterial species usually associated with bacterial pneumonias secondary to influenza but there is also a long history of NTHi also being associated with secondary pneumonias under its previous designation of *Bacillius influenzae*.[24]

A murine model of virally induced bacterial pneumonia has demonstrated that administering Spn to mice previously infected with influenza resulted in bacterial invasion beyond the epithelia into the mesenchyme.[25] This invasion was accompanied by a drastic increase in neutrophil recruitment compared to mice exposed to influenza or bacteria alone. Furthermore, these increased neutrophil populations exhibited impaired antimicrobial activity compared to single-infected equivalents, and the increased presence of these cells appears to drive pathology to influenza.[26] Such observations suggest excessive neutrophil recruitment as a major factor in secondary bacterial mortality. A further study demonstrated that influenza infection appears to drive massive alveolar macrophage depletion (in some cases up to 90% depletion); after which secondary bacterial pneumonia occurs.[27] Taken together, these results show that influenza infection has a severe impact upon the lung, promoting a permissive environment for bacterial invasion beyond its normal niche in the lung lumen. Additionally, influenza infection of macrophages compromises their ability to phagocytose Spn and NTHi.[28,29] The loss of alveolar macrophage function and number as well as the considerable epithelial cell death as a consequence of influenza infection impair frontline immunological

barriers, resulting in bacterial invasion of the mesenchyme. As fibroblasts are a key mesenchymal cell, fibroblast responses to invading bacteria may be a key immune mechanism contributing to defence and/or immune pathology under these circumstances.

Fibroblast function is mediated by origin and tissue environment

Fibroblasts arise from the mesoderm during embryonic development and inhabit the lung submucosal zones, where they are responsible for the production of extracellular matrix (ECM) components such as elastin, proteoglycans, fibronectin and the collagens that make up the lung stroma. The ECM forms the structural framework and provides the means by which inflammatory cells can migrate through the organ. Upon culture, fibroblasts themselves are characterised by an elongated, spindle-like cell body and are morphologically similar between different tissues within the body.[30]

Fibroblasts play an important role in wound healing and are also capable of differentiating into myofibroblasts. In this situation, fibroblasts express myosin and alpha-smooth muscle actin filaments, which allows them to exert contractile force and physically close wounds.[31,32] Thus, fibroblasts and myofibroblasts have not only been considered a cell type involved in the production and maintenance of the scaffold that hold tissues together, but also in scar formation and subepithelial fibrosis.[33] Despite the ultrastructure of fibroblasts being incredibly similar between different tissue zones,[34] there are major differences in the behaviour and response to inflammatory mediators by fibroblasts from different organs. This suggests that fibroblasts are not a universal cell type, but rather that fibroblast phenotype is determined by the tissue specific milieu. As a result, fibroblasts from different tissues can exhibit starkly different levels of involvement in inflammatory reactions. Furthermore, it has been established that there are different phenotypes of fibroblasts even within the same

tissue.[30,35] For example, in the case of the lung, the proximal airway fibroblasts and the distal, parenchymal fibroblasts have notable differences in their ECM production, their cytokine response to inflammatory mediators as well as differential responses to steroids.[36-38]

This situation is further complicated by the fact that lung fibroblasts can be derived from different sources.[35] The resident populations derived from the mesoderm include interstitial fibroblasts, lipofibroblasts and pericytes but there is also evidence for mesothelial fibroblasts that can arise as a result of epithelial to mesenchymal transition.[35,39] Furthermore, there are fibroblastic cell types that are derived from bone marrow; the mesenchymal stem cells and fibrocytes. Both these cell types can be recruited to the tissue in response to injury, but as fibrocytes are haematopoietic in origin and appear monocyte-like it is tempting to speculate that these may have a greater capacity to present antigen to CD4+ T cells.[40,41] However, understanding the level of involvement of fibroblasts in adaptive immune responses is still a developing field.

Fibroblast innate immunological functions

Fibroblasts can themselves respond to both bacterial and viral infection as they express numerous PRRs, such as the Toll-like receptors.[42] Thus, fibroblasts can be activated either as a result of infection or in response to PAMPs such as LPS, poly(I:C) and flagellin. A common response to such innate stimuli is the release of soluble factors such as the potent neutrophil chemotactic factors CXCL8, as well as CXCL10 and CCL2, demonstrating a general ability to recruit innate responder cells in response to stimulation.[43,44] Furthermore, the release of these pro-inflammatory factors can be enhanced in disease associated fibroblasts. For example, opthalmopathy-associated fibroblasts produced greater amounts of CXCL8, CCL2 in addition to the pro-inflammatory cytokine IL-6 when stimulated with CD154 and IL-1β.[45] Additionally,

fibroblast-like synoviocytes of the rheumatoid joints can also produce an increased amount of an array of inflammatory mediators that create a highly inflammatory microenvironment; recruiting and activating immune cells, including T helper cells (reviewed in[46]). These examples demonstrate disease-specific phenotypic changes in fibroblasts.

