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ALLERGIC DISORDERS IN EARLY CHILDHOOD - Prevalence, Risk Factors and Prevention

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ABSTRACT
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Doctor of Medicine

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by Syed Hasan Arshad

Two studies were carried out between 1989 and 1991. The first was an epidemiological, observational study (n=1518) to estimate the prevalence of allergic disorders and to identify genetic and environmental risk factors important in their development. The second was a randomized, controlled study to assess the effectiveness of allergen avoidance measures in the prevention of allergy in high risk infants.

Cord IgE was measured using a new enzyme-linked immunoassay, EIA ULTRA^{R} test. Cord IgE levels were higher in infants with a history of atopy in the immediate family. Male infants had a higher IgE than females. There was no effect of length of gestation, birth weight, maternal smoking or month of birth on cord IgE values.

At one year 282 of 1342 (21%) infants had developed one or more allergic disorders. At two years 275 of 1174 (23%) children were considered to have allergic disorder. The prevalence varied from 3.2% for rhinitis to 10.9% for asthma. Maternal and sibling allergy were significant risk factors for various allergic disorders. Paternal allergy had no effect on allergy in the infant. Cord IgE was not related to the development of allergic disorders during the first two years of life. Male sex was a risk factor for asthma and definite allergy. Low birth weight infants developed total and definite allergy, asthma and food intolerance significantly more often than those with normal birth weight. Breast feeding offered some protection against asthma at one year but this effect disappeared by the age of two. Maternal smoking and summer births were significant risk factors for asthma. A significantly higher percentage of children born during summer and autumn months developed rhinitis. Infants belonging to the lower socio-economic group were at a higher risk to develop asthma and rhinitis.

The effect of food and house-dust mite allergen avoidance on the development of allergic disorders in infancy was assessed blindly in a prenatally randomised, controlled study of 120 high risk infants. In the prophylactic group (n=58) lactating mothers avoided allergenic foods (milk, egg, fish and nuts). Infants in addition to these foods avoided soya, wheat and orange up to 12 months. *Der p* I levels were significantly lower in the prophylactic group following treatment with Acarosan^{R}. In the control group (n=62) lactating mothers and infants had an unrestricted diet. At one year 25 (40 %) infants had one or more allergic disorders in the control group compared with 8 (13 %) in the prophylactic group (OR=6.34, CL=2.0-20.1). The prevalence at one year of asthma and eczema was significantly higher in the control group.

Chapter 1: **A HISTORY OF ALLERGY**

Some historian believe that King Menes of Memphis who ruled Egypt about 3000 B.C. was killed by a hornet. If this is true then he must be the first person known to have an allergic disorder. There are some vague clues in the Chinese and Roman history that suggest that allergic problems existed in those times. The clinical symptoms and signs of asthma were well described by ancient Greek scholars although several types of breathing difficulty were probably called asthma. One of the Greek physician (? Hippocrates) writes about intolerance to cheese, *that the odd patient here and there could not consume even the tiniest piece of cheese without promptly becoming severely ill.*

The case of the Archbishop of St. Andrew's in 1552 is very interesting. The Archbishop suffered from severe asthma and sent for Jerome of Carden from Italy. The Italian consultant, after observing the archbishop for a few weeks, suggested, among other things he should get rid of a feather quilt and pillows as it causes "overheating of the brain". The archbishop's asthma was cured and Dr. Carden returned to Italy with great fame and fortune. A contemporary of Carden, Leonardo Botallo from the university of Pavia wrote a clear description of *Rose Fever.* He had a patient who *could not abide roses, since they caused his nose to itch intolerably, made him sneeze and gave him nasty headaches.* Another Italian doctor Pietro Mattioli in 1570 described the case of a patient who developed agitation, sweating and pallor on exposure to cat. In 1586 Marcello Donati in Germany described a young count *whose lips swelled up, accompanied by dark red flecking of the face, whenever he indulged in eggs.*

The first datable skin test under medical auspices seems to have been carried out by Pierre Borel in 1656. He confirmed a patient's hypersensitivity to egg by applying some to his skin, raising a blister. In 1665 Philipp Jacob Sachs described a case of generalised urticaria following ingestion of strawberry and another of shock after eating

fish. During the 17th century German authors wrote not only of cats but also of mice, dogs and horses that in certain subjects produced weakness, fainting and asthma. In the middle of the 17th century William Cullen witnessed asthma attacks suffered by the wife of a pharmacist while her husband was preparing ipecacuanha. About the same time the town doctor of Ulm reports a case of asthma arising from the pharmaceutical use of pumpkin seeds. These may be the first reported incidences of drug allergy.

Dr. Bostock in 1819 described his own "Periodical Affection of the Eyes and Chest", which was a good description of hay-fever. Dr. John Elliotson in the London Medical Gazette in 1831 laid the blame *upon the flower of grass, and probably upon the pollen*. The classic experiments of Charles Blackley, in his paper of 1873, finally provided undeniable proof that grass pollen is the cause of hay-fever. Ragweed pollen was identified by Morrill Wyman as the pre-eminent cause of autumnal hay-fever in the United States.

It seems that Edward Jenner was the first person to produce a hypersensitivity reaction under experimental conditions. In the "Treatise on vaccination" published in 1798 he observed that *the inflammation at the site of a smallpox inoculation was more rapid in a subject who had previously had smallpox than in one who had not*. In 1839 the French physiologist Magendie described anaphylactic shock and death in dogs repeatedly injected with foreign proteins. Simon Flexner in 1894 clarified the phenomenon of anaphylaxis by showing that *a second dose of dog's serum into rabbits after a lapse of some days or weeks could be lethal*. The German dermatologist Joseph Jadassohn demonstrated in 1895 that eczema could be caused by hypersensitivity to certain substances by using what is now known as "patch test". Von Behring coined the term *hypersensitivity* to describe the *exaggerated response and even death following a second dose of diphtheria toxin into animals, too small to injure normal untreated animals*. Portier and Richet in 1902 first used the term *anaphylaxis* when they described *a clinical shock syndrome encountered in dogs given otherwise innocuous doses of toxin, after previous experience with the same substance*. In 1906, Von Pirquet and Schick analysed the observations that some patients receiving diphtheria or tetanus antitoxic serum might suffer strange systemic and local symptoms which they named serum sickness. Von Pirquet coined the term

allergy (altered reactivity) to describe these and related phenomena. In the subsequent years the mechanism of anaphylactic reaction was further expanded by the experiments of Schultz and Dale on intestinal and uterine muscles. A cellular involvement in the process of anaphylaxis was proposed stating that *the small amounts of antibody required were in fact affixed to the surface of appropriate target cells*. Any subsequent interaction with specific antigen would result in cell damage and a consequent shock like syndrome. The pathogenesis of allergic disease was further elucidated with the demonstration that an anaphylactic shock-like syndrome can be induced by intravenous administration of histamine and the knowledge of the role of histamine in mediating many of the typical local symptoms of allergic reaction (1910-1915). The conditions of hay fever and asthma were brought into the immunological fold of anaphylaxis and allergy. In 1906, Wolff-Eisner made the connection between hay fever and a hypersensitivity state in the immunological sense and in 1910 Meltzer did the same for asthma.

The first study of genetic predisposition to allergic disease was published by Cooke and Vander Veer. In 1916 they studied large series of cases (621, including identical twins) and concluded that *inheritance is a definite factor in human sensitisation*. The antibody responsible for this sensitisation was named *reagin*. Their data permitted them to conclude that the predisposition to sensitisation is inherited as a dominant mendelian characteristic. Further, by comparing the allergens to which their patients (and especially identical twins) were sensitive, they were able to show that *specific sensitisation is not inherited but an unusual capacity to react to foreign proteins*. In 1923 Coca and Cooke employed the term *atopy* to designate those human hypersensitive conditions that are genetically inherited. This view was challenged by Adkinson who believed that asthma but not the hypersensitive state itself is genetically transmitted and is recessive in the majority of cases. In a subsequent study Spain and Cooke questioned Adkinson's conclusions and suggested instead that a multifactorial inheritance might explain why all children of atopes are not themselves allergic. This was widely accepted until recently when Cookson and Hopkin revitalised Cooke's original suggestion that *atopy is inherited as an autosomal*

dominant character although its clinical expression depends on interaction with other factors.

Cooke introduced intradermal skin test in 1915 to aid the diagnosis of allergic conditions. His colleague Arthur F. Coca first purified allergenic extracts for use in such tests. Together Coca and Cooke attempted to classify the various hypersensitivity states and to distinguish among such conditions as hay fever, contact dermatitis, serum sickness and experimental anaphylaxis in animals. The American doctors Schloss and Walker developed the scratch test and in Britain a variant, called the prick test was introduced by Pepys who carried out fundamental work on the method during the fifties. Provocation in the shock organ had been used sporadically for centuries. Recently these provocation tests have been standardised and used widely in the diagnosis of allergic diseases.

Mites in house dust were observed as far back as 1694, and in the 18th century it was reported that dust could cause asthma, but it was not until the 1920s that the mite came under suspicion as an allergenic factor in house dust. In early 1960s Voorhorst and Spieksma proposed that mites were one of the most important allergens in house dust in certain countries. In 1924 Cadham reported the first case of mould allergy. The first reported case of anaphylactic reaction and death following bee-sting in the medical literature came from Desbret in France in 1765. In 1833 German scientists Brandt and Ratzeberg noted that the effect of a bee-sting was akin to the reaction produced by viper poison. Waterhouse, writing in the Lancet in 1914, reports anaphylactic reactions in a bee-keeper following stings. In 1930, Benson and Semonove demonstrated the occurrence of allergy to bee protein and suggested treatment with specific hyposensitisation.

Besredka endeavoured to neutralise hypersensitivity in animals by injecting a highly dilute solution of antigen and then successively increasing the doses. In 1911, Noon injected extracts of various pollens into hay-fever patients and assessed the value of the treatment by provoking the eye. Cooke, who introduced the treatment into the U.S. in 1914, proposed the name *hyposensitisation* for this injection treatment in 1922.

A significant contribution to the understanding of human allergic reaction came in 1921 from Carl Prausnitz and Heinz

Kustner. Kustner was exquisitively sensitive to the cooked flesh of certain fish, but fish extracts failed to demonstrate the presence of precipitating antibodies in his serum. However, when a little of his serum was injected into Prausnitz's skin, a typical wheal and erythema hypersensitivity reaction could be elicited 24 hours later by local administration of the appropriate allergen. This demonstrated the ability to transfer passively the human allergic condition, which strongly implicated antibody by analogy with other passive transfer reactions.

Attempts to isolate and characterise the antibody responsible for allergic reaction (reagin) remained unsuccessful until 1966 when Kimishige and Teruko Ishizaka prepared an antiserum to a reagin-rich fraction from the serum of a person showing extreme hypersensitivity to ragweed and demonstrated that this antibody would neutralise the Prausnitz-Kustner transferability of allergy with the patients serum. Upon purification of the antibody, it was found that it would not react with any other known immunoglobulin class and was given the name gamma-E (for erythema) globulin. Johanssen and Bennich independently isolated an atypical immunoglobulin from a myeloma patient which they called IgND (after the patient's initials). They went on to demonstrate that the serum of patients suffering from asthma or hay fever exhibited elevated levels of IgND. It was concluded at a WHO international conference in 1968 that this new class of immunoglobulin was identical to gamma-E globulin and was the true mediator of the biological and immunological features formerly ascribed to reaginic antibodies. It was renamed Immunoglobulin E.

Mast cells were discovered by Paul Ehrlich in 1877 as a distinctive cell found in connective tissue. In 1953 Riley and West demonstrated the presence of histamine in the mast cell. Following the discovery of IgE, mast cells have been extensively studied for their relationship to IgE and their role in allergic reactions.

With improved methods for the isolation and purification of various allergens, it has recently become possible to work out many of the mechanisms and pharmacological pathways involved in human allergic conditions. IgE antibodies have the specialised ability to bind tightly to basophils and mast cells, and their interaction with specific antigen has been shown to result in degranulation and the release of

histamine and other mediators that produce symptoms of the disease.

Further Reading

This chapter was compiled from the following two books:

- A History of Allergy by Arthur M Silversteine. Academic Press Inc. 1989; Sandiego, California 92101, USA

- Footnotes on Allergy by D Simon Harper. Pharmacia AB, Uppsala, Sweden; 1980.

Chapter 2: **LITERATURE REVIEW**

Immunoglobulin E

The presence of reaginic antibodies in the serum of allergic patients was first demonstrated by Prausnitz and Kustner in 1921. The nature of the antibody remained unknown for 40 years until IgE was discovered by the Ishizakas[1]. In 1967 Johansson[2] reported elevated IgE concentration in patients with exogenous asthma. In these patients the mean IgE concentration was six times higher than in healthy persons. On the other hand, asthmatic patients in whom allergy tests had given negative results showed no higher mean IgE levels than their non-asthmatic counterparts. Similar results were shown by Berg and Johansson[3] for children with atopic diseases (allergic asthma, allergic rhinitis and atopic eczema). They also demonstrated a significant rise in serum IgE during the pollen season in children with pollen allergy. These and other studies provide evidence that the IgE antibody plays a role in the pathogenesis of atopic diseases. Regulation of IgE antibody synthesis has been studied extensively in experimental animals[4].
Stevenson et al[5] in their study of 34 maternal-neonatal pairs concluded that maternal IgE does not cross placenta and serum IgE detected in the cord blood is foetal in origin. In 1973 Miller[6] demonstrated synthesis of IgE by foetal lung and liver as early as 11th week of gestation.

Measurement

The most commonly used commercially available method for the determination of serum IgE is a paper disc radioimmunoassay technique called PRIST^{R} described by Ceska and Lundkvist[7] in 1972. This is a direct radioimmunoassay system using solid phase coupled antibodies and immunosorbent purified antibodies labelled with a radioactive isotope. The IgE antibody (in the serum to be assayed) is first bound to the solid-phase (paper-disc) coupled antibody. After washing it is incubated with labelled (immunosorbent purified) anti-IgE antibody. Other solid-phase radioimmunoassays use sephadex

or cellulose particles instead of a paper-disc. The sandwich techniques were shown to be more sensitive and gave lower values than the original competitive inhibition radioimmunoassay[8,9]. The lower limit of the assay was said to be 1.5 ku/l in the original paper[7] but Johansson et al[8] when comparing radioimmunoassay methods claimed it to be as low as 0.1 ku/l for PRIST. However other investigators[10,11] found that some modification in the procedure is necessary to reliably measure IgE values below 0.5 ku/l.

The vast majority of cord IgE values lie below 0.5 ku/l. Eisenbrey et al[10] reported a comparison of 4 methods of radiometric immunoassay (RIA) for measurement of IgE with sensitivity below 300 pg/ml. The methods were double antibody RIA, ultrasensitive enzymatic RIA, sensitive paper radioimmunosorbent test (modified PRIST) and microtitre solid-phase RIA. They concluded that microtitre solid-phase RIA is superior to other methods for measuring minute quantities of IgE in serum samples where concentrations fall below 1 ng/ml (0.42 ku/l).

With recent advances in methodology of the enzyme-linked immunosorbent assay (ELISA) technique as well as a desire to reduce radioactive exposure attention has been directed towards enzyme-linked assays. The introduction of a commercially available kit also stimulated interest. Bayne and Mathews[12] in 1982 reported determination of total IgE by ELISA in tubes and plates. It correlated very closely with PRIST but the lower sensitivity limit was only 0.5 ku/l. Kimpen et al[13] in his large study used a conventional microtitre sandwich ELISA with a detection limit of 0.01 ku/l. Kemeny et al[14] recently described a modification of a two-site ELISA which increases the sensitivity of the assay 10-20 fold. By using the Fab fragment of either rabbit or mouse monoclonal anti-IgE conjugated to alkaline phosphatase (AP) as the detector, the background of the assay was reduced sufficiently to permit signal amplification, using a commercially available amplified AP substrate. With this assay as little as 10 pg/ml (0.004 ku/l) of IgE could be detected.

Genetic and Environmental Factors

Serum IgE levels in healthy persons (excluding those with atopy, helminthic infection and rare immune disorders with high IgE levels) are affected by a wide variety of genetic

and environmental factors. The levels depend on a family history of atopy[15-19], age[19-22] sex[19-22], ethnic origin[22,23] smoking[24-27], type of infant feeding[28,29] and viral infections[30]. It has been suggested that basal serum IgE levels are determined by genetic factors[15,16] possibly by two alleles at a single locus. More recently Cookson[17] proposed that atopy as defined by the propensity to produce IgE in response to common, usually inhaled allergens, is inherited as an autosomal dominant character. In their study 10% of the atopic children did not have atopic parents which is not compatible with simple autosomal dominant inheritance.

In the study by Gerrard et al[16] when both parents had low IgE level (<150 u/ml), 19% of their offspring had high IgE levels (relative to their age). If one parent had a high IgE level, 43% of the children had high levels. When both parents had high IgE, 77% of their children had high levels. Lebowitz et al[18] showed that serum IgE in children is related to sibling, maternal and paternal IgE in that order of significance. Sears et al[19] also showed the effect of family history of atopy on serum IgE levels. In this study Sears also defined reference IgE levels for adults (age range: 17-30) without personal or family history of atopy. However, in this study information on family history was based on a questionnaire completed by the subjects which was not verified in each case. Kjellman et al[20] estimated serum IgE in 226 children without family history and defined "normal" values for different age groups.