Being located in the interstitium, lung fibroblasts are also in a prime location to receive conditioning from the overlaying epithelial cell layers and studies have demonstrated lung fibroblasts exhibit strong responses to epithelial alarmins such as IL-33 (factors released by dead or stressed cells). Lung fibroblasts respond to epithelial-derived IL-1α in a co-culture model, again exhibiting robust release of IL-6 and CXCL8.[47] Intriguingly, the same response was not mirrored by macrophages, suggesting IL-1α release represents a specialized epithelial-fibroblast mechanism to recruit immune cells in cases of epithelial death.[48]

From this growing body of work, it is clear that fibroblasts are not immunologically inert, background cells to the immune response in the lung, but are capable of interacting with epithelial cells and amplifying inflammatory reactions. What is not fully established is the nature of the CD4+ T cell-fibroblast interaction, particularly the ability of fibroblasts to present antigen to T helper cells within the lung.

CD4+ T cell activation

The standard paradigm of CD4+ T cell activation is predicated on the activation of naïve T cells in a lymph node. T cell activation is mediated via the T cell receptor (TCR) recognizing and binding a specific antigen when presented within a major histocompatibility complex (MHC) class II molecule. The MHC II complexes are constitutively expressed by professional APCs such as DCs, macrophages and B cells. TCRs can be formed of alpha and beta subunits

(which allow the T cell to bind MHC-presented protein antigen) or gamma and delta subunits (possibly imparting invariant antigen binding properties in the context of CD1d or recognition of epithelial-based tissue-specific factors).[49-51] However, antigen presentation without adequate co-stimulation results in the T cell becoming anergic or inducing apoptosis.[52] This level of control prevents immature APCs from activating T cells unless the APC has encountered PAMP signals. The co-stimulatory molecules CD80 and CD86 are upregulated upon maturation of professional APCs, which bind CD28 upon the interacting T cell and deliver a potent activation signal. This CD80/86 signalling through CD28 is absolutely essential for correct naïve T cell activation.[53,54] Additional stimulatory molecules such as CD40 and cell-adhesion molecules such as intercellular adhesion molecule 1 (ICAM-1) also have roles in delivering activation signals and stabilizing the cell-to-cell interaction. Full stimulation results in T cell proliferation through autocrine action of interleukin (IL-)2 and upregulation of the IL-2 high affinity receptor (CD25) on T cells.[55] Soluble cytokine factors released by the activated APC will also affect the outcome of T cell activation, dictating the nature of the response T cells will go on to promote (e.g. IL-4, IL-7, IL-12, IL-13, IL-15, IL-18). From our own work, the frequency of primary CD4+ subsets in the human lung are Th1>Th17>T-regulatory (Treg) cells>Th2.[56] Additionally, this work confirmed that naive cells are rare within the peripheral lung environment and that CD45RO+CCR7- effector memory cells predominated, making up approximately 80% of T cells found in the lung, with the majority of the remainder being central memory cells.[7,56,57]

An important difference between naïve and memory T cells is their requirement for co-stimulatory signals. While naïve T cells are recognised as having an absolute requirement for signalling through CD28-CD80/86 interaction, it has been shown that memory T cell populations do not necessarily require this interaction to reactivate.[58,59] CD40-CD40L interactions in particular have been shown to be required for proliferation of CD4+ memory T

cells.[60] More recently the co-stimulatory molecules of the TNFR receptor family including OX40, CD30, CD70 and 4-1BB have also been shown to control effector memory CD4+ T cell expansion.[61-63] This would allow effector memory T cells to interact with APCs that do not express the CD80 and CD86 molecules that are so critical for naïve T cell activation, and suggests memory cells are able to interact with cells that can inducibly-express MHC class II, not just professional APCs.