In a large population study of 2743 subjects age 6 years and over, Barbee et al[21] showed the distribution of serum IgE and the effect of age and sex. In both sexes highest levels occurred among those aged 6 to 14 years and males were found to have higher IgE values than female at any age. The effect of sex (male > female) was confirmed in another large study of serum from 4,440 blood donors in South Africa[22]. Ethnic differences were revealed with higher IgE levels in coloured and blacks compared to whites. Grundbacher[23] confirmed the conclusion that blacks have higher IgE values than whites.

Several reports of higher serum IgE values in cigarette smokers have appeared in the literature[24-26]. A significantly higher IgE value at 9 and 36 months of age in children of smoking parents was reported by Kjellman[27]. Juto and Bjorksten[28] reported that serum IgE in infancy is

influenced by the type of feeding. Serum IgE at the age of 12 months was significantly higher in infants weaned after the age of 6 months than those weaned before the age of 6 months. In another report of the same study Juto[29] points out that serum IgE during infancy is related to the number of T cells assessed at the age of 1 month and type of feeding. Cow's milk fed babies with low T cell counts had higher IgE at the age of 3 and 6 months than breast fed babies with low T cell counts. Of the babies fed cow's milk those with low T cell counts had higher IgE levels than those with normal T cell counts. Perelmutter et al[30] noted a drop in serum IgE level in the convalescent phase of infection compared to the acute phase.

Cord Blood Total IgE

Maternal but not paternal IgE was related to cord IgE level in a study by Michel[31]. In an earlier study by Kjellman and Johansson[32] no relationship was found between the IgE level in maternal and respective cord sera. However the cord IgE values used for the purpose of correlation were obtained from a sequential addition modification of RIST which tends to give higher values because of non-specific serum factors. Croner et al[33] found that infants with an immediate family history showed a significantly higher incidence (7.2%) of raised cord IgE (>1.3 ku/l) than those with no family history (3.8%). The incidence of high cord IgE did not differ significantly between children with a history of maternal or paternal atopic disease. A significant relationship between cord IgE and family history of atopy was found by Magnusson[34]. Chandra[35] in his study of prediction of atopy found high (>0.7 u/ml) cord IgE in 44.3% of infants with a positive family history of atopy compared to 16% in those with negative family history. Haus[11] concluded that a positive family history of atopy influenced the cord blood IgE concentration in the white and mixed ethnic groups but not in the black groups.

Cord IgE values are generally higher in male infants compared to females[33,36-38]. A higher percentage of male infants was found to have high IgE (>1.3 ku/l) compared to female infants (7.3% vs. 3.2%; $p<0.001$) by Croner[33]. Kimpen confirmed a higher cord IgE values in male compared to female[36]. Magnusson[37] found a significantly higher geometric mean cord IgE (ku/l) in boys than in girls (0.46 vs. 0.33)

in European infants. Halonen[38] made similar observation that boys have higher cord IgE (ku/l) than girls (0.10 vs. 0.08; p=0.002) in both Anglos and Hispanics.
Magnusson[37] reported a higher level of cord IgE in neonates of African-Asian origin compared to European neonates. This was confirmed by Haus[11] who found the highest cord IgE in blacks followed by mixed race followed by whites after excluding infants with a family history of atopy and maternal ascariasis. Halonen[38] found higher cord serum IgE levels in Hispanics compared to Anglos.
Michel[31] showed an increased percentage of detectable cord IgE with increase in gestational age and with prenatal administration of progesterone. Kimpen[36] in his large study failed to detect any influence of gestational age on cord IgE. Maternal smoking caused a significant rise in cord IgE values in infants with a negative family history of atopy[39]. Paternal smoking did not have any effect. However Halonen[38] could not confirm a relationship between maternal smoking and cord IgE levels although he did find an effect of month of birth with lowest values in infants born in September. A significant effect of month of birth on cord IgE level was also reported by Kimpen et al[13] with values more than 1.0 showing a cyclic trend. The maximum was around late April and the minimum around late October. Prenatal sensitisation to helminth antigens in offspring of parasite-infected mothers was claimed by Weil et al[40].

Cord IgE and Prediction of Atopy
Orgel et al[41] followed a sample of 34 infants throughout the first year of life with serial IgE measurement and monitored the development of allergy. They proposed that elevation of serum IgE preceded the manifestation of atopy and a high serum IgE level at or before 1 year of age was highly correlated with the development of atopic disease in the first two years of life. Thus the measurement of IgE in infants would have predictive value to the subsequent development of atopic disease. In a Swedish study[42] of 206 healthy infants and children followed up for 18 months, a more than ten fold increase (47% Vs. 3%) of subsequent atopic disease was found if the IgE level exceeded the geometric mean+ 1 SD limit in healthy subjects of the same age. In another study from the same group[32] 30 infants with single and 38 with dual heredity were followed for 18 months

from birth. Cord serum IgE was elevated significantly more often in those who subsequently developed atopic disease in comparison with those who did not. Out of 21 children with subsequent atopic disease, 7 (33%) could be identified at birth by elevated (mean+2 SD) cord serum IgE level. Thirty six children of atopic mothers and 17 of healthy mothers were followed for a period of 2 years by Dannaeus et al[43]. All five children with a cord IgE level >0.4 ku/l developed atopic symptoms.

Michel et al[31] followed 83 infants for 9 months age and suggested that cord IgE is more predictive for the development of allergy in infancy than the family history. Of 17 infants who developed some allergic manifestation 71% had detectable cord IgE (0.5 ku/l) compared to 21% of 66 symptom-free infants. Only 9 infants developed definite allergy so the conclusion drawn may not be of significant value. Moreover, out of 136 infants included in the study only 83 were followed up and the selection criteria for this group was not specified.

In a large study of 1701 un-selected infants followed for 18 months, Croner and Kjellman[33] found that 70% of infants with a high cord IgE (>1.3 ku/l) developed obvious or probable atopy compared to 5% with low cord IgE. The corresponding figures for family history of atopy were 10.5% and 5.3%. A high IgE in cord blood was associated with a high IgE and a positive radioallergosorbent test between ages 18 months and 24 months more often than was a low cord IgE. The main criticism of this study was that infants were examined clinically if atopic disease was suspected from the questionnaire (n=193) but also if their cord IgE was high (n=51). Moreover cord IgA was not done to exclude possible contamination of cord with maternal blood during sampling.

A follow-up of 1651 children of the same cohort to seven years of age was reported[44]. This seemed to confirm earlier results but the IgE cut-off was brought down from 1.3 ku/l to 0.9 ku/l. Eighty per cent of those with high cord IgE (>0.9 ku/l) developed obvious or probable atopy compared to 30% with a low cord IgE. Only 3.5% children with no atopic disease showed high IgE at birth. Most of the data at this follow-up was obtained from questionnaire filled by the parents and scrutinising medical records rather than direct clinical assessment. On the basis of these results Hjalte[45] claimed that cord IgE screening to select infants for

preventive measures is economically worthwhile whereas selecting on the basis of family history alone was not. Eleven year follow-up of 1654 of the original 1701 infants was less supportive of predictive value of cord IgE as the sensitivity was only 26% (specificity 94%)[46]. Obvious atopic disease developed in 67% children with cord IgE >0.9 ku/l and a further 15% developed probable atopic disease.

Businco et al[47] followed 101 infants of atopic parents from birth to two years of age. Cord blood IgE levels were significantly higher in the group of infants who developed atopic disease (1.06 Vs. 0.34 ku/l). In each feeding group (Breast, Soy and Cow's milk) more infants developed atopy with high cord IgE (>0.8 ku/l) than those with low cord IgE. There were several problems with this study. Student's t-test was used for analysing cord IgE data which were highly skewed. Possible maternal contamination was not excluded. The reason for a cut-off at 0.8 ku/l was not indicated. Duchateu and Casimir[48] claimed significantly more atopy in infants with high IgE at day 5 if they were fed cow's milk but not if they were breast-fed. Only a small number of infants were assessed with a short follow-up (one month) and the cut-off at 1.0 ku/l was arbitrary. Hattevig et al[49,50] followed 86 children (all female with Rh-negative blood group) from birth to 7 years of age. At 4 years follow-up, all 4 children with cord IgE more than 1.3 ku/l had developed atopy. At 7 years follow-up, the cut-off was lowered to 0.9 ku/l. The specificity of cord IgE for the development of atopy in the first 7 years of life was 95% but the sensitivity was only 14%.

Chandra[35] reported that cord IgE higher than 0.7 ku/l was associated with a high risk of development of atopic eczema and wheezing, 52.8% and 58.8% respectively compared with 13.4% and 1.1% in the group with IgE less than this level. In the study by Strimas and Chi[51] 4 of 6 infants (67%) with high cord IgE (>0.5 ku/l) developed allergy within the first year of life compared to 15 of 76 (19.7%) with low cord IgE. The paper does not identify if these differences were statistically significant. There was no correlation between amniotic fluid IgE and the development of allergy. In a study by Magnusson[34] mean cord IgE levels and the incidence of elevated cord IgE (> 1.2 ku/l) were significantly higher in groups of infants classified as having definite or probable allergy at 18 months of age compared to those with

doubtful or no allergy. In a recent study by Halonen et al[38] infants who developed eczema in the first year of life had higher cord IgE levels than infants who did not [geometric mean (CL); 0.16 (0.10-0.25) Vs. 0.09 (0.08-0.10); $p<0.002$]. A significant correlation between IgE levels at birth and 9 months was observed ($r=0.44$; $p<0.001$).
There were two studies which failed to confirm the relationship of cord IgE and development of allergy. A follow-up of 487 infants born to allergic parents did not reveal any difference in the incidence of asthma or eczema with varying levels of cord blood IgE[52]. In another study of 79 infants with a bi-parental history of atopy followed-up for 12 months using a cut-off of 0.7 ku/l, Ruiz et al[53] found that the sensitivity of cord IgE test was only 10% with a specificity of 97%. The positive and negative predictive values were 80% and 50% respectively. High levels of cord IgE did not distinguish the most atopic infants.

Summary: PRIST^R is the most commonly used commercially available method for the measurement of cord IgE. Enzyme-linked immunoassays (ELISA) have certain advantages and there is a need for a sensitive, commercially available ELISA method. Serum IgE levels in children and adults are influenced by a host of genetic and environmental factors. Serum IgE levels at birth are determined primarily by genetic factors. However there is some evidence to suggest that maternal factors acting through placenta may influence the level of cord IgE. The overwhelming evidence so far suggests that cord IgE is a good predictor of atopy but there is some indication that the sensitivity may be too low to use it as a screening test.

Genetics of Atopy

The first detailed study of the genetics of allergic disease was carried out by Cooke and Vander Veer[54] They obtained family histories from 504 allergic and 76 nonallergic subjects and concluded that allergy was inherited as an autosomal dominant trait. Other workers have suggested an autosomal recessive trait and more recently a polygenic mode of transmission has been favoured[55]. Several studies evaluated the question of allergic inheritance by determining the prevalence of a family history of allergic disease in individuals who did or did not have a particular allergic disease. Although there are differences between the studies as many as 40 to 80% of individuals with allergic rhinitis or asthma had positive family histories compared to 20% or less in individuals without allergic disease[56].
Many studies have assessed the risk of a child becoming allergic based on the parental history of allergy. In a study by Gerrard et al[57] 9 of 38 children born to parents, one of whom had asthma, developed asthma compared to 11 of 437 whose parents did not have asthma. There was a significant increase of rhinitis and eczema in the child when a parent had the disease. Individual allergic diseases (asthma, eczema and rhinitis) were most frequent when parents suffered from the same disease except for urticaria which was most common when parents had asthma or eczema. A prospective study of 543 newborns up to the age of 5 years[58] showed the difference in allergy prevalence in three family history groups. With atopic parents 51% infants developed allergic disease, with atopic grandparents (non-atopic parents) the prevalence was 36% and with negative family history only 19% developed one or more allergic disease. When both parents were atopic there was an 80% prevalence of atopy in children. The occurrence of asthma was 8% in children of asthmatic parents compared to 1% in children of non-asthmatic parents. Similarly 18% of children with allergic rhinitis had rhinitis and 56% of children of parents with atopic eczema had eczema. There was a large drop out in this study (127 families) and not all children with possible atopic symptoms were assessed.

In a large retrospective study[59] the mean age of onset of allergic disease was said to be 3.7 years with bi-parental heredity, 6.1 with single heredity and 8.7 with no family history. In a questionnaire study of 7 year old Swedish children Kjellman[60] reported 43% allergy when both parents were allergic, 20% when one parent was allergic and 13% when both parents were non-allergic. When both parents had an identical type of atopic disease, the incidence of that disease in their children was higher (72%) than when non-identical types occurred in the parents (21%). The results from a questionnaire study can not be as conclusive as those with clinical assessment. In a recent study from Italy[61] 930 children between 9 and 15 years were skin tested to a range of 18 allergens. A subject was defined as atopic if at least one SPT caused a wheal greater than 3 mm. No effect of family history of atopy was observed.

More relevant to this work are studies where infants are followed-up from birth to determine the prevalence of allergic disease and relationship to family history of atopy and other risk factors. In 1963 Pugh[62] followed-up 199 infants from birth to 2 years of age. Thirteen of 16 infants with eczema had an immediate or remote family history of atopy. Similarly all six infants with wheezing and 57 of 73 infants with positive skin tests had such a history. In a study by Halpern et al[63] the incidence of allergy by family history showed significant differences: immediate, 15.6%; remote, 12%; and negative, 8.8%. In the immediate group allergy occurred earlier and asthma and allergic rhinitis more often. Orgel et al[41] followed 28 infants from birth to 2 year of age. Of 11 infants who developed definite atopic disease 8 (73%) had a history of atopy in the immediate family. Kaufman[64] followed 94 infants of allergic mothers from birth to 24 months of age. The incidence of allergic disease was 80% in infants with bi-parental atopy and 40% when one parent was allergic. Thirty six infants of atopic mothers (group A) and 17 infants of healthy mothers (group B) were followed for 24 months from birth[43]. Eighteen (50%) infants in group A but none in group B developed atopic symptoms. Jakobsson and Lindberg[64] found that 70% infants who developed cow's milk intolerance during the first year had a positive family history compared to 35% for the total group of 328 infants. Michel et al[31] concluded that 40% of infants of allergic mothers (as defined by positive RAST

test) developed obvious or probable atopy compared to 20% with no maternal allergy. Father's allergy was not related to infants allergic disease. Hide and Guyer[66,67] reported that at one year 22% infants with parental allergy and 14% with no parental allergy developed asthma or eczema. The corresponding figures for the 4 year follow-up were 26% and 18%. In a recent study by Strimas and Chi[51] allergy developed in 29% infants with, and in 14% infants without, a family history of allergy. A highly significant difference was seen in the incidence of atopy at 18 months in infants with (13%) and without (5%) family history of atopy in the study by Croner and Kjellman[33]. In infants with bi-parental allergy the incidence of atopic disease was 23%. At 7 year follow-up[44] obvious or probable atopic disease developed in 30% infants with no family history and 50% with positive family history of atopy. In the same cohort the cumulative incidence of obvious atopy at 11 year of age was 45% in children with and 26% without a family history of atopy.

The influence of genetic factors on the level of serum IgE in cord blood[33-38], and in children and adults[15-19], was reviewed in the previous chapter which highlights the relationship between heredity and atopy. These and other studies are relatively consistent in finding that serum IgE levels are predominantly genetically controlled but the mode of inheritance is not clear. Earlier studies by Marsh et al[68] and Rao et al[69] suggested recessive inheritance of high IgE levels. Happle and Schnyder[70] evaluated the children of men and women with atopic asthma. In children of atopic fathers, 32 of 98 (33%) were affected by some form of atopic disease compared to 45 of 93 (48%) of children of atopic mothers. They believed that this difference in hereditary risk was due to the Carter effect[71] providing evidence for polygenic inheritance in allergy. Even an X-chromosome linked effect was proposed by Turner et al[72] who studied 1,016 school children and their families. The mother's serum IgE concentration was more closely related to her son than to her daughter and the converse relationship existed for girls. This led them to suggest that the X-chromosome of man carries genes which influence IgE synthesis. A single gene linked to X-chromosome could not explain inheritance of IgE and allergy in both sexes.

A recent study form Oxford[17]has proposed that atopy is inherited as an autosomal dominant character. The same group

of investigators further studied transmission of atopy in 7 families with the help of molecular genetic linkage analysis and assigned the gene locus to chromosome 11q[73].

An interesting method for examining the importance of genetic influence is to evaluate twins reared together compared to twins reared apart. In a review, Blumenthal[56] stated that no differences in concordance for asthma or seasonal rhinitis were noted in monozygotic twins reared together or apart, suggesting a dominant genetic effect. Some studies have attempted to link allergic diseases and the major histocompatability complex in man but none of these association has been strong enough to be clinically useful[55,56].

The Christchurch child development study[74] confirmed the increased risk of childhood asthma in children of allergic parents. The risk of developing asthma was markedly different between sexes. 14.3% boys had developed asthma by age 6 years in contrast to 6.3% of girls ($p<0.0001$). Several other epidemiological studies have confirmed the male preponderance in asthma[75-77]. Some studies have found that males are at a higher risk for allergic disease[33,34,59,61] although other studies could not find a difference in the incidence between sexes[58,60,72].

Summary: Studies have consistently demonstrated a significant genetic influence on the risk of developing allergic disease. The inheritance of allergy is more likely to be polygenic with gene locus at chromosome 11q being one among several others. Boys have a 1.5-2 times higher risk of developing asthma compared to girls but there is less agreement on male preponderance for other allergic disorders.

Infant Diet and Allergy

Since the pioneer study of Grulee and Sanford[78] on the beneficial effects of breast feeding on the development of eczema a large number of trials have been done with conflicting results and the subject remains controversial. The studies can be divided into those which confirmed the protective effect of breast feeding and those which did not.

Studies reporting benefit

Studies on un-selected infants: The first and largest of all the studies was the prospective study of Grulee and Sanford[78]. They studied 20,061 infants from birth to 9 months age between 1924 and 1929. They concluded that the incidence of infantile eczema was lowest in the breast fed infants, double in the partially breast fed and seven times greater in the formula fed infants. Saarinen et al[79] followed 237 babies for 1 year and re-examined 178 of these at 3 years of age. Eczema was less in breast fed babies at one year than formula fed but significance was taken at 10% level. In a long-term follow-up study of 649 children to 15 years Gruskay[80] showed a reduced incidence of allergy in breast fed infants compared to either cow's milk or soy milk fed infants. In the negative family history group incidence of atopy was 5% in the breast fed compared to 16% in the formula fed. In the positive family history group the percentages for breast, cow's milk and soy were 28%, 53% and 53%. The value of this study is diminished because of a large drop-out (40%) during the follow-up. In a study by Chandra et al[35] of 226 infants followed up to 2 years of age the incidence of eczema and wheezing was significantly lower (12%) in breast fed infants compared to formula fed infants (32%). The difference was even more pronounced for infants with high (>0.7 ku/l) cord IgE levels. Moore et al[81] attempted a controlled trial of the effect of infant feeding on eczema but did not succeed because of non-compliance in the experimental group. The results were analysed as observational study in 475 infants. Exclusive breast feeding for 4 weeks protected against eczema at 3 and 6 months but

there was no significant difference in the two groups at 12 months follow-up. Soy feeding was not associated with reduction in eczema. Subsequent follow-up of these children up to the age of 5 years revealed no difference in atopy between the two groups[82].

Studies on selected (high risk) infants: Chandra[83] followed 74 infants with a history of allergy in the sibling for 3 years. Thirty seven were exclusively breast fed for at least six weeks and the other 37 infants were given formula milk before that age. The incidence of eczema and recurrent wheezing was significantly less in breast fed compared to formula fed children (11% vs. 57%). Moreover there were significant differences in serum IgE levels and IgE antibodies to cow's milk in favour of the breast fed group. Matthew et al[84] followed 49 infants up to the age of 12 months. Eczema developed in 3 of 20 (13%) infants who were breast fed and 9 of 19 (47%) who were formula fed. There were no significant differences in positive skin test responses in the two groups. Mean serum IgE level was lower in the breast fed group at 6 weeks but this difference disappeared by the age of 6 months. Businco et al[85] evaluated 101 newborns of allergic families from birth to two years. Breast feeding mothers were said to be on dietary allergen avoidance regime but they were allowed 200 ml of cow's milk and 2 eggs per week. The incidence of atopic disease was less in the breast fed (for 6 months) and soy milk fed babies (6 of 34 and 6 of 25) respectively compared to 15 of 41 in cow's milk fed babies. However these deferences were not statistically evaluated.
In another study of 246 children of atopic parents followed up to the age of 7 years Businco[86] found a significant benefit of breast and soy milk feeding compared to cow's milk feeding (15% vs. 39%) on the development of atopy. In this study some dietary and environmental avoidance measures were practised. The control group was formed by mother who did not continue to breast feed their babies. The two groups were also different with regard to family history of atopy. Miskelly et al[87] followed 487 infants of allergic families for 12 months. Breast feeding even for a short period protected against wheeze (22% vs. 43%), and diarrhoea (59% vs. 76%) but not against eczema. The replacement of soy milk in some breast fed infants instead of cow's milk for

supplement had no effect. In this study the duration of breast feeding was not relevant to the outcome.

Some other studies: In a long term follow-up of children with asthma Blair[88] determined the effect of method of feeding on the prognosis 5 to 20 years later. Breast feeding for more than 8 weeks improved the prognosis for mild and severe onset asthma groups. No data were presented on the effects of breast feeding for 1-8 weeks. In a retrospective study Wittig et al[59] analysed patients records (allergic disease and skin prick tests responses) on 2,190 patients in relation to various risk factors. Mean age of onset of allergic disease was earlier in patients who were formula fed compared to those who were breast fed (7.1 vs. 4.5 years). Formula fed patients had significantly more positive skin tests to cat dander. Wright et al[89] in their study of 1022 infants followed for one year found a beneficial effect of breast feeding on wheezy respiratory tract illness in the first four months. Two studies[90,91] reported a protective effect of breast feeding against respiratory syncytial virus infection.

Studies not reporting benefit

Studies on un-selected infants: Gerrard et al[92] followed 787 consecutively born infants for allergic symptoms related to cow's milk and other food allergy. The proportion with allergy was similar in those who were breast fed to that in the cohort as a whole, showing no protective effect of breast feeding. The authors did not distinguish between exclusive and partial breast feeding. In a study by Halpern et al[63] 753 infants fed breast, soy or cow's milk from birth to 6 months of age were followed for varying periods up to 7 years. The original groupings were not adhered to and data were analysed according to what infants received. Diet did not affect the incidence of allergic disease although allergy developed earlier in the cow's milk group compared to the breast fed group, the soy group being intermediate. Jakobsson and Lindberg[65] followed up 1079 infants during their first year. Infants who became cow's milk intolerant were entirely breast fed for the same duration as other infants (13 weeks vs. 12 weeks) showing no protective effect of breast feeding. Information on 751 of these infants was

obtained solely from hospital records and may not be complete.
In a study by Juto and Bjorksten[28] serum IgE during the first year of life was related to the type of feeding. Out of 400 eligible mothers only 68 agreed to participate and 2 mother were included on their own initiative when their babies were three months old. During the first 6 months of life there was no effect of method of feeding on serum IgE level. Serum IgE at 12 months was significantly higher in infants breast fed for longer than 6 months (Geometric means: 3.1 vs. 9 ku/l). The longer the period of breast feeding the higher the serum IgE levels were at 12 months. Hide and Guyer[66] followed up 843 infants during the first year of life. The incidence of eczema and rhinitis was not different in infants who were initially breast fed compared to those who were started on formula feed. However there was less asthma (defined as one or more episode of wheezing) and consequently a reduction in the overall incidence of allergy. Four hundred and eighty six of these children were assessed at 4 years[67]. The definition of asthma was revised to more than one episode of wheezing. There was no difference in any of the allergic manifestations in the two feeding groups.
In a case control study of 470 children from a dermatology clinic Kramer and Moroz[93] could not find an association between method of feeding and eczema. Fergusson et al[94] followed 1123 infants for 2 years. No difference was found in the incidence of eczema between infants who were exclusively breast for 4 months (15.4%) and to the rest of the group (16.6%). A national cohort of 13,135 children followed up to 5 years showed a higher incidence of eczema (as reported by parents) in children who were breast fed[95]. There was no effect of method of feeding on asthma or wheezing not labelled as asthma.

Studies on selected (high-risk) infants: Kaufman and Frick[64] followed 94 infants of allergic mothers for 2 years. There was no significant difference between the two feeding groups as regards the development of asthma and eczema or positive skin prick tests (although the authors claimed a beneficial effect of breast feeding on asthma and eczema). In a small study by Dannaeus et al[43] no relationship between the development of allergic symptoms and time of onset of

artificial feeding could be demonstrated. Gordon et al[96] identified 250 infants with a history of asthma or eczema in a parent or sibling. On following up the infants for 2 years they found no difference in the incidence of eczema or asthma between infants who were breast fed and those who were formula fed. Van Asperen[97] examined the relationship of diet to the development of atopic manifestations in a group of 79 infants with an immediate family history of atopy followed from birth to 20 month of age. They could not find a relationship between the duration of breast feeding or introduction of cow's milk to the development of eczema, rhinitis or wheeze. Seventy three children born to atopic parents were followed up to 5 years of age by Cogswell et al[98]. No protective effect of breast feeding could be demonstrated on the development of eczema, asthma or positive skin tests. The numbers were small so no definite conclusions could be drawn.

Soy versus Cow's milk

Randomised controlled trials of various types of formula milk are possible. In 1966 Johnston and Dutton[99] reported one such trial of 292 infants with a family history of allergy, randomly chosen to receive either a soya preparation or a cow's milk formula. Ten years later allergic disease had occurred in 18% of the soy group and in 50% of cow's milk group. The incidence of asthma and perennial allergic rhinitis was significantly less in the soy group but there was no difference in the incidence of eczema or hay fever. Kjellman and Johansson[100] reported a small study of 48 infants with bi-parental history of atopy randomised to soy based or cow's milk based formulae. Obvious and probable atopic disease developed in 66% infants by the age of 36 months. No significant difference was observed between the two groups in the incidence or the time of appearance of allergic disease. Chandra et al[101] studied the effect of feeding whey hydrolysate, soy, cow's milk formula and exclusive breast feeding on incidence of atopic disease in infants with a family history of atopy. The incidence of total atopy was 7.4%, 35.8%, 36.7% and 20% in that order for whey hydrolysate, soy, cow's milk formula and exclusive breast feeding. Skin test responsiveness was less in whey hydrolysate and soy group compared to breast fed and cow' milk fed group.

Sensitisation through breast milk

Talbot[102] in 1918 was the first to describe a case report of a breast fed infant whose eczema occurred after his mother had eaten chocolate. The lesion disappeared when chocolate was eliminated from the mother's diet. Since then there have been several sporadic reports of allergic symptoms in infants provoked by food ingested by their mothers. Matsumura et al[103,104] reported ten cases of eczema due to egg sensitivity and 5 of cow's milk and soya bean sensitivity in infants who were solely breast fed. Eczema disappeared when food was removed from mother's diet and recurred on direct and indirect (through mother's diet) challenge. Jakobsson and Lindberg[105] demonstrated that colic in 12 of 19 infants disappeared when their mothers were put on a cow's milk free diet and re-appeared on at least two further indirect challenges. Gerrard[106] reported eighteen exclusively breast fed infants who developed sensitisation and allergic symptoms to foods ingested by the mother.

Bjorksten and Saarinen[107] followed 95 infants up to 12 months. Cow's milk specific IgE was detected in only breast fed infants. The authors suggested that small amounts of cow's milk protein transferred through breast milk are more antigenic than large amounts (in cow's milk fed infants) which can actually suppress antibody response in the infants. This possibility was investigated by Firer et al[108] in a group of 54 infants with cow's milk allergy. Breast fed infants (minimal exposure to cow's milk) had decreased titres of IgG, IgA and IgM milk antibodies and increased total and milk specific IgE compared to those who were fed substantial amounts of cow's milk. These results provide evidence that large amounts of allergen suppress rather than stimulate IgE production.

Van Asperen et al[109] reported 8 infants with immediate hypersensitivity reactions (confirmed by positive skin tests) on the first known exposure to the foods (milk, egg or peanut). Eighty six infants were prospectively studied by Hattevig et al[49] through the first four years of life. Total and specific IgE levels to common foods were demonstrated at 3, 8, 25 and 48 months. Nine infants developed IgE antibodies to egg or cow's milk before the introduction of these nutrients into the food.

Detection of food proteins in breast milk

Stuart and Twiselton[110] were the first to detect cow's milk proteins (beta-lactoglobulin and casein) in breast milk by a a double-antibody sandwich ELISA. Beta-lactoglobulin was detected in the milk of 5 of 28 mothers and casein in 13 of 28 mothers. Of 57 exclusively breast fed infants, 11 developed allergic symptoms in a study by Machtinger and Moss[111]. Beta-lactoglobulin was detected in 45% of breast milk specimens and persisted up to 3 days after maternal dietary milk exclusion. The breast milk from these mothers was also found to have lower serum total IgA and specific IgA to whole cow's milk and casein than milk from mothers whose infants had no symptoms. Cant and Marsden[112] detected ovalbumin in breast milk from 14 of 19 mothers tested after ingestion of egg. Axelsson et al[113] measured beta-lactoglobulin in 232 milk samples from 25 mothers during the whole lactation period. Detectable amounts (5-800 ug/l) were found in 93 (40%) milk samples. The presence of diarrhoea, vomiting and colic was significantly correlated to high levels of lactoglobulin in the milk. In a prospective study[114] of 1749 newborns, 39 developed cow's milk allergy during first year. Nine of 39 were said to be exclusively breast fed but all of these infants were inadvertently exposed to cow's milk in the nursery. Detectable amounts of beta-lactoglobulin were found in 3 of 9 milk samples against which the infants reacted clinically.

Delay in the introduction of solid food

In the late 1920's Glaser[115] observed frequent intolerance of egg yolk in infant when the food was introduced at the age of 3 months and rare intolerance when the food was introduced at the age of nine months or later. Kajosaari and Saarinen[116] claimed that delay in the introduction of solid food to six months prevents the development of eczema and food allergy. They followed 135 exclusively breast fed infants of atopic parents up to one year. The infants were defined as delayed solid feed group according to the time of introduction of solids by the parents. This self selection is likely to produce important differences in the two groups with regard to development of allergy. Eczema occurred significantly more often in infants given solid food at 3 months (35%) compared to those where no solids were given during the first 6 months. The occurrence of food allergy

was similarly reduced (37% vs. 7%). A reduction in the incidence of eczema was shown by Fergusson et al[94] by delaying solid foods to 4 months. In a retrospective study Kramer and Moroz[93] could not detect an effect of age of introduction of solid food to the development of eczema. In a prospective study, Van Asperen[97] did not find a difference in the incidence of eczema in infants given solid foods before the age of 4 months and in those where solid foods were withheld.

Summary: More than 50 years have passed since the first study on the effect of breast feeding on allergic diseases and the subject remains controversial. The main problem is a lack of randomised trials but to randomise mothers to breast feed or formula feed their babies is neither practical nor ethical. As Sauls[117] points out mothers who chose to breast feed or formula feed their babies differ in several variables which effect the incidence of allergic disease in the two group of infants. There was also lack of blind assessment in most of these studies. An alternative would be to follow a large number of un-selected infants prospectively with blind assessment and adjust for confounding variables during analysis. However at this stage one can safely conclude that if breast feeding does protect against allergic disease the effect is not highly significant. It has been known for some time that infants can be sensitised through breast milk and recently cow's milk protein and other food proteins have been detected in the breast milk. Some argue that these small amounts of protein are more antigenic to the immune system of the infant than large amounts given directly in cow's milk preparations. Hydrolysed milk may prove a useful alternative to cow's milk or soy milk in the treatment and prophylaxis of allergic disease. Delay in the introduction of solid food may help to reduce allergic disease but the evidence is not conclusive.

Environmental Factors

The foetus can be influenced by environmental factors such as drugs, tobacco smoke and food antigens through the placenta. The effect of these factors on the level of cord IgE has been discussed in the previous chapters. Intra-uterine sensitisation is rare and specific IgE is not found in cord blood in more than 2% infants. Once the infant is born he/she is exposed to a wide variety of food and inhalant allergens and adjuvants. These factors may influence the infant's immune system depending on the kind and intensity of exposure. This antigenic stimulus may be of critical importance in those infants who are genetically predisposed.

Social Factors

In a questionnaire study of the parents of 5,301 between the ages of 6 months to 16 years Andrae et al[118] investigated the association of bronchial hyperreactivity and allergic asthma to a host of environmental factors. Children living near a paper pulp plant more often had symptoms suggesting bronchial hyperreactivity (relative risk: 1.3) and allergic asthma (relative risk: 1.3). In children living in a damp house the relative risk for bronchial hyperreactivity and asthma was 1.9. Children living in a damp house with parents who smoked had the highest risk, 2.8 for bronchial hyperreactivity and 2.5 for allergic asthma. In a preliminary study Martin et al[119] investigated the effects of housing conditions on the health of 358 children. Children living in damp houses, especially where fungal moulds were present, had higher rates of respiratory symptoms after adjusting for smoking and number of other children in the house. However these symptoms were self reported and were open to bias.

Strachen[120] attempted to validate parental reports of wheezing with exercise induced reduction in FEV_1 in 873 seven year old children. Wheeze in the past year was the symptom most closely associated with reported dampness and particularly with mould (adjusted relative risk: 3.00) but there was no significant difference in the degree of bronchospasm measured among children from homes with and

without mould. The authors concluded that awareness of dampness or mould may effect the parental reporting of symptoms. In the Christchurch child development study Horwood et al[74] were unable to find an association between the development of asthma and family social background, stress in the family, breast feeding, parental smoking habits and pets in the child's family.
Burr et al[121] studied the relationship of allergic symptoms to various environmental factors in 483 infants of atopic families followed up for 1 year. The incidence of wheezing was significantly related to low social class, number of siblings and houses with open coal fires. There was a non-significant trend for increased wheezing episodes with the presence of dampness and moulds. The number of prolonged colds was associated with number of siblings in the house and cow's milk feeding. Eczema was not related to any of the environmental factors. A sample of 930 children, between 9 and 15 years, was studied by Astarita et al[61]. Atopy, as defined by at least one positive skin prick test, was significantly associated with high density housing and exposure to high environmental allergens (each individual was classified as exposed to high or low environmental allergen depending on overcrowded living conditions, living on a farm etc.). Social class did not affect the incidence of atopy.
Wittig et al[59] in an analysis of 2,190 patients records found that a rural population had earlier age of onset (3.8 vs. 6.5 years; $p<0.0001$) of allergic disease and more frequent positive skin tests to birch pollen, *Alternaria, Rhizopus, Trichoderma* and horse dander (all $p<0.05$). Other environmental factors may be associated with the development of respiratory allergy such as humidity levels and air pollution (especially sulphur dioxide and various kinds of sprays and fumes).