These long-lived resident memory T cells appear to lodge within the lungs and do not recirculate as other effector-memory T cells are thought to.[64,65] Tissue resident T cells do not rely upon replenishment from circulatory sources and exist in distinct microenvironmental niches within the lung, where they are responsible for rapid and protective responses to lung pathogens.[8,66] Generation of these highly specialized T cell subsets appears to require respiratory rather than systemic infection.[67] Perhaps most interestingly of all, resident memory T cells do not seem to exist in ectopic, organized lymphoid structures (such as bronchial-associated lymphoid tissue), which form during certain acute infections.[68,69] Rather, resident memory cells appear to be more diffusely stationed within the tissue during acute viral infection, particularly in the submucosal and immediate subepithelial mesenchymal zones where fibroblasts are resident.[8]

Fibroblasts as APCs

Fibroblasts are thought to contribute to the immunosuppressive steady-state environment in the lung through suppression of T cell activation. This immunosuppressive state is thought to be brought about by contact-dependent mechanisms that appear to suppress recently activated T cell proliferation and reducing the proportion of TNFα+ cells while maintaining the proportion of immunoregulatory IL-10+ T cells.[70] This immunosuppressive ability is in line with

observations of other lung resident professional antigen-presenting cells (APCs) such as macrophages and dendritic cells (DCs), and non-professional APCs including epithelial cells that can express Programmed Death Ligand (PDL)-1 that acts via interaction with the PD-1 receptor, as a key break on T cell activation.[57,71] Interestingly, fibroblast suppression of T cell proliferation is impaired in patients with COPD, indicating that fibroblasts respond to the local environment and have variable phenotypes with regard to immune capability.[72] While lung fibroblasts are immunosuppressive in the resting state, they are able to alter their function in response to local environmental cues.

Fibroblasts have been demonstrated to express detectable levels of MHC II molecules *in situ* in the eye, skin and lung.[56,73-75] However, analysing the ability of fibroblasts to act as APCs to CD4+ T cells was hampered by the lack of fibroblast expression of MHC II molecules when these cells were cultured *in vitro*. Such differences could be explained by the very act of culturing these cells as there are substantial differences in fibroblast phenotype dependent upon whether they are cultured in 2D tissue culture plates or 3D matrix cultures.[76] The discovery that the cytokine IFNγ could induce expression of the class II molecule, HLA-DR in cultured human fibroblasts paved the way for *in vitro* modelling of antigen presentation by these cells.[77] Early work demonstrated that IFNγ stimulated dermal fibroblasts could express HLA-DR upon their surface and present tetanus toxoid to antigen-specific CD4+ T cell clones resulting in their proliferation *in vitro*.[78] Further work by the same group demonstrated that these IFNγ-treated fibroblasts could not stimulate naïve T cell proliferation unless exogenous IL-2 was added.[79] These two studies reinforce the idea that fibroblasts can only activate antigen-experienced memory T cells and not naïve cells.

Treatment of fibroblasts with recombinant IFNγ is now routinely used to enable investigation of interactions with T cells; demonstrating that in addition to dermal fibroblasts, synovial and lung fibroblasts can also activate CD4+ T cells particularly those T cells

inhabiting tissue zones with an activated memory phenotype.[56,79,80,81] However, addition of exogenous IFNγ is not always necessary to model these effects, again demonstrating the range of responses that can be observed from fibroblasts derived from different sources. Indeed, corneal fibroblasts have been shown to be able to make their own IFNγ when exposed to LPS derived from the lung pathogen *Pseudomonas aeruginosa*.[82] IFNγ is also not the only cytokine that can induce fibroblast expression of MHC II. Both FGF2 and PDGF have been demonstrated to induce functional expression of HLA-DR on human mesenchymal stem cells.[83] Furthermore, cultured gastric fibroblasts/myofibroblasts could express HLA-DR when exposed to *Helicobacter pylori* resulting in IL-17A production by naïve CD4+ T cells.[84]

Additionally, despite CD80 and CD86 not being essential for activation of memory T cells, that is not to say that co-stimulatory molecules do not also play a role in fibroblast-T cell interactions. IFNγ can not only be used to recapitulate HLA-DR expression, but can also increase *in vitro* cultured lung fibroblast expression of OX40L and CD70, both co-stimulatory molecules associated with activation of effector memory T cells.[56] The caveat to these observations is that the evidence for antigen-presentation by fibroblasts is limited to *in vitro* work and human *in vivo* evidence remains associative. For example, recent work has demonstrated that expression of OX40L by fibroblasts was a key pathway in SSc.[85] This human data regarding OX-40L expression was further complemented by investigation of the functional role of this molecule in a murine model of SSc.[85] Addition of OX40L blocking antibodies prevented development of fibrosing alveolitis, suggesting a functional role of fibroblast-mediated antigen presentation in lung autoimmunity. In order to answer the specific question as to whether lung fibroblasts can stimulate CD4+ T cells in response to bacteria, we set up a model whereby we could expand antigen-specific CD4+ T cells from human lung obtained from cancer resection surgery and at the same time culture fibroblasts from the same tissue (Figure 1). Recombining the expanded CD4+ T cells with their autologous fibroblasts