Passive Smoking
As previously discussed maternal smoking has been associated with increased cord IgE level[39] and exposure to passive cigarette smoke may increase the serum IgE level in children[27]. The effect of smoking on sensitisation to occupational allergens was investigated in 175 non-atopic subjects by Zetterstrom et al[122]. The geometric mean IgE concentration was higher in smokers. Evidence of

sensitisation (RAST and positive SPT) was more common in workers who smoked. Riedel et al[123] showed increased sensitisation (IgG_1-antibody) and bronchial responsiveness in guinea pigs exposed to tobacco smoke. In an analysis of 2,190 patients records[58], age of onset of allergic disease was found to be lower in children exposed to tobacco smoke compared to those who were not (5.3 vs. 6.5 years; $p< 0.001$). Children who lived in houses with smoking also had more skin test reactions to pollens and lactalbumin ($p< 0.05$).

Ronchetti et al[124] claimed that children of smoking parents had increased serum IgE level. However no significant relationship was found between passive smoking and IgE levels with multivariate analysis controlling for various confounding variables. Ównby and McCullough[125] studied two groups of children (age range: 2-17). One group of 100 children was selected from well-child clinic and the other group of 91 children was referred for allergy related symptoms. Children of smoking parents were more likely to be referred for allergy evaluation but did not show evidence of increased sensitisation (total or specific IgE) in either group. The authors concluded that passive smoking does not cause allergic sensitisation.

Pedreira[126] followed 1,144 infants during the first year of life. In infants exposed to cigarette smoke tracheitis (10.3% vs. 7.1%) and bronchitis (30.6% vs. 21%) occurred more frequently. The authors do not indicate how the information on smoking habits was obtained and there was a large drop out (24%) from the study. Maternal smoking imposed greater risks on the infant than paternal smoking.

Harlap and Davies[127] reported analysis 10,672 infants admission with respiratory illness to hospital and related this to maternal smoking habits. The infants of mother who smoked had significantly more admissions for bronchitis and pneumonia. All the information was obtained from hospital records and may not be complete. Moreover the effect was limited to the infants aged 6-9 months while at older and younger ages there was no significant effect of maternal smoking. In the study by Burr et al[121] of the effect of environmental factors on allergic symptoms in infants, maternal smoking was significantly associated with wheezing episodes. In a survey of 1058 infants Chen et al[128] found a significant relationship between admission for respiratory

illness and exposure to passive smoking. The data were obtained from self administered questionnaire and were not validated. Said et al[129] found a significant correlation between parental smoking and infantile colic.
Fergusson et al[130] examined the relationship between parental smoking and respiratory illness in a birth cohort of 1180 one year old children. Maternal smoking was significantly related to increased incidence of lower respiratory illness but it did not affect the overall incidence of respiratory illness (upper and lower). No effect of paternal smoking was demonstrated. When this cohort was followed up to 6 years parental smoking was not found to be related to childhood asthma[74]. Cogswell et al[98] followed up 73 children born to atopic parents for 5 years. At this age 62% of parents who smoked had children who had experienced episodes of wheeze compared to 37% in families where parents did not smoke ($p<0.05$). No significant difference was found in serum IgE levels in children with or without parental smoking. Kershaw[131] found significantly higher prevalence of household smoking in 6 years old asthmatic children (n=91) compared to the general population. In the study by Andrae et al[118] incidence of exercised induced cough was significantly higher in children whose parents smoked (7% vs. 5%). Murray and Morrison[132] reported a study of 94 consecutively observed asthmatic children. The 24 children whose mother smoked had 47% more symptoms, a 13% lower mean FEV_1 and a four fold greater responsiveness to histamine. The smoking habits of the fathers were not correlated. In another study[133] a higher percentage of children with atopic dermatitis develop asthma if the mother smokes (79% vs. 52%; $p=0.001$). It was thought that atopic dermatitis signified a predisposition to atopy while smoking acts as an adjuvant factor. Colley et al[134], in a follow-up study of 3,899 twenty years old subjects, investigated the relation between cough in winter to events recorded during infancy and childhood. Exposure to cigarette smoke in infancy was found to be most significantly related to prevalence of cough (14.6% vs. 7%) at the age of 20. The selection criteria for the study could be questioned such as including children of all non-manual workers but only one of four of manual workers and there was a large drop out from the study.

Season of Birth

Bjorksten and Suoniemi[135] described a retrospective study of 1421 patients allergic to pollens and 728 allergic to animals. There was a significantly higher risk in male born in March-May and September-November for both pollen allergy and animal epithelium allergy. They postulated that boys have a sensitive period early in life when environmental factors are more likely to influence the development of subsequent allergy. In another report[136] they examined the records of 2171 patients (age: 0-29 years) with positive SPT to birch pollen and correlated with exposure to birch pollen. The risk was highest for infants born in February-April (soon before the birch flowering season in May) and lowest in those born in July-August. This risk was also dependent on the quantity of birch male flower and the number of non-rainy days in the first birch flowering season met in infancy. Similar results were reported by these investigators[137] for grass and mugwort pollen allergy. Suoniemi et al[138] investigated the relationship of risk factors encountered in infancy to the development of allergy in adolescent period. Seven hundred and eight un-selected 15-17 year old adolescents were examined and skin tested to common allergens. The development of positive SPT and respiratory allergy correlated positively to exposure to pollen and cat epithelium during the first six months of life, eczema in infancy and atopic heredity. The study was retrospective and recall of events as long ago as 20 years may not be accurate. Soothill[139] followed 58 infants of atopic heredity up to the age of 12 months and examined the relationship of risk factors to the development of allergy and skin test reactivity. Positive skin tests to any allergen were significantly more in infants born in September and October. Similar trends for positive SPT to HDM and grass pollen were not significant.

Morrison and Springett[140] analysed the effect of month of birth in 1715 children with asthma. There was a significantly higher proportion of asthmatic children born in May to October (55%) than would be expected (50%). Children with positive SPT to HDM also showed a higher proportion born in May-October (56%). David and Beards[141] analysed month of birth of 4,520 children with asthma born over a 15 year period. Although there were statistically significant excesses of asthmatic births in certain months

there was no definite pattern and peak months varied from year to year. Duffy and Mitchell[142] examined the relationship between month of birth and episode of wheezing, productive cough, eczema, hay fever and mid-expiratory flow (FEF_{25-75}) in a representative sample of 4,549 Australian primary school children (mean age 10 years). Children experiencing frequent wheeze were more likely to be born in spring and summer (odds ratio=1.6). No effect of month of birth was demonstrated on other illnesses or lung function.

House-dust Mite (HDM)

House-dust mite antigen has been shown to be causative in the development of mite-sensitive asthma. Korsgaard[143] related the concentration of mite in the house dust to the prevalence of asthma in a case-control study. House dust was collected from the homes of 25 newly diagnosed patients with mite asthma (positive skin tests and RAST to HDM) and 75 matched controls subjects. The difference in exposure corresponded to a relative risk of 7.0 and a clear dose-response relationship could be demonstrated. Turner et al[144] investigated the dramatic rise in the prevalence of asthma in South Fore area of Papua New Guinea (from 0.1% to 7.3%). They compared the mite concentration in the blankets of residents of South Fore area and those of adjacent Asaro valley where asthma prevalence was still very low (0.3%). The mite concentration was significantly lower in blankets from Asaro valley (283/g dust) compared to South Fore area (1371/g dust).

In a study by Platts-Mills et al[145] 9 mite sensitive, asthmatic patients avoided exposure to house-dust mite for 2 months or more. In all patients symptoms and early morning peak-flow improved. In 7 patients treatment could be reduced and 5 showed increased tolerance to histamine on challenge. However this was an uncontrolled study and factors other than allergen avoidance (related to prolonged hospitalisation) could have contributed to the benefit observed. Murray and Alexander[146] in a controlled study of 20 patients with mite sensitive asthma showed that avoidance of house-dust mite results in significant improvement in symptoms and signs of asthma and a reduction in bronchial hyperreactivity. However the measures taken to keep rooms free of dust in the intervention group were presumed to have resulted in house-dust mite allergen avoidance and

measurement of house-dust mites or *Der p* I was not performed.
Lau et al[147] determined concentration of *Der p* I and *Der f* I in 183 dust samples from mattresses of 133 atopic and 50 non-atopic children. Children living in houses with high level of *Der p* I and *Der f* I (>2ug/g dust) were found to have significantly higher serum IgE level to these antigens compared to patients with low mite allergen exposure. The relative risk for sensitisation in the highly exposed group versus the group with very low exposure was 7 to 32 fold. In a prospective study of 67 children followed-up from birth to 11 years of age Sporik et al[148] investigated the relationship between house-dust mite exposure to the development of mite-sensitisation and asthma. Of the 17 with active asthma 16 were atopic, all sensitive to house-dust mite (positive SPT and RAST). There was a trend towards an increasing degree of sensitisation at the age of 11 with greater exposure at the age of 1. The development and severity of asthma was directly related to the degree of exposure to house-dust mite antigen during infancy. Although the relationship of house-dust mite sensitivity was strong with diagnosed asthma the same was not true for wheezing. The authors do not point out that almost 50% of children with a history of wheezing were not sensitised to house-dust mite.

Viral Infections
There are several reports of increased bronchial hyperreactivity several years after bronchiolitis or viral lower respiratory tract infection during infancy. Gurwitz et al[149] found increased bronchial hyperreactivity at methacholine challenge and low peak flow rates 9-10 years after admission to hospital with bronchiolitis. Sims et al[150] examined 35 children at the age of 8 who had respiratory syncytial virus (RSV) bronchiolitis in infancy. Mean exercise bronchial lability of these children was significantly higher and peak flow at rest significantly lower than 35 matched controls. Mok and Simpson[151] assessed 200 seven year old children after acute lower respiratory infection during infancy and their matched controls. The index patients had higher prevalence of cough, wheeze, respiratory illnesses, asthma and bronchitis. On examination impaired ventilatory function and increased bronchial

hyperreactivity was found in index cases compared to the controls. Pullan and Hey[152] assessed 130 children 10 years after their admission to hospital with proved RSV lower respiratory tract infection and compared with matched controls. Wheeze was more common in index patients (42% vs. 19%) and a three fold increase in bronchial lability was detected.
Cogswell et al[153] followed 92 children from birth to a maximum of 3 years of age. Wheezing was a clinical feature in 12 children during lower respiratory tract infection in infancy. Of these 12, 6 were atopic in the first year (positive SPT), one developed eczema in the second year and the other 5 developed raised serum IgE by the age of 3 years. Welliver et al[154] in an un-controlled study reported the appearance of IgE antibodies bound to exfoliated nasopharyngial epithelium following RSV infection in 42 infants. Perelmutter[30] reported an increase in the IgE level during the acute phase of viral respiratory infection which decreased significantly in the convalescent phase. Frick et al[155] followed 13 infants with bi-parental history of atopy from birth to 4 years of age. Eleven of these 13 children became allergic clinically and immunologically (raised serum IgE and positive RAST). Upper respiratory infection occurred in all 11 children 1-2 months prior to the onset of allergic sensitisation. The authors concluded that certain viruses may contribute to the allergic sensitisation process. The numbers were small and matched controls were not studied.

Animal Dander:
There are very few reports in the literature of the effect of exposure to animal dander on the development of subsequent allergy. A cat in the house during the first six months of life was significantly ($p=0.01$) related to the positive skin test reaction to cat in the adolescent period[138]. No such relationship was detected for dogs. Horwood et al[74] in the Christchurch development study, were unable to find a relationship between exposure to cat or dog dander and the development of asthma. In a follow-up study to one year, Burr et al[121] were unable to find an effect of presence of cats, dogs, other mammalian pets and birds in the house on the incidence of allergic symptoms. In a questionnaire study Andrae et al[118] did not find an

association between the presence of furred pets or birds on the incidence of bronchial hyperreactivity or asthma.

Summary: Conclusive evidence that presence of moulds and dampness in the house are involved in the development of allergic asthma is lacking. Respiratory symptoms such as wheezing and cough are worse in children of smoking parents especially during the first year of life. Exposure to passive smoking early in life may increase the likelihood of sensitisation in genetically predisposed children.
Children born in spring and summer are more likely to develop asthma and pollen allergy. Those born in autumn are probably at a higher risk of being sensitised to HDM. Exposure to house-dust mite antigen is causally related to the development of mite sensitisation and asthma. Avoidance of HDM antigen improves asthmatic symptoms and reduces the need for treatment. Exposure during infancy may be particularly relevant to the development of subsequent atopy and asthma.
There is little doubt that lower respiratory tract viral infection during infancy is a risk factor for bronchial hyperreactivity and asthma in later childhood. It is not certain if bronchiolitis in infancy increases the likelihood to develop atopy in the genetically predisposed infants. There is no convincing evidence that exposure to animal dander in early life increases the risk of subsequent development of allergy.

Prevention of Allergy

Intra-uterine sensitisation in guinea pigs was first demonstrated by Ratner et al[156]. They concluded that the passage of antigen from a mother guinea pig to her foetus can result, in certain instances, in a definite active sensitisation of new-born, having received its sensitising dose in utero. Intra-uterine sensitisation has been shown to occur in the human infants[40,103,109]. Some investigators have attempted to restrict mothers' diet during pregnancy to protect infants from this source of sensitisation.
Grulee and Sanford[78] were able to show a seven times reduction in eczema during the first year of life in infants who were breast fed compared to those who were cow's milk formula fed. Although a large number of studies have produced conflicting results[78-98], no one since has been able to reproduce such a huge reduction in eczema or other allergic disorders. This lack of protective effect of breast feeding is thought to be due to the small amount of protein ingested by the mother which is secreted unaltered into the breast milk[110-114]. Jarrett[157] from her experiments on animals argued just the opposite that regular exposure to milk proteins induce tolerance by suppressing synthesis of IgE, whereas limited intake may be associated with allergic sensitisation. These findings in rats have not been confirmed in humans. Inhalant allergens are even more difficult to control and few investigators have attempted to reduce inhalant allergen exposure as a prophylactic measure.

No Restriction on Maternal Diet

In a randomised controlled trial Johnston and Dutton[99] reported a significant reduction in the incidence of asthma and perennial allergic rhinitis in infants who were fed soya milk and avoided egg and wheat up to nine months. There was no difference in the incidence of eczema or hay fever. Matthew et al[84] reported a significant reduction in eczema during first year of life in infants on allergen avoidance diet (cow's milk, egg and fish) for the first 6 months. Soya milk was given as supplement to breast milk. They also claimed that exposure to inhalant allergens from pets and house-dust mites was avoided but no details are given as to

how this was achieved. Other studies[100,101] have reported a reduction in allergy by using soya milk or protein hydrolysate as substitute to cow's milk though the usefulness of soya milk has been questioned[63].
In a study[158] of fish and citrus allergy 177 children avoided fish and citrus up to the age of 1 year whereas 145 other children were given fish before the age of 6 months and citrus before the age of 3 months. At 3 years there was no difference in the incidence of allergy to fish or citrus (defined by positive challenge) in the two groups. The authors claimed that food allergy could be delayed but not prevented by dietary elimination in infancy. However lactating mothers did not avoid fish or citrus and possibility of sensitisation through breast milk was not considered.

Some Restriction on Maternal Diet
In 1953 Glaser and Johnstone[159] looked at the possibility of prophylaxis of allergic disease by completely withholding cow's milk from the diet of the infant. Ninety three infants of allergic families were fed breast milk or soybean milk if required. Lactating mothers avoided egg and cheese and limited their daily intake of cow's milk to one pint a day boiled for 10 minutes. Intake of milk and egg was also restricted during pregnancy. Development of allergies was compared to 65 siblings of infants in the experimental group and 175 unrelated children with similar family histories. These children were followed for a maximum of 10 years. Allergic disease developed in 15% of the experimental group, 64% of sibling controls and 52% of unrelated control group ($p<0.01$).

Complete Maternal Avoidance of Allergenic Foods During Pregnancy: Lilja et al[160] studied the effect of maternal diet during the third trimester of pregnancy on the immune response in the foetus. One hundred and sixty three atopic mothers were randomly divided into four groups of diet ranging from no cow's milk and egg to one egg and one litre of cow's milk daily. Although mothers' IgG levels to ovalbumin, ovomucoid and betalactoglobulin were influenced by their diet, total IgE levels and specific IgG to these foods in cord blood were not different in the different groups. Specific IgE to these food proteins was not detected

in the cord blood of any group. The authors concluded that maternal diet during pregnancy is unlikely to sensitise the foetus. As an extension of this study[161] a sub-group of women continue to practice diet during the lactation period. Serum IgE and specific IgG and IgG antibodies were not found to be different in infants from different groups up to the age of 18 months. In a prospective randomised study Falth-Magnusson and Kjellman[162] evaluated the effect of maternal abstention of cow's milk and egg during the third trimester of pregnancy on the development of allergy (as judged by symptoms and signs, skin test and serum IgE) up to 18 months of age. No statistically significant differences were detected between the diet and the non-diet groups. It was concluded that maternal diet during pregnancy does not protect the infant against allergy.

During Pregnancy and Lactation: In a randomised controlled study Chandra et al[163] claimed a beneficial effect of maternal food antigen avoidance during pregnancy and lactation on the development of eczema during infancy. Fifty five infants in an intervention group and 54 in a control group completed the trial. In the intervention group dairy products, egg, fish, beef and peanut were excluded from the mothers' diet during all the pregnancy and lactation if she breast-fed her infant. Eczema was significantly reduced in the intervention group infants only if they were breast fed. Zeiger et al[164] looked at the effect of food antigen avoidance in a prenatally randomised controlled trial of infants of atopic parents. Mothers in the prophylactic group (n=103) completely avoided cow's milk, egg and peanut during the third trimester of pregnancy and lactation. Casein hydrolysate was given to the infant for supplements. Solid foods were not introduced for 6 months and allergenic foods were avoided for more than 12 months. The cumulative prevalence of atopy at 12 months was 16% in the prophylactic group and 27% in the control group ($p=0.03$). The prevalence of eczema, urticaria, food reactions and positive SPT to milk was reduced but the prevalence of rhinitis, asthma and inhalant skin tests was not affected. This was a carefully designed study but suffered from non-compliance in the prophylactic group and the blind assessment was not complete.

During Lactation: Hattevig et al[165] in a randomised, controlled study followed two groups of infants with a family history of atopy up to the age of 18 months. In one group mothers had a diet free of eggs, cow's milk and fish during the first three months postpartum and in the other group mothers had a normal diet. In both groups infants were not given cow's milk for 6 months and egg and fish for 9 months. The incidence of eczema was significantly lower in the maternal diet group during the first 6 months (11% vs. 28%) but not after that age. Other allergic manifestations and positive SPT did not differ in the two groups. Serum levels of IgE and specific IgE, IgG and IgA to egg and cow's milk protein were measured at intervals[166]. There was no significant difference between the two groups. Chandra et al[167] evaluated the effect of various dietary regimes on the development of eczema in infants at risk of allergy. Mothers who planned to breast feed were randomly allocated to dietary restriction (avoiding cow's milk, egg, soyabeans, peanut and fish) or a normal diet. Mothers who planned to bottle feed were randomised to cow's milk, soy milk or casein hydrolysate groups. Eczema was significantly less common and milder by the age of 18 months in breast fed infants with dietary restriction and in infants on casein hydrolysate (22% and 21% respectively) compared to infants on breast feeding without maternal dietary restriction (48%), infants fed soy milk (63%) and cow's milk (70%).

Summary: These studies show a clear benefit of breast-feeding on the development of allergy, especially eczema and food allergy, if the lactating mother eliminates allergenic foods from her diet. Protein hydrolysates may prove an acceptable alternative but the usefulness of soya milk is doubtful. There is a need for randomised trials which attempt to reduce inhalant as well as food allergen exposure to the infant at high risk.

Chapter 3: **INTRODUCTION**

A number of common disorders are associated with high serum levels of IgE. These include allergic asthma, atopic dermatitis, allergic rhinitis, allergic urticaria, and some forms of food allergy. These disorders are major causes of ill health and asthma is responsible for about 2000 deaths in England and Wales every year[168]. They are important causes of absence from school and work-place and make great demands on the medical resources of the community. If it were possible to identify the allergically predisposed infant at, or shortly after, birth and implement measures that should reduce or prevent the development of IgE mediated allergy a significant advance in preventive health would have been made.

According to various estimates at least 20% of the population in the United Kingdom is atopic and the incidence is increasing[169]. The illnesses vary from a mild degree of eczema that clears in infancy or mild rhinitis at the peak of the grass pollen season to life threatening asthma or disfiguring eczema. There is a wide variation in the reported prevalence of various allergic disorders. There may be a number of explanations. Prevalence varies according to the population studied but it also depends on epidemiological methods and diagnostic criteria. Therefore direct comparison between various studies spaced in time is often difficult. In spite of these problems it seems likely that the prevalence of allergic disease particularly extrinsic asthma is increasing in the western world[77]. In fact prevalence could change over a very short period of time. A study of asthma in adults in Papua New Guinea in 1972 gave a prevalence of less than 0.1%. However in 1980 a study of the same population showed a prevalence of more than 7%[170]. Various studies from New Zealand, United Kingdom and other western countries have shown a definite though less dramatic rise in the prevalence of asthma[171].

It is generally agreed that genetic factors are important in the development of allergic disorders. During the last decade several studies claimed that cord IgE could be a good

predictor of allergic disease. Other genetic markers which have been studied included the number and type of lymphocytes in the cord blood and certain HLA haplotypes. These markers, along with family history, could identify infants at high risk of atopy and thus be suitable for preventive measures.

At present there is little that can be done to influence the genetic aspects of allergic disease so attention must be turned to influencing the environment. There is evidence to suggest that environmental factors play a major role in the sensitisation and clinical expression of atopic disease[172]. Maternal factors may play an important role during pregnancy and the first few years of life. Numerous studies have produced conflicting results on the protective effect of breast feeding[173]. As the child grows aeroallergens become more important. In this country house-dust mite is the most common allergen followed by pollens, animal dander and moulds. The most obvious means of preventing allergic disease is to avoid exposure to known allergens. That may be possible if the trigger is a pet that can be removed from the home although dander may linger in the carpet dust for years. It is difficult to achieve with dust-mite and almost impossible with pollen unless the individual moves to another part of the world. It is now possible to reduce the level of major house-dust mite antigen *Der p* I in the carpets or mattress with physical barriers and/or use of chemicals that neutralise the antigen[182,184]. Avoidance of allergen in a sensitised person, when achieved, is usually effective in relieving symptoms (secondary prevention). The question of primary prevention, that is reducing the likelihood of sensitisation in genetically predisposed individual, is not so easy. Obviously one can not avoid exposure to allergens throughout a life-time. Allergen exposure during early infancy may be crucial to the development of subsequent allergic disease[138]. In late 1920s Glaser[115] observed frequent intolerance to egg yolk in infants when the food was introduced at the age of three months and rare intolerance when it was introduced at the age of nine months or later. Glaser and Johnstone[159] in 1953 postulated that an immunological immaturity exists in the early months of life that results in sensitisation in the potentially allergic children. Cow's milk, egg and wheat are important food allergens in the first year of life but

inhalant allergens are equally important. Bjorksten[136] has demonstrated that the maximum risk of birch pollen sensitivity is associated with birth between February and April and the maximum risk for grass pollen allergy is for April and May. The minimum risk for all pollen is birth in July or August. Mite sensitive asthma was found by Warner and Price[174] to be more likely in London in infants born between July and December. A recent study from Poole in England[148] supports a direct correlation between exposure to house-dust mite antigen in infancy and the development of sensitivity to house-dust mite and the symptoms and severity of asthma in later childhood. Maternal smoking, viral infections and exposure to animal dander during infancy may all be important in this context.

When food allergen avoidance was practised by lactating mothers or infants were given hydrolysed milk, reduction in eczema and food allergy was achieved which outlasted the actual period of abstinence[167]. There has been less stress on avoidance of inhalant allergens and adjuvants presumably because it is more difficult to achieve. In any programme of prevention of allergy, attempts should be made to minimise exposure to both food and inhalant allergen during infancy.

The Isle of Wight is situated off the south coast of England in the English Channel. It is about 24 miles from east to west and 12 north to south with a resident population of 130,000. It lends itself to epidemiological survey particularly into the commoner disorders. Two studies were carried out between 1989 and 1991. The first was an epidemiological, observational study to estimate the prevalence of allergic disorders and to identify genetic and environmental risk factors important in their development. The second was a randomized, controlled study to assess the effectiveness of allergen avoidance measures in the prevention of allergy in high risk infants. The results of two years follow-up for the first study and one year follow-up for the second study are presented in this work.

Aims

1. To assess the influence of genetic and environmental factors on the level of total IgE at birth measured by a new, sensitive, enzyme-linked immunoassay EIA-ULTRA.

2. To assess the value of the cord IgE and the family history in predicting the risk of allergic disorders in the first two years of life.

4. To assess the effect of environmental factors on the development of allergic disorders.

5. To assess the value of food and house-dust mite allergen avoidance in the early months of life in high risk infants on the subsequent development of allergic disorders.

Chapter 4: MATERIALS AND METHODS

An epidemiological study was planned to look at genetic and environmental factors influencing the development of allergic manifestations in infancy and early childhood in an entire infant population. Approval for the study was given by the ethical committee of the Isle of Wight Health Authority.

Recruitment

There are about 1300 deliveries in Isle of Wight per year. Apart from 15-20 home deliveries, all are delivered at the maternity department of St. Mary's Hospital, Newport. Fifteen hundred and thirty six infants were born in the Isle of Wight in the fourteen months between 1st January 1989 to 28th February 1990. The Research Fellow visited the maternity department every morning and interviewed mothers (and fathers if available) of the infants born during the previous 24 hour. The nature and purpose of this study was fully explained to the parents. Eleven parents declined to participate. Parents of 1525 infants agreed to participate and signed an informed consent (Appendix I).

Family History: Information was obtained on the history of atopic disorders in the immediate family (Appendix II). A diagnosis of asthma, eczema and allergic rhinitis was accepted when it had been confirmed by a doctor. Additionally, food allergy was considered when there was a history of typical symptoms such as rash or wheezing immediately following ingestion of a recognised food allergen. The infant was regarded as having a positive family history when either parent or sibling suffered from one or more atopic disorder. Allergy in distant relatives was not considered.

Parental Smoking: Information was obtained on the parental smoking habits separately for mother and father. A note was made if any other person was likely to smoke inside the house. Those who smoked regularly (one or more cigarettes a day) were regarded as smokers. Occasional smoking (less than one cigarette per day) was disregarded.

Pets: Information was also obtained on the presence or otherwise of pets in the house. Pets living primarily outside the house or in the garden such as rabbits, were not included as infants are unlikely to be exposed to them.

Maturity: Data on the estimated date of delivery (EDD) and birth weight were taken from the record books of the labour ward. If there was a discrepancy between the EDD stated by the mother and that estimated from the ultrasound examination, the latter was chosen. Week of gestation was calculated from the EDD and the date of birth.

Socio-economic class: Data on the occupation of father and mother were obtained from hospital maternity notes. Unfortunately these entries were incomplete and the data were available for only 890 infants. As these infants were un-selected they were considered a representative sample for analysing the effect of socio-economic class. The infants were classified by the fathers' occupation; the mothers occupation was coded if she was a single parent or if her husband was unemployed and she was employed. The social classes were formed according to the Registrar General's classification.

Class 1: Professionals (doctors, solicitors and directors).
Class 2: Farmers, managers, teachers, engineers etc.
Class 3: Skilled workers such as nurses, technicians.
Class 4: Low-skilled workers such as factory workers.
Class 5 (a): Non-skilled workers such as labourers.
Class 5 (b): Unemployed

To highlight the effect of socio-economic class, part of the analysis was done with classes 1, 2 and 3 grouped together as a higher socio-economic group (professional and skilled workers) and classes 4 and 5 (semi-skilled, Unskilled and unemployed) grouped together as a lower socio-economic group.

Laboratory Methods

Cord Blood Total IgE

At the time of birth midwives routinely take blood from the umblical cord vein for a haematology screen (haemoglobin and blood group). They kindly agreed to take an extra 5ml of blood in a specimen bottle containing dipotassium EDTA. The mid-wives were given detailed instructions on sampling of cord blood to avoid contamination with maternal blood. Briefly, they were asked to identify umbilical vein in the cord, clean the area on the cord with a piece of cotton and aspirate blood with a syringe and needle. The blood was centrifuged at 3000 rpm for 5 minutes. Serum was then separated and stored at -20^{o}C until analysed for total IgE and IgA levels. Cord blood was obtained from 1405 (92.1%) infants.

IgE EIA ULTRA^{R}

Introduction: Total IgE was measured in cord blood using EIA ULTRA (Pharmacia Diagnostics AB, Uppsala, Sweden) kit unmodified, designed to measure IgE between 0.2 and 50 ku/l on 0.1 ml. of serum or plasma. EIA ULTRA is a solid phase enzyme immunoassay using monoclonal anti-human IgE covalently bonded to plastic tubes.

Materials:

Plastic microtitre plates with 96 tubes (wells) coated with monoclonal anti-IgE antibodies (Pharmacia G.B., Milton Keynes, U.K.).

B-galactosidase labelled polyvalent second antibody.

Substrate: 4-nitrophenyl-B-galactoside.

Wash Solution: 0.9% NaCl + Tween 20

Tween 20: Polyoxyethylene (20) Sorbitan mono-laurate (a non-ionic surfactant which blocks vacant binding sites on the microtitre plate).

Stop Solution (Pharmacia G.B., Milton Keynes, U.K.).

'Vari' laboratory Shaker (Dynatech, England)

Ultra-Spek-K Spectrophotometer.

Method: Plastic tubes (wells) labelled with monoclonal anti-IgE antibodies were washed with 'wash solution' and the excess fluid was drained. 0.1 ml of cord serum (or calibration/quality control serum) was added to each tube and the tubes were incubated for 1 hour at room temperature

with continuous shaking (in the laboratory shaker). The tubes were then washed three times with the 'wash solution' and 0.1 ml of enzyme (beta galactosidase) labelled polyvalent anti-IgE was added in each tube and the tubes were incubated for 2 hour at room temperature with continuous shaking. The tubes were again washed three times with 'wash solution'. 0.2 ml of substrate (4-nitrophenyl-B-galactoside) was added and the tubes incubated for 2 hours at 37°C. One ml of stop solution was added to inhibit the enzyme action and absorbance was read in each tube at 420 nano-meter in Ultra-Spek-K.

Each batch was run with 6 calibration sera (from 0.2 to 20 ku/l), 3 quality control sera (high= 18 ku/l, low= 1.0 ku/l and blank= 0 ku/l) and up to 41 patients sera. All samples were measured in duplicate.

Reliability: A standard curve was constructed by plotting the absorbance values of the standards against the IgE concentration on a log-linear scale. Human IgE was diluted in equine serum at 20, 7.5, 2.5, 1.0, 0.5 and 0.2 ku/l (Fig. 4.1).

Reproducibility: Reproducibility of the assay is shown in table 4.1. Six 'zero' (diluent blank) and 6 'low' quality control assays were run for within batch variation. Between batch variation was calculated for 'low' and 'high' quality control sera. Means of best and worst coefficient of variation are given.

Table 4.1. Reproducibility of IgE EIA Ultra.

	Number of assays	Mean (SD) (ku/l)	CV
Within Batch:			
Zero (diluent blank)	6	0.02 (0.001)	5.77%
Low control	6	0.95 (0.08)	8.3%
Between Batch:			
Low control:	36	0.99 (0.12)	12.3 %
High control:	34	6.1 (1.0)	16.8%

SD: Standard Deviation, CV: Coefficient of Variation

Correlation with Phadebas IgE PRIST^R

IgE EIA Ultra correlates well with PRIST (personal communication, Klobas O. Product Manager, Pharmacia Diagnostics AB, Uppsala, Sweden). The correlation was studied with 48 human sera in range 0.5-50 ku/l (Fig. 4.2). Phadebas IgE PRIST was run on five different occasions according to the manufacturer's instructions, each serum in 3-replicates. IgE EIA Ultra was run on six different occasions as described above, each serum in duplicate. Linear regression analysis was performed on the values obtained with the two procedures. Mean values from each method for each sample have been compared and the mean difference between the x-method values and the y-method value was calculated as ln y - ln x and expressed in percent (x 100). The mean difference for all values (delta) was then calculated.

Phadebas IgE PRIST = IgE EIA Ultra x 0.92 + 0.05 r = 0.99

Delta = -1.4% (Phadebas IgE PRIST gives -1.4% lower sera values.

ELISA IgA

Introduction: Sometime during sampling, umbilical cord venous blood (foetal blood) gets contaminated with arterial blood (maternal blood). As the IgE level in maternal blood is much higher than the foetal blood it spuriously raises the level of IgE in the cord blood. To make certain that a high IgE level in the cord blood is foetal in origin cord blood IgA was measured in all samples where cord IgE was more than 0.3 ku/l. Although foetus had the capability of making IgA the amount present in foetal blood is small. Its presence in larger amounts therefore indicates contamination of foetal with maternal blood at the time of sampling. Cord blood IgA was measured using an 'in-house' enzyme immunoassay designed to measure IgA between 4 and 40 mg/l.

Materials:

Nunc polysorp microtitre plates with 96 tubes (wells) (Nunc, Denmark).

Rabbit anti-human IgA alpha chain antibody (code: A-262, Dakoplatts, Denmark).

Alkaline phosphatase conjugated rabbit anti-human IgA (code: D-338, Dakoplatts, Denmark).