pre-treated with heat-killed NTHi and IFNγ caused the T cells to express IFNγ and IL-17.[56] Moreover, this T cell cytokine expression was abrogated in the presence of a HLA-DR blocking antibody. In light of data indicating the existence of cytotoxic CD4 T cells, a question that remains is whether these CD4 cells can go beyond releasing cytokines in this scenario and directly kill the bacterially-infected fibroblasts, and thus the pathogen, via cytotoxic granule release as has been shown for both influenza and *Francisella tularensis*, the causative agent of tularaemia.[86,87]

The ability of fibroblasts to impact upon T cell function is perhaps best understood within the lymph nodes themselves.[88] Not only do the fibroblast reticular cells provide the structural framework of the lymph node, they play a substantial role in attracting and promoting survival of T cells, as well as B cells and DCs.[88] Moreover, the fibroblasts can proliferate during infection to accommodate lymph node hypertrophy as a result of T and B cell proliferation.[88] Whilst in response to acute infection memory T cells do not seem to associate with organised lymphoid structures,[8] increased numbers of tertiary lymphoid organs (TLOs) have been found in the lungs of COPD patients as well as patients with Cystic Fibrosis (CF).[69,89-91] These TLOs seem to be a result of the chronic bacterial infection associated with these diseases.[89] TLOs consist of T cells, B cells and type 3 innate lymphoid cells and are primarily located in the peribronchial and submucosal regions where fibroblasts are resident and most importantly these fibroblasts play a substantial role in the formation of TLOs.[92] There also appears to be some antigen-presentation capacity of these stromal cells which in secondary lymph tissue such as lymph nodes leads to tolerance but in TLOs seems skewed to chronic inflammation and autoimmunity.[92] However, a full understanding of the role of the TLOs and their associated fibroblasts in lung disease remains to be elucidated.

Effect of activated T cells on fibroblasts

Whilst the evidence points to a role for lung fibroblasts in activating T cells, cellular communication is two-way traffic and factors released from T cells can also impact upon fibroblast function. As already discussed, CD4 cells are a major source of IFNγ that could lead to further expression of HLA-DR and co-stimulatory molecules upon the fibroblast, possibly contributing to a positive feedback loop. IFNγ exposure also drives fibroblast production of CCL5, a potent T cell chemotactic factor.[93] Additionally, opthalmopathy-derived fibroblasts also express the T cell co-stimulatory molecule CD40, ligation of which by T cell expressed CD40L causes further IL-6 and CXCL8 production from the fibroblasts.[45] These interactions may also not be limited to Th1-type interactions as exposure to IL-4 promotes the release of eotaxin, allowing fibroblasts to possibly contribute to type 2 immune reactions though eosinophil recruitment.[93]

Summary

The human lung contains a substantial number of T cells that are involved in responding to bacteria. These adaptive immune cells rely upon interaction with tissue resident APCs in order to receive antigen-specific activation signals. Through this interaction, CD4+ T cells are able to direct the wider immune response via potent cytokine release, affecting macrophage function as well as B cell activation, maturation and immunoglobulin isotype switching.[29,94,95] IgG1 in particular appears to be relevant to control of NTHI infection.[96] We provide evidence for the ability of lung fibroblasts to present bacterial antigens to CD4+ T cells, which may represent a mechanism contributing to responses against bacterial invasion of the mesenchyme. This would be particularly relevant in situations where epithelial and macrophage defences are impaired, such as is seen in pneumonia. Further work is required to investigate if fibroblast-T

cell interactions are also relevant to other lung PPMs, particularly invasive lung pathogens that are known to disrupt epithelial tight junctions, such as *F. tularensis* or *Burkholderia pseudomallei* (the causative agent of melioidosis).[97] The interaction between T cells and bacterially-activated fibroblasts may also be relevant to the formation of TLOs in COPD and CF, both of which are associated with chronic bacterial infection.[89] Furthermore, there is increasing evidence of both a role for bacterial infection and CD4+ T cells in idiopathic pulmonary fibrosis pathology.[98,99] An integrated analysis of the fibroblast:T cell:bacteria triad may therefore also shed further light on the processes involved in either IPF disease progression or exacerbations. However, bacteria do not exist in isolation being part of not only a wider bacterial community but also interacting with the lung mycome and virome.[100] Modelling of these complex interactions is required to gain a deeper understanding of not only the role of these microorganisms in lung health and disease but also of the impact of co-colonisation/co-infection on the host immune system. This complex milieu will undoubtedly impact on the interplay between the memory CD4+ T cells and structural cells, including the fibroblast.