Substrate solution: 4-nitrophenyl phosphate 1mg/ml in ethanolamine buffer (BCL, Germany).
[Ethanolamine buffer: Diethyleamine: 97 ml + Magnesium chloride: 0.1 g + sodium azide: 0.2 g + distilled water: 800 ml. 1 M HCl added to give PH of 9.8, made to 1000 ml with distilled water.]
Standard: Beckman's ICS calibrator 1 (Beckman instruments, USA).
Buffer A: NaH_2PO_4 0.0025 M, Na_2HPO_4 0.0075 M, NaCl 0.145 M

Buffer B: NaH_2PO_4 0.0025 M, Na_2HPO_4 0.0075 M, NaCl 0.5 M + Tween 20 1 ml/l.
Stop reagent: 1 M NaOH
'Vari' laboratory Shaker (Dynatech, England)
Plate reader (Dynatech, England).
Method: Rabbit Anti-human IgA was diluted 1:600 in coating buffer A and 0.1 ml was added to each tube on the microtitre plate which was covered and incubated overnight in a wet chamber at $4^{o}C$. The tubes were washed 6 times with buffer B. Cord serum (or calibration/quality control serum) was diluted 1:100 in buffer B and 0.1 ml of this added to each tube in the microtitre plate. The tubes were incubated for 2 hours at room temperature with continuous shaking and then washed 6 times with buffer B. 0.1 ml of alkaline phosphatase conjugated anti-IgA (pre-diluted 1:1000 in buffer B) was added to each tube. The tubes were again incubated at room temperature for 1 hour with continuous shaking and then washed 6 times with buffer B. 0.1 ml of substrate was added to each tube and incubated for 15 minutes at room temperature. 0.1 ml of stop reagent was then added and absorbance was read at 405 nano-meter using Dynatech plate reader.
Each batch was run with 10 calibration sera (from 4 to 40 mg/l), a quality control serum and up to 39 patients sera. All samples were measured in duplicate.
Reliability: A standard curve for ELISA IgA assay was constructed by plotting the absorbance values against known concentrations of IgA (mg/l). Dilutions of Beckman's calibration sera were used at 4, 8, 12, 16, 20, 24, 28, 32, 36 and 40 mg/l (Fig 4.3).
Reproducibility: Reproducibility of the assay is shown in table 4.2. Nine quality control assays were run for within

batch and six for between batch variation. Means of best and worst coefficient of variation are given.

Table 4.2. Reproducibility of ELISA IgA assay.

	Number of assays	Mean (SD)	CV
Within Batch:	9	7.7 mg/l (0.56)	7.3%
Between Batch:	6	6.8 mg/l (0.84)	12.5%

SD: Standard Deviation, CV: Coefficient of Variation

In previous studies a cut-off of IgA to indicate contamination has been taken at 20 or 30 mg/l[13,38]. To find out a level of IgA most indicative of contamination the cord IgA (mean and Mean+2SD) were calculated for various groups of infant with regard to their IgE values (Table 4.3). There was a parallel rise in IgA with rise in IgE values. In samples with cord IgE >0.3 ku/l, the cord IgA rose sharply raising the possibility that a significant number may be contaminated with maternal blood. An un-selected population of infants had mean+2SD of 8.4 mg/l and this was thought to represent approximately the upper limit of normal values. Therefore a cut-off of cord IgA to indicate contamination was taken at 10 mg/l rather than 20mg/l or 30mg/l which may be too high. All cord samples (n=73) with IgA more than 10 mg/l were considered as contaminated by maternal blood. In 13 infants, cord sample was insufficient for IgA assay. Serum IgE levels in these 86 infants were excluded from further analysis.

Table 4.3. Cord blood IgA and IgE (mean and mean+SD) at various cord IgE levels.

Cord IgE (ku/l) levels	IgA (mg/l)		IgE (ku/l)	
	Mean	Mean+2SD	Mean	Mean+2SD

Un-selected (n=116):	5.03	8.4	0.38	2.56
> 0.2 (n=125):	5.62	11.4	0.77	3.85
> 0.3 (n=395):	8.48	26.1	1.05	4.75
> 0.5 (n=202):	10.6	30.9	1.7	6.5
> 1.0 (n=97):	14.9	38.4	2.8	9.1

SD: Standard Deviation

Maternal Blood Total IgE

Blood samples were obtained from mothers within a week of delivery to measure total IgE levels. This was combined with the routine blood sampling by mid-wives on the third or fourth post-natal day to check the haemoglobin level. An extra 5 ml blood was taken in a specimen bottle containing dipotassium EDTA. This was centrifuged at 3000 rpm for 5 minutes. Serum was then separated and stored at -20^{o}C until analysed for total IgE. Maternal blood was available for 1056 mothers.

IgE EIA 100^{R}

Introduction: Total IgE in maternal serum was measured using Pharmacia IgE EIA 100 (Pharmacia Diagnostics AB, Uppsala, Sweden) kit. IGE EIA is a solid phase enzyme immunoassay based on the sandwich technique designed to measure IgE from 2 ku/l to >1000 ku/l.

Materials: (As used for IGE EIA ULTRA assay)

Method: Plastic tubes (wells) labelled with mouse monoclonal anti-IgE antibodies were washed with 2 ml of 'wash solution' and the excess fluid was drained. 0.05 ml of maternal serum (or calibration/quality control serum) and 0.05 ml of enzyme (beta- galactosidase) labelled anti-IgE was added to each tube and the tubes were incubated for 3 hour at room temperature with continuous shaking. The tubes were washed three times with 'wash solution' and 0.2 ml of substrate (4-nitrophenyl-B-galactoside) was added. The tubes were incubated for 30 minutes at 37^{o}C. One ml of stop solution was added and the absorbance was read in each tube at 420 nano-meter in Ultra-Spek-K.

Each batch was run with 6 calibration sera (from 2 to 1000 ku/l), 2 quality control sera (low and high) and up to 42 patients sera. All samples were measured in duplicate.

Reliability: A standard curve was constructed by plotting the absorbance values of the standards against the IgE concentration on a log-linear scale. Human IgE was diluted in horse serum at 2, 5, 20, 100, 400 and 1000 ku/l (Fig. 4.4).

Reproducibility: Reproducibility of the assay is shown in table 4.4. Eight 'low' and eight 'high' quality control assays were run for between batch variation. Means of best and worst coefficient of variation are given.

Table 4.4. Reproducibility of IgE EIA 100.

	Number of assays	Mean (SD) (ku/l)	CV
Between Batch:			
Low control:	8	17.8 (1.34)	7.52 %
High control:	8	80.2 (8.96)	11.17%

SD: Standard Deviation, CV: Coefficient of Variation

Follow-up

First Year Follow-up

Health visitors from the department of community medicine on the Isle of Wight were given detailed information on the study with written information and copies of protocol including criteria for the diagnosis of allergic disorders. Their monthly meeting was attended by the research fellow and consultant to answer any queries.

Health visitors routinely see all infants between 9 months and 1 year of age for developmental assessment. They completed a questionnaire for the study (Appendix III). Height and weight were recorded and information was sought on the duration and type of feeding and any symptoms that might indicate allergic disorder. Information on the presence of smoking in the house was updated. If any other person apart from father and mother smoked this was coded as smoking by the father (non-maternal smoking). Information on the presence of pets in the house was also updated. Health visitors were asked to take into account neighbours' pets which might spend time in the infant's house. If the infant spent significant time in other locations where he or she might be exposed to passive smoking or pets, this too was recorded.

Parents were invited to bring the infant to attend the hospital clinic if one or more symptoms related to allergy were reported by the health visitors. A clinical assessment was made by the research fellow and skin prick tests were performed for all infants attending the clinic.

Clinic Assessment

History: A detailed history, relevant to allergic manifestations in the infant, was taken from the mother (Appendix IV). Information regarding respiratory symptoms was obtained on the presence and severity of any cough or wheezing, whether symptoms were worse at night and any treatment given. Information regarding skin manifestations included the duration, severity and extent of any rash or eczema, whether the lesions were itchy and if any trigger factors had been recognised. Similarly for the upper respiratory tract, symptoms such as recurrent runny or blocked nose, sneezing and irritation of the nose or eyes were recorded. If there was any suggestion of food

intolerance, information was obtained on the suspected food, the type of reaction, number of episodes and any temporal relationship between the ingestion of food and the appearance of symptoms. This information was considered in the light of strict diagnostic criteria (described below) for the diagnosis of allergic disorder in the infant.

Physical Examination: Apart from a general examination, the skin was examined for any rashes or scratch marks, eyes and nose were inspected and the chest was auscultated for any sign of wheezing.

Hospital records were scrutinised on all infants who were hospitalised during the first year for additional information.

Allergic disorders were defined as follows.

Definitions:

Asthma: Three or more separate episodes of cough and wheezing.

Eczema: Chronic or chronically relapsing (lasting more than six weeks), itchy dermatitis with characteristic morphology (areas of scaly, erythematous, pruritic lesions) and distribution (face, post-auricular area, scalp, extensor surface of extremities and flexural creases).

Rhinitis: Recurrent nasal discharge or blockage with attacks of sneezing and itchy eyes.

Food reactions: A history of vomiting, diarrhoea, colic or rash within four hours of ingestion of a particular food on at least two occasions.

Skin Prick Test: All skin prick tests were performed by the same person (research nurse) using standard allergen extract (Soluprick, ALK, Denmark). All infants were tested against Timothy grass pollen, cat dander, house dust mite, egg white and cow's milk. The inhalant allergens were selected as over 90% of atopic adults would react to one or more of these allergens. Egg and milk were included in the standard battery as these are common allergens during infancy. Additional prick tests were carried out if the history suggested them. Positive (histamine: 1 mg/ml) and negative (saline: 0.9%) were used as standard. Mean wheal diameter was taken as half the sum of largest diameter and its perpendicular.

Negative: No reaction or flare only.

One plus (+): Wheal diameter less than 2 mm
Two plus (++): Wheal at least half the size of the histamine reaction and not less than 2 mm.
Three plus (+++): Wheal at least the size of the histamine reaction.
Four plus (++++): Wheal at least twice the size of the histamine reaction.
Two plus and greater tests were considered positive.
On the basis of clinical features and the result of skin tests a conclusion was reached whether the infant had:
No Allergy: Minor symptoms, not fulfilling the diagnostic criteria.
Probable Allergy: One or more disorders as defined above but negative skin prick test.
Definite Allergy: One or more disorders as defined above with positive skin prick test to a relevant antigen.

Second Year Follow-up

One of the research nurse contacted all parents between the ages of 21 months and 24 months. The nurse filled a questionnaire over the telephone which was very similar to the one filled by the health visitors at one year (Appendix III). Information was obtained regarding any allergic symptoms since the first follow-up. For parents who were not available by telephone a simplified version of the same questionnaire was sent by post in a reply paid envelop. If there was no reply from the first questionnaire, a second questionnaire was sent. A list was made every two months for those who did not reply to the second questionnaire. To locate these infants a computer search was done in the Isle of Wight community health centre for possible change of address.

If any symptoms were present the parents were invited to attend the hospital clinic. History, examination and skin tests were carried out as described for the first year follow-up (Appendix IV).

STATISTICAL METHODS

Cord IgE was below the detection limit (0.2 ku/l) in 58.5 % samples. This caused problems with computation. Kimpen et al[13] in a large study used an immunosorbent assay with a detection limit of 0.01 ku/l. We used their displayed data in the range 0.01 to 0.19 ku/l to find the mean IgE for values below 0.2 ku/l which was 0.06. Thus cord IgE values below the detection limit were assigned a value of 0.06 ku/l. This value was used for computation purposes only as this may not be a totally correct figure for our data. The data were analysed using statistical software SPSS (SPSS Inc. Chicago, Illinois). A 5% significance level was used for all comparisons.

Logarithmic Transformation: The distribution of cord and maternal IgE values is positively skewed. In the logarithmic transformation one variable, x is changed to another variable, y, by the equation:

$y=\log x$

We used natural logarithm (log n) for IgE values. The logarithm transformation tends to stabilise variance therefore the scatter is reduced and the distribution becomes more 'normal'. This was the case with maternal IgE values which normalised after log transformation. Cord IgE values were highly skewed and did not normalise after the transformation. Moreover there was concern over the use of a hypothetical value (0.06) assigned to all undetected values. Distribution free methods were therefore used such as comparing the median and percentile for sub-group analysis.

Comparison of two means: Wherever the distribution allowed un-paired and paired 't' tests were used to compare the means of two samples. Un-paired 't' test was used to evaluate the effect of maternal allergy and smoking on maternal IgE (after log transformation). The distribution of *Der p* I values were reasonably 'normal'. Un-paired 't' test was used to compared the mean *Der p* I between prophylactic and control group infants. Paired 't' test was used to compare mean *Der p* I at intervals in the same group.

Linear Regression: When observations are made on two variables (x and y) and the object is to know how y changes

on the average as x assumes different values, the method of linear regression is used. The regression equation is:

$Y_i = a + bx_i$

Linear regression was used to correlate cord IgE values obtained on the same samples using two different methods (EIA ULTRA and PRIST). Cord IgE was also related to maternal IgE values using this method.

Mann-Whitney U test: This test was used to compare two groups of observations from two sample. The observations are ranked together in order of increasing magnitude. The null hypothesis is that the distribution of observations in one sample is exactly the same as the distribution of observation in the other sample. The test is sensitive in situations where two distributions differ in location so that one is greater than the other. Normal distribution is not assumed for this test. Cord IgE values were compared with this test in the two groups depending on the presence or absence of a risk factor.

Kruskal-Wallis one way analysis of variance (ANOVA): This is again a distribution free method which compares the sum of group ranks. Several groups can be tested. The null hypothesis is that the distribution of observation in various groups is the same (that is, all the groups are drawn from the same population). This test was used to evaluate the effect of month of birth on cord IgE.

Chi-square (X^2) test: The influence of risk factors on the development of allergic disorders was assessed with the help of X^2 test, usually in the form of a 2 X 2 table. In this test the observed numbers are tabulated with and without the risk factor to those who did or did not develop the disease. Expected number in each cell is calculated and difference is taken of the expected from the observed values. This gives a X^2 value in each cell which is summed up. From the X^2 table the significance of the difference is noted that is whether the observed values differs significantly from the expected value. Continuity correction (Yates's correction) for X^2 statistics was used throughout although it is more relevant for small samples. When at least one expected frequency was less than 5, the exact test for 2 X 2 table (Fisher's exact test) was used.

Relative Risk: Relative risk was calculated for the group of infants exposed to a possible risk factor compared to those

who were not exposed to that factor. Relative risk can be defined as:

Relative risk = I_E/I_{NE}

that is incidence of the disease in those who are exposed to incidence of the disease in those who are not exposed to the factor in question. This needs to be calculated for the number of subjects in each group. From these proportions standardised normal deviate and confidence intervals are calculated as described by Armitage and Berry[175].

Multivariate Logistic Regression: This method describes the relationship of a dependent variable to a set of explanatory variables (independent variables). The effect of the independent variables on the dependent variable is adjusted (weighted) to the confounding effect of each other. This test is used when the dependent variable is dichotomous such as the presence or absence of a disease. The logit transformation is preferred as the logit is the logarithm of odds, and logit difference are logarithm of odds ratio. The method of maximum likelihood was used to calculate the regression coefficient (log of odds ratio) for each independent variable. The method also gives standard error of the estimated regression coefficient and the confidence interval is then calculated. The influence of genetic and environmental risk factors on the development of various allergic manifestations was analysed using logistic regression.

Sensitivity, Specificity and Predictive value: The term sensitivity of a test is used to characterise the incidence of positive results obtained when a test is applied to patients known to have the disease. Specificity is used to characterise the incidence of negative results obtained when a test is applied to subjects known to be free of disease. The predictive value of a positive test result is defined as the percentage of positive results that correctly identify subjects with disease.

True positive: Subjects with positive test who have the disease.

False Positive: Healthy subjects with positive test.

True negative: Healthy subjects correctly classified by the test.

False negative: Subjects with negative test who have the disease.

Sensitivity: True positive divided by all subjects with disease.
Specificity: True negative divided by all healthy subjects.
Predictive value: True positive divided by those with positive test.
The sensitivity, specificity, and predictive value of cord IgE, at various cut-off levels, to the development of allergic disorders in the first two years of life was calculated.

Sample Size: Sample size would depend on the incidence of the disease, expected difference between the groups, required power to detect a difference and the significance level. For the intervention study size of the sample was calculated to give a reasonable probability to detect a difference at 5% significance level between the two groups. A 50% reduction in allergic disorder was sought in the prophylactic group. This combined with an expected high incidence of allergy in these high risk infants meant that at least 60 infants were required in each group to give 80% power of detecting a difference at 5 % significance level.

Chapter 5: **DEMOGRAPHIC DATA**

Introduction

Out of 1536 infants born during a period of fourteen months from January 1989 to February 1990, 1525 infants entered into the study. Some characteristics of the study population are given:

Twins: There were 20 pairs of twin born during this period.

Prematurity: Information on week of gestation was available on 1497 infants. Seventy nine (5.3%) infants were premature (< 37 week of gestation).

Low Birth Weight: Sixty one (4.1 %) infants had a birth weight of less than 2.5 Kg. Information on birth weight was available on 1494 infants.

Sex: There were 781 (51.3%) males and 741 (48.7%) females (n= 1522).

Family History of Allergy (n= 1518)

One or more first degree relative of 865 infants (57 %) had a history of allergic disorder. Five hundred and eighty nine infants (38.8 %) had single heredity (allergic disorder in one parent or one sibling) and 276 (18.2 %) had dual heredity (allergic disorder in both parents or one parent and one sibling or two siblings). Details of history of allergic disease for mother, father and sibling are outlined in table 5.1.

Table 5.1. History of allergic disorders in the immediate family (n=1518).

Allergic Disorder	Mother	Father	Sibling*
Asthma:	163 (10.74)	150 (9.88)	107 (12.04)
Eczema:	186 (12.30)	99 (6.52)	205 (23.06)
Rhinitis:	306 (20.16)	222 (14.62)	59 (6.64)
Food Reactions:	30 (1.98)	13 (0.86)	71 (7.99)
Any Disorder:	503 (33.14)	371 (24.44)	304 (34.20)

*n= 899. Percentages are given in brackets.