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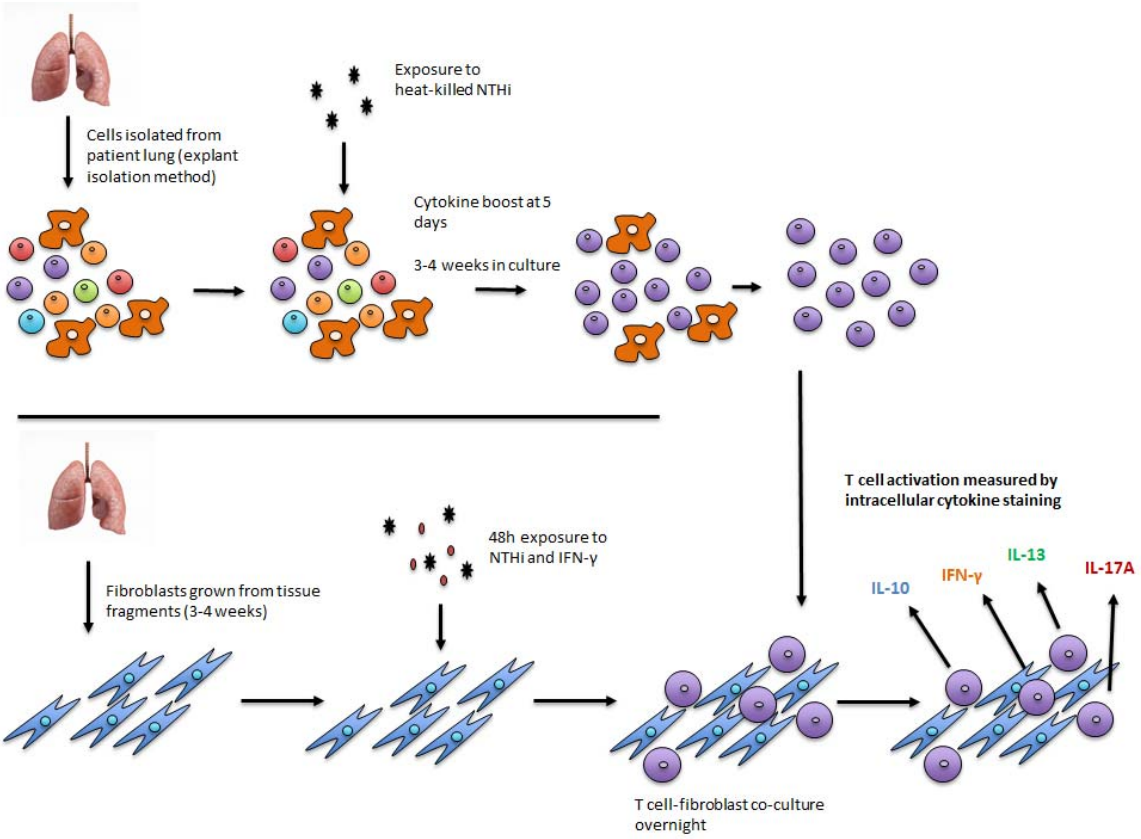
Figure Legends

FIG 1: Isolation and culture of autologous human lung fibroblasts with CD4+ T cells for co-culture in order to examine the ability of fibroblasts to act as an antigen-presenting cell. Both lung fibroblast and lung T cells were isolated using *in vitro* fibroblast outgrowth and T cell egression methods (54). Briefly, fibroblasts were outgrown from lung fragments over a 3-4 week period until sufficient numbers were attained in culture. Concurrently, patient-matched lung derived immune cells were isolated and subsequently exposed to heat killed NTHi in order to drive the generation of a NTHi-responder enriched T cell line. This provided a pool of T helper cells with a known Ag-specificity that can be used to test the ability of matched lung fibroblasts to present NTHi-Ag and reactivate this line. This represents activation of T helper cells by a professional, bonafide APC population. T helper cell activation after co-culture was measured by flow cytometry intracellular cytokine staining.


Exposure to
heat-killed NTHi
Cells isolated from
patient lung (explant
isolation method)
Cytokine boost at 5
days
3-4 weeks in culture
T cell activation measured by
intracellular cytokine staining
48h exposure to
NTHi and IFN-γ
Fibroblasts grown from tissue
fragments (3-4 weeks)
IL-10
IFN-γ
IL-13
IL-17A
T cell-fibroblast co-culture
overnight