Environmental Factors Information was obtained from all parents on exposure of infant to passive smoking and animal dander during the first year. Data on the occupation of father and mother were obtained from hospital maternity notes. Unfortunately these entries were incomplete and the data were available for only 890 (58.4%) infants. As these infants were un-selected they were considered a representative sample for analysing the effect of socio-economic class.
Table 5.2. shows the distribution of these possible risk factors in the study population.

Table 5.2. Distribution of environmental risk factors in the study population.

	Number	Percent
Parental Smoking (n= 1521)		
None:	816	53.65
Father (alone):	321	21.10
Mother (alone):	103	6.77
Both Parents:	281	18.47
Maternal Smoking:	384	25.25
Paternal Smoking:	602	39.58
Pets in the House (n= 1519)		
No Pets:	614	40.42
Cats:	363	23.90
Dogs:	282	18.56
Cats and Dogs:	186	12.24
Other Pets:	74	4.87
Social Classes (n= 890)		
I:	23	2.58
II:	106	11.91
III:	275	30.90
IV:	371	41.69
V:	46	5.17
Unemployed:	69	7.75

Cord and Maternal IgE:

Cord IgE

Cord blood was analysed for total IgE levels in 1405 (92.1%) infants. All cord samples (n=73) with IgA greater than 10 mg/l were considered as contaminated by maternal blood. In 13 infants, the cord sample was insufficient for IgA assay. Serum IgE levels in these 86 infants were excluded from further analysis. Valid IgE data were available for 1319 infants.

Maternal IgE

Maternal blood was available for 1056 (70.1%) mothers. For administrative reasons, sampling for maternal IgE had to be discontinued before completion of the study (in December 1989).

Follow-up

No information was available for 101 (6.6 %) infants. Thirteen infants died either within the first few days or during the first year and 41 moved from the Island. Completed questionnaires were available for 1370 (90%) infants. One or more symptoms were present in 394 of 1370 (28.8%) infants who were then asked to attend the hospital clinic. Twenty eight infants did not attend the clinic despite two or three reminders. Three hundred and sixty six infants were assessed in the hospital clinic by the research fellow.

The follow-up information was available on a maximum of 1370 infants. The average age of the infant at which health visitors filled the questionnaire was 10 months (range 7-15). Clinic follow-up was generally a month later and consequently the average age was 11 months (range 8-17).

Mean height was 72 Cm (range 56.4-97.7) at 10 months and mean weight 9 Kg (range 5.9-18).

Infant Feeding Details of methods of infant feeding is given in table 5.3.

Table 5.3. Duration of total and exclusive breast feeding (n=1359).

Breast Feeding	Total duration (%)	Exclusive (%)
Initiated:	1044 (76.82)	-
3 months (13 weeks):	536 (39.44)	471 (34.66)
6 months (26 weeks):	363 (26.71)	238 (17.51)
9 months (40 weeks):	160 (11.77)	56 (4.12)

Solids were introduced to 615 infants (46.9 %) by the age of 3 months and 1289 infants (98.2 %) by the age of 6 months. This information was available on 1312 infants.

Prevalence of allergic disorders in infancy

Information was available on 1342 of 1525 infants (88 %) entered into the study at birth. Nine hundred and seventy six infants had no symptoms that might be related to allergy during the first year. Allergic symptoms were thought to be present in 366 infants who were then assessed and skin tested in the hospital clinic. Two hundred and eighty two of these were confirmed to have allergic disorder. Fig. 5.1. shows the prevalence of various allergic disorders with positive (definite) and negative (probable) skin tests. The prevalence varied from 3.2 % for rhinitis to 9.9 % for asthma. Twenty six percent of infants with allergic symptoms had skin tests positive to one or more allergens.

Eighty four infants were intolerant of 94 foods. The most frequent was cow's milk in 61 infants followed by egg (19), wheat (6) and other foods such as orange, fish and peanut (8).

Seventy four infants reacted positively on skin prick test to 101 allergens. The distribution of skin test positivity is shown in fig. 5.2. Egg was the commonest reaction in 32 infants followed by house-dust mite (18), cat (16) and cow's milk (14). Other reaction included one each for budgie, fish, strawberry and peanut.

Chapter 6: **CORD BLOOD TOTAL IgE**

Results

Fig. 6.1 shows the distribution of cord IgE which was highly skewed with 772 0f 1319 (58.5%) values less than the detection limit of the assay [(0.2 ku/l). Frequency distribution (percentiles) and statistical comparison of cord IgE in relation to various heredity and environmental factors is shown in table 6.1. Cord IgE was significantly higher in infants with family history of atopy (p= 0.005) and in the sub-group with single heredity (p= 0.007) compared to those with negative family history. There was a trend for higher values in infants with dual heredity compared to those with no family history but this did not reach statistical significance (p= 0.07). There was no difference in cord IgE between infants with single and dual heredity (p= 0.7). Cord IgE was significantly raised in infants whose mothers had a history of allergic disease (p= 0.0002). Male infants had a significantly higher cord IgE than female (p= 0.005).

Cord IgE values were not different significantly in infants considered premature by gestational age (< 37 weeks) or with low birth weight (< 2.5 kg) (Table 6.1). There was no significant difference in proportions of detectable and high cord IgE for each week of gestation from <35 to >43 weeks (data not shown).

There was no significant effect of month of birth on cord IgE values (p= 0.1; Kruskal-Wallis one way ANOVA test). Percentage of high cord IgE (> 0.5 ku/l) are shown in figure 6.2 for each calendar month. Maternal smoking did not alter cord IgE levels (p= 0.5).

Table 6.1. Cord IgE values (percentiles) in relation to various heredity and environmental factors.

	n =	Percentiles				p (two tailed)
		60	75	90	95	(Mann-Whitney U tes
TOTAL:	1319	0.21	0.29	0.53	0.85	
FAMILY HISTORY:						
Positive:	745	0.22	0.32	0.60	0.95	0.005*
Single heredity:	499	0.22	0.33	0.60	0.96	0.007*
Dual heredity:	246	0.21	0.29	0.61	0.92	0.07*
Negative:	560	<0.2	0.27	0.43	0.61	
MATERNAL ALLERGY:						
Allergic:	424	0.23	0.34	0.60	0.87	0.0002
Non Allergic:	881	<0.2	0.27	0.47	0.85	
SEX:						
Male:	676	0.22	0.32	0.55	1.00	0.005
Female:	638	<0.2	0.27	0.51	0.73	
GESTATIONAL AGE:						
=> 37 Week:	1229	0.21	0.29	0.52	0.82	0.4
< 37 Week:	64	<0.2	0.27	0.95	1.44	
BIRTH WEIGHT:						
=> 2.5 Kg:	1240	0.21	0.29	0.54	0.85	0.3
< 2.5 Kg:	49	<0.2	0.27	0.43	1.14	
MATERNAL SMOKING:						
Smoker:	326	0.21	0.31	0.57	1.02	0.5
Non Smoker:	982	0.20	0.28	0.53	0.82	

* Comparisons made against negative family history.

Cord IgE data were analysed at various cut-off levels (as dependent variables) to obtain the adjusted odds ratios for each factor using logistic regression models (Table 6.2). The baseline for each factor was defined as:

Family History: Negative family history
Sex: Female
Maturity: Premature
Smoking: No maternal smoking

All data were available for 1274 infants. Family history of allergy and sex of the infant had prominent independent influence on the level of cord IgE.

Table 6.2. Risk factors for high cord IgE (ku/l) at various cut-off with logistic regression analysis (n= 1274).

Cord IgE	Risk Factors	Odds Ratio	Confidence Limits
> 0.2:			
	Positive History:	1.33	1.06 - 1.67
	Single Heredity:	1.36	1.06 - 1.74
	Dual Heredity:	1.27	0.93 - 1.73
	Male sex:	1.39	1.11 - 1.74
	Maturity:	1.46	0.84 - 2.55
	Maternal Smoking:	1.13	0.87 - 1.46
> 0.5:			
	Positive History:	1.92	1.31 - 2.82
	Single Heredity:	1.97	1.30 - 2.96
	Dual Heredity:	1.83	1.11 - 3.01
	Male sex:	1.15	0.81 - 1.65
	Maturity:	0.78	0.36 - 1.70
	Maternal Smoking:	1.12	0.75 - 1.67
> 0.8:			
	Positive History:	2.02	1.19 - 3.44
	Single Heredity:	2.12	1.21 - 3.72
	Dual Heredity:	1.83	0.92 - 3.66
	Male sex:	1.40	0.86 - 2.28
	Maturity:	0.44	0.19 - 1.01
	Maternal Smoking:	1.35	0.80 - 2.28
> 1.0:			
	Positive History:	1.66	0.89 - 3.12
	Single Heredity:	1.61	0.82 - 3.18
	Dual Heredity:	1.77	0.80 - 3.94
	Male sex:	2.07	1.11 - 3.87
	Maturity:	0.33	0.13 - 0.82
	Maternal Smoking:	1.51	0.81 - 2.82

The distribution of maternal IgE is shown in Fig. 6.3. There was a weak though statistically significant correlation between cord and maternal IgE ($r = 0.3$, $p < 0.001$). Descriptive statistics of maternal IgE and its relation to cord IgE, maternal allergy and maternal smoking are given in table 6.3. Maternal IgE was significantly related to high cord IgE and maternal allergy but not to maternal smoking.

Table 6.3. Descriptive statistics and comparisons of maternal IgE (ku/l) in relation to Cord IgE, Maternal allergy and Maternal smoking.

Groups	n=	Mean	Median	GM	SD	p*
TOTAL:	1056	127.90	33	34.40	5.74	
CORD IGE:						
High (=>0.5):	96	327.20	66	80.96	7.14	<0.001
Low (<0.5):	897	104.97	30	30.16	5.50	
MATERNAL ALLERGY:						
Allergic:	354	204.61	75.5	67.12	5.85	<0.001
Non Allergic:	698	89.29	23	24.42	5.16	
MATERNAL SMOKING:						
Smoker:	257	127.25	40	39.88	5.13	0.5
Non Smoker:	795	128.37	32	32.69	5.95	

GM: Geometric mean, SD:Standard deviation

*Two tailed p values using un-paired t test.

Discussion

IgE synthesis by the human foetus was noted as early as the 11th week of gestation[6]. If it is assumed that the level of cord IgE is influenced by the maturity of the foetal immune system one might anticipate a relationship with gestational age. Although this was said to be the case in a small study by Michel[31] who could not find IgE in cord blood of premature (<37 week) infants, the detection limit of his assay was 0.5 ku/l. We were unable to demonstrate any effect of prematurity (<37 weeks of gestation) or low birth weight (<2.5 kg). This conclusion agrees with Kimpen[36] who in a large series could not find any effect of gestational age on cord IgE values.

We confirmed previous reports[33-35] of high cord IgE in infants with a family history of atopy. There is a male preponderance in atopic disorders; accordingly cord IgE levels were higher in male infants. The effects of positive family history and sex on cord IgE levels were independent of each other and other variables such as prematurity and maternal smoking.

The effect of positive family history and sex of the infant points towards a genetic influence on the immune system of the foetus. Kjellman[32] did not find a correlation in cord and maternal IgE levels. Although we found a statistically significant correlation between cord and maternal IgE values as a group there was no individual relationship in sera from maternal-cord pairs. It was common to find high IgE levels in mothers with undetectable IgE in cord blood or relatively high cord IgE with low (<10 ku/l) values in the mother. The correlation in cord and maternal IgE therefore represents a hereditary effect on their offspring of allergic mothers with high IgE values rather than passive transfer of IgE through placenta.

IgE in the cord blood is said to be non-specific as specific IgE has rarely been found in cord blood[31,34]. If this is true then environmental factors such as maternal smoking and month of birth should not effect the level of cord IgE. In this series we could not find a seasonal effect or cyclical variation according to month of birth (Fig 6.2), or any effect of maternal smoking on cord IgE values (Table 6.2).

The evidence from this and some of the previous studies suggests that the foetus produces IgE primarily under the influence of genetic factors. Whether it is all non-specific is debatable as it is possible that tests such as the RAST^{R} lack the sensitivity required to detect very small amounts of specific IgE present in the cord blood. With the advent of new more sensitive assays it might be possible to detect specific IgE in cord blood. It would be interesting to measure specific IgE against common allergens in maternal-cord paired sera to clarify the influence of maternal dietary and environmental allergen exposure on the foetal immune system.

We conclude that genetic factors regulate the production of foetal IgE and thereby determine the IgE level at birth. We could not find any significant effect of environmental factors, which would presumably act through the placenta, on foetal IgE production and its level in the cord blood.

Chapter 7: **GENETIC FACTORS**

Results

Two hundred and eighty two (of 1342; 21 %) infants developed one or more allergic disorders. Seventy four of these 282 infants also had skin prick test positive to one or more allergens (definite allergy). There was no difference in cord IgE values between infants with or without allergic disorders (p= 0.76, Mann-Whitney-U test).
Table 7.1 shows percentages of infants (with odds ratios and confidence limits) who were considered to be allergic in relation to various risk factors. Family history of atopy and male sex were significant risk factors. High cord IgE at various cut-off levels was not found to be related to the development of allergy in infancy. Maternal and sibling allergy affected the prevalence of allergic disorder in the infant but paternal allergy did not. Figure 7.1 shows percentages of infants with allergy in relation to allergy in the immediate family members. Sibling allergy with or without parental allergy seems to be the most significant factor. When one or more sibling were affected with allergic disorders (irrespective of allergy in parents), 92 of 269 infants (34.2%) developed allergy compared to 26 of 113 infants (23%) with bi-parental atopy. The difference was highly significant (p=0.001).

Table 7.1: Genetic Risk Factors for the development of allergy in infancy

Groups	Number (%)*	Odds Ratio (95% CL)
FAMILY HISTORY OF ATOPY		
Positive:	203 (26.6)	2.31 (1.73-3.07)
Single Heredity:	131 (25.1)	2.13 (1.56-2.90)
Dual Heredity:	72 (29.9)	2.71 (1.88-3.90)
Negative:	78 (13.6)	
Maternal Allergy:	117 (26.6)	1.62 (1.23-2.12)
No Maternal Allergy:	164 (18.3)	
Paternal Allergy:	77 (23.4)	1.20 (0.89-1.62)
No Paternal Allergy:	203 (20.3)	
Sibling Allergy:	92 (34.2)	2.23 (1.59-3.11)
No Sibling Allergy:	98 (18.9)	
LEVEL OF CORD IGE		
0.2 ku/l		
=> 0.2:	93 (19.7)	0.91 (0.68-1.22)
< 0.2:	145 (21.1)	
0.5 ku/l		
=> 0.5:	26 (21.3)	1.05 (0.67-1.67)
< 0.5:	212 (20.4)	
0.8 ku/l		
=> 0.8:	11 (18.0)	0.85 (0.43-1.65)
< 0.8:	227 (20.7)	
1.0 ku/l		
> 1.0:	9 (22.0)	1.09 (0.51-2.32)
< 1.0:	229 (20.5)	
SEX OF THE INFANT		
Male:	164 (24.1)	1.46 (1.12-1.90)
Female:	118 (17.9)	

* Number (%) with one or more allergic manifestations
CL: Confidence Limits

Total and definite allergy and various allergic disorders were analysed (as dependent variables) to obtain the adjusted odds ratios for each factor using logistic regression models (Table 7.2). The baseline for each factor was defined as:

Family History: Negative family history
Cord IgE: Low IgE (< 0.5 ku/l)
Sex: Female

All data were available for 1133 infants. Family history of allergy had a prominent independent influence on the development of allergic disorders in infancy. Male sex was a risk factor for asthma but not for other allergic disorders.

Table 7.2: Adjusted odds ratio (95 % confidence limits) for various risk factors to the development of allergic disorders (n= 1133).

	Positive FH	High cord IgE	Male Sex
Total Atopy:	2.13 (1.56-2.91)	0.94 (0.59-1.50)	1.30 (0.97-1.75)
Definite Atopy:	1.32 (0.77-2.28)	1.22 (0.56-2.66)	1.30 (0.77-2.20)
Asthma:	1.17 (0.78-1.77)	0.80 (0.40-1.60)	1.67 (1.11-2.51)
Eczema:	2.66 (1.68-4.21)	1.03 (0.55-1.91)	1.29 (0.86-1.92)
Rhinitis:	2.47 (1.19-5.11)	0.38 (0.09-1.59)	1.10 (0.58-2.07)
Food Reactions:	1.92 (1.11-3.32)	1.83 (0.94-3.54)	0.78 (0.47-1.29)

FH: Family history, High cord IgE: >0.5 ku/l

Cord IgE is said to predict the development of allergy. The sensitivity, specificity and predictive value of cord IgE at various cut-off was estimated and compared with positive family history (Table 7.3). When detectable IgE (0.2 ku/l) was considered as high IgE, the sensitivity and specificity were both low and predictive value was only 19 %. As the cut-off is increased specificity improves but sensitivity becomes too low to be of any value. The sensitivity of cord IgE (cut-off: 0.5 ku/l) and family history of atopy was compared for total and definite allergy and various allergic disorders (Fig. 7.2). All were highly significant in favour of family history.

Table 7.3: Sensitivity, Specificity and predictive value of cord IgE and positive family history in relation to development of allergy in infancy.

	0.2	0.5	0.8	1.0	Pos.FH
Sensitivity:	39.1	10.9	4.6	3.8	72.2
Specificity:	58.8	89.6	94.6	96.5	47.0
Pos. Pred.Value:	19.7	21.3	18.0	22.0	26.6
Neg. Pred.Value:	78.9	79.6	79.3	79.5	86.4

IgE: ku/l, +FH: Positive family history of atopy
(All values are percentages)

In our study cord IgA value to indicate contamination by maternal blood was taken at 10 mg/l. Some of the previous studies have taken a higher level of IgA (20mg/l and 30mg/l). This may affect the predictive capacity of cord IgE by including samples with high cord IgA (and usually high IgE). Data were analysed with various cut-off for IgA but little improvement in predictive capacity was observed (Table 7.4).

Table 7.4: Predictive capacity of cord IgE for the development of allergy at various cut-off of cord IgA and IgE.

IgA cut-off:	20 mg/l		30 mg/l	
IgE cut-off:	0.5	1.0	0.5	1.0
Sensitivity:	15.2	7.2	16.9	9.2
Specificity:	87.7	95.5	85.6	93.7
Pos. Pred.Value:	24.7	30.0	23.9	28.2
Neg. Pred.Value:	79.6	79.5	79.3	79.3

(All values are percentages)

When family history was positive 25.5% infants developed allergy. This did not improve for infants in the sub-group where IgE (cut-off: 0.5 ku/l) was also high (26%). Only 9 % infants developed allergy where cord IgE was high but family history was negative.

Discussion

This study shows that cord IgE is not a useful screening test, primarily because of its poor sensitivity. Most of the previous studies were not population based or infants were pre-selected[31,32,34,35,47,51]. To assess the value of a screening test a large un-selected population is needed as in the study of Croner et al[33]. The results also depend on the method of measuring IgE, cut-off limit, definition of atopic disorder and length of follow up.

A history of atopy in the immediate family was found in 763 (57%) infants. This is somewhat higher than previous reports, 31 %[33], 44 %[34] and 48 %[51]. Care was taken to avoid overestimation and diagnosis of atopic disease in parents and sibling was only accepted if it had been diagnosed and treated by a doctor unless the history was very obvious, for example hay fever symptoms during grass pollen season.

Disagreement on precise definition of allergic disorder in infancy makes direct comparison between studies difficult. None of the previous studies has used identical criteria[31-35,47,51]. Most have distinguished between probable and obvious atopy[31-34,44,51]. In this study if the clinical assessment suggested allergy this was called probable allergy and when the assessment was backed by relevant skin prick tests this was called definite allergy. Standard criteria were used for atopic eczema. Although not all infants who have recurrent wheezing episodes during infancy develop asthma in later life, in the absence of an objective parameter this remains the most commonly used criterion[31-35]. Strict criteria for rhinitis and food reactions were employed to avoid over diagnosis.

Total accumulated incidence of allergic manifestations in infancy was 21 %. This is similar to other studies[34,51] but less than the large study by Croner et al[33]. Only 122 (10.5%) infants had high cord IgE (>0.5 ku/l). 26 of them became allergic (positive predictive value 21%). The predictive value depends on prevalence of the disease. When disease prevalence is 21%, a predictive value for positive result of the same percentage is no better than simple chance occurrence. Only 26 of 238 infants with allergy had high cord IgE (Sensitivity 10.9%). If this test is used for screening not only a majority (89%) of infants who show

signs of allergic disorders during first year will be missed (false negative), but also 79% of infants indicated to be at risk by this test will not develop allergic symptoms (false positive). When results were analysed using stricter criteria of definite allergy, the sensitivity improved to 13%, and specificity remained at 89% but positive predictive value was further reduced to 7% as the disease prevalence was only 5%. Results were also analysed using various cut-off limits from 0.2 ku/l to 1.0 ku/l. Obviously as the cut-off limit is lowered sensitivity improves slightly but specificity becomes unacceptably low (Table 7.3). Even at a detection limit of 0.2 ku/l the sensitivity is no higher than 39%, with specificity of 59%. With a cut-off of 1.0 ku/l the sensitivity was a mere 3.8% (specificity 96.5%).

203 of 763 (26.6%) infants with positive family history developed allergy compared to 78 of 574 (13.6%) with no family history ($p<0.001$). This compares well with previously reported figures[34,51]. The risk of developing allergy doubled with atopy in the family and was highest when one or more siblings were affected.

In line with previous studies male sex was found to be significant risk factor for asthma[74-77]. Positive family history and male sex were independent risk factors for various allergic disorders (Table 7.2). High cord IgE (>0.5 Ku/l) was not found to be significant for any of the allergic disorders.

This study shows that cord serum IgE is an insensitive method of detecting infants at risk of atopy. History of atopy in the immediate family is a superior indicator and little is added by knowledge of cord IgE.

Results

Information on the development of allergic disorders was available on 1342 infants at one year. Table 8.1 gives the percentage of infants with allergic manifestations according to various risk factors. Breast feeding offered some protection against asthma ($p < 0.01$). Asthma was more common in infants whose mothers smoked ($p < 0.001$). Infants born in the summer months (June, July and August) suffered from asthma ($p < 0.01$), rhinitis ($p < 0.05$) and definite allergy ($P < 0.05$) more frequently than those born in winter months (December, January and February). A higher proportion of low birth weight infants ($p < 0.05$) and those belonging to the lower S-E group suffered from asthma ($p < 0.01$) but not other allergic disorders. No difference in any allergic disorder could be shown between children with or without pets (cats and/or dogs) at one year.

Table 8.1: Effect of various environmental factors on the development of allergic disorders in infancy.

	Total	Definite Asthma	Eczema	Rhinitis	Food Int.
Method of Feeding					
Formula: (n=861)	21.7	5.6	11.7**	9.5	3.0	6.0
Breast: (n=465)	19.6	5.6	6.0	10.8	3.4	6.9
Maternal Smoking					
Smoking: (n=315)	26.7**	5.4	18.1***	10.5	3.5	8.3
No Smoking: (n=1022)	19.3	5.6	7.3	9.7	3.1	5.7
Season of Birth					
Summer: (n=307)	25.4	6.8*	14.7**	12.0	4.6*	7.8
Winter: (n=426)	17.8	2.8	6.8	9.4	1.9	4.5
Birth Weight					
Low: (n=46)	26.1	8.7	19.6*	4.4	2.2	8.7
Normal: (n=1260)	21.0	5.4	9.7	10.2	3.3	6.0
S-E Group					
Lower: (n=433)	22.4	5.8	13.4**	9.5	3.7	7.6
Higher: (n=353)	20.1	8.2	6.5	11.1	3.7	6.8
Cats and/or Dogs					
Yes: (n=758)	20.5	5.0	9.2	9.0	2.8	5.9
No: (n=579)	21.8	6.2	10.7	11.1	3.8	6.7

*p<0.05, **p<0.01, ***p<0.001 (X^2 test with Yates's correction)
S-E: Socio-Economic, Food Int.: Food Intolerance
All figures are percentages

366 infants were referred to the clinic by the health visitors and all were skin tested. The percentage of infants with skin reactivity at one year to common inhalant and food allergens for each risk factor is given in table 8.2. Reaction to house-dust mite (HDM) occurred significantly more often ($p < 0.05$) in infants who were formula fed and in infants exposed to maternal smoking. A significantly higher proportion of infants whose mothers did not smoke showed positive reaction to egg ($p < 0.05$). Infants born in spring and summer were more likely ($p < 0.05$) to develop skin sensitivity to grass pollen than those born in autumn and winter. Low birth weight infants developed skin test reactivity to dog dander more often than normal birth weight infants ($p < 0.01$).

Table 8.2: Effect of environmental factors on skin test reactivity to common inhalant and food allergens.

	GP	HDM	Cat	Dog	Egg	Milk	Others
Method of Feeding							
Formula: (n=242)	1.2	7.0*	4.6	1.2	8.3	3.3	2.1
Breast: (n=120)	2.5	0.8	4.2	1.7	10.0	5.0	3.3
Maternal Smoking							
Smoking: (n=99)	1.0	9.1*	6.1	2.0	3.0	2.0	2.0
No Smoking: (n=265)	1.9	3.4	3.8	1.1	10.9*	4.5	2.6
Season of Birth							
Summer: (n=105)	1.9*	4.8	4.8	1.0	6.7	3.8	3.8
Autumn: (n=82)	0	4.9	6.1	0	11.0	7.3	2.4
Spring: (n=82)	4.9*	6.1	2.4	3.7	12.2	2.4	2.4
Winter: (n=97)	0	4.1	4.1	1.0	6.2	2.1	1.0
Birth Weight							
Low: (n=15)	0	6.7	0	13.3*	6.7	0	0
Normal: (n=1260)	1.8	4.7	4.7	0.9	8.8	3.8	2.6
S-E Group							
Lower: (n=131)	0.8	5.3	2.3	1.5	8.4	1.5	3.8
Higher: (n=95)	5.3	5.3	9.5	1.1	12.6	6.3	4.2
Cats and/or Dogs							
Yes: (n=193)	0.5	4.2	6.2	1.6	7.8	4.2	2.6
No: (n=171)	2.9	5.9	2.3	1.2	9.9	3.5	2.3

GP: Grass Pollen, HDM: House-dust mite, S-E: Socio-Economic
'Others' include Tree pollen, Budgie, Wheat, Peanut, Strawberry and Fish. All figures are percentages.

Some of these environmental factors are inter-related. There were highly significant differences in smoking habit and socio-economic class between groups of mothers who chose to breast feed and those who formula fed (Table 8.3). Moreover in the higher S-E group only 14% mothers smoked compared to 33% in the lower S-E group ($p< 0.001$). The prevalence of maternal asthma was similar in various sub-groups (Table 8.3). Unfortunately entries of parent's occupation were incomplete in the maternity notes and the data on social class were available for only 890 infants. However infants where social class was known were compared to those where it was not known for various confounding variables (Table 8.3). There were no significant differences.

Table 8.3: The relationship of various confounding variables to method of feeding, maternal asthma and known/unknown social class.

	Formula vs. Breast	Mat. Asthma vs. No Mat. Asthma	S.C. Known vs. S.C. Unknown
Male Sex:	436:251 (49.1:53.3)	82:691 (50.6:51.3)	438:343 (49.8:53.4)
Pos. F.H.:	510:268 (57.8:57.0)	_	495:370 (55.9:58.5)
Mat. Asthma:	98:49 (11.1:10.4)	_	106:57 (12.0:9.0)
Mat. Smoking:	276*:57 (31.2:12.1)	41:343 (25.2:25.4)	215:169 (24.3:26.6)
Lower S-E Group:	301*:135 (60.2:45.8)	60:424 (56.6:54.4)	_
Formula Feeding:	-	_	500:388 (62.9:68.8)

S.C.: Social Class, Pos. F.H.: Positive Family History of atopy, Mat.: Maternal, S-E: Socio-economic
Percentages are given in brackets

Because of the confounding variables the net effect of the factors may not be as is outlined in tables 8.2 and 8.3. The logistic regression model was used to obtain the adjusted odds ratios for each factor. The baseline for each factor was defined as:

Method of Feeding:	Breast feeding
Smoking:	No maternal smoking
S-E Groups:	Higher S-E group
Birth Weight:	Low birth weight
Season of Birth:	Winter births
Pets:	No cats or dogs
Maternal Asthma:	Mother not asthmatic
Sex:	Female
Skin Prick Test:	Negative

The predominant effect of environmental factors was on the development of asthmatic symptoms. Logistic regression was performed with asthma as the dependent variable (Table 8.4).

Table 8.4: Effect of various risk factors on the development of asthma in infancy.

Risk Factors	(First Analysis n=1320) O.R. (95% C.L.)	(Second Analysis n=223) O.R. (95% C.L.)
Formula Feeding:	1.66 (1.06-2.61)*	1.82 (0.92-2.52)
Maternal Smoking:	2.53 (1.71-3.75)***	2.63 (1.35-5.11)**
Season of Birth:*		
Spring:	1.42 (0.82-2.44)	0.71 (0.25-2.01)
Autumn:	1.40 (0.80-2.46)	0.77 (0.27-2.17)
Summer:	2.12 (1.28-3.51)**	1.44 (0.56-3.68)
Cats & Dogs:	0.80 (0.55-1.16)	1.30 (0.70-2.41)
Maternal Asthma:	2.30 (1.40-3.76)***	1.36 (0.62-2.99)
Birth Weight:	2.27 (1.11-4.83)*	2.14 (0.51-8.90)
Lower S-E Group:	_	2.27 (1.17-4.41)*
Positive SPT:	_	2.98 (1.46-6.05)**

In the first analysis maximum number (1320) of infants were included (S-E group and skin test sensitivity were not tested as data were not available for all infants). Formula feeding, maternal smoking, summer births, low birth weight and maternal asthma were significant risk factors. When paternal smoking was included as a separate factor it was not significant (OR=0.91, CL=0.4-1.86) and had no effect on the significance of other factors.

Twenty four percent of infants with asthma had skin test positive to one or more allergens. Skin test sensitivity might have an effect on the relationship of asthma to various risk factors. To adjust for this, skin test sensitivity was added to the next regression model alongwith S-E group to include all factors of interest (Table 8.4). All data were available on 223 infants. Lower S-E group ($p= 0.02$) and positive skin test ($p< 0.005$) both proved to be significant risk factors for asthma. Maternal smoking remains highly significant but the significance of formula feeding, low birth weight and summer births were reduced.

The influence of risk factors on the prevalence of total allergy (infants with one or more allergic disorder) and definite allergy (sub-group with positive skin tests to one or more allergens) was analysed using logistic regression (Table 8.5). With regard to total allergy, maternal smoking, summer births and maternal asthma were the significant risk factors. For infants with definite allergy season of birth and maternal asthma were the only significant risk factors.

Table 8.5: Effect of various risk factors on the development of total and definite allergy in infancy (n=1320).

Risk Factors	Total Allergy O.R. (95% C.L.)	Definite Allergy O.R. (95% C.L.)
Formula Feeding:	1.04 (0.78-1.39)	1.00 (0.60-1.67)
Maternal Smoking:	1.50 (1.10-2.04)**	0.98 (0.55-1.75)
Season of Birth:		
Spring:	1.13 (0.78-1.65)	2.33 (1.13-4.83)*
Autumn:	1.24 (0.84-1.81)	2.63 (1.26-5.48)**
Summer:	1.45 (1.01-2.09)*	2.47 (1.19-5.11)**
Maternal Asthma:	2.19 (1.49-3.20)***	2.01 (1.09-3.72)*

Other variables evaluated but non-significant were S-E group, low birth weight and pets.

With positive skin tests to HDM, cat, dog and grass pollen as the dependent variables logistic regression was performed to test the significance of method of feeding, maternal smoking, S-E group, season of birth, low birth weight and presence of cat or dog in 366 infants where skin test were done. For skin test sensitivity to house-dust mite, formula feeding was the significant risk factor (OR=7.92, CL=1.02-61.39, p=0.05) but maternal smoking failed to reach statistical significance (OR=2.39, CL=0.89-6.39, p=0.08). Low birth weight infants were more likely to be sensitised to dog dander (OR=17.95, CL=2.67-120.63, p=0.003). There was a significant risk for infants with cats in the house to have positive skin test to cat dander (OR=2.81, CL=1.21-8.95, p=0.01).

Discussion

Allergic symptoms are extremely common during the first year of life. They do not always represent IgE mediated, type I allergy. Adverse food reactions, infantile eczema and rhinitis could all have different immunological mechanisms. A sub-group of infants with definite allergy was defined whose symptoms were backed by relevant positive skin prick tests. In infancy bronchial hyper-reactivity reveals itself by recurrent cough and wheeze, usually following a viral respiratory tract infection. It has been termed wheezy bronchitis, infantile wheezing, pseudoasthma or asthma. There is controversy in the literature as to the nature and outcome of recurrent wheezing in infancy[176]. A longitudinal study by Williams and McNicol[75] concluded that wheezing in response to viral infections and asthma have the same underlying basic disorder. Park et al[177] found that 87% of infants who wheezed during the first year did not have asthma at the age of 10 although they were more likely to have asthma with increasing number of wheezy attacks during the first year. We preferred to use the term asthma for wheezy infants as in this group there was strong genetic component (significant relation to maternal asthma and male sex). This is not to imply that most of these infants will continue to wheeze or they are necessarily atopic. Indeed evidence for atopy (positive skin prick test) was found in only 24% of infants with recurrent wheezing during their first year.

The environmental factors studied in this cohort were method of feeding, passive smoking, social class, season of birth and exposure to pets. The first three risk factors are closely related and any individual effect would have to be adjusted for these and other possible variables such as maternal asthma. None of the environmental factors had any significant effect on eczema, rhinitis or food intolerance (Table 8.1). The effect on "total allergy" was primarily due to the effect on prevalence of asthma.

In line with several previous studies[63,66,178] we could not detect an association between mode of feeding and incidence of total allergy. Some studies have found a protective effect of breast feeding on wheezing[52,89]. As there are so many inter-related confounding variables these must be taken

into account when assessing the effect of method of feeding on the development of allergic disorders. In this study there seemed to be a protective effect of breast feeding against wheezing episodes but when adjusted for other variables this effect became less significant. It is said that breast feeding provides immunological protection against infections with transfer of IgA and IgG through breast milk. The effect of breast feeding was probably a result of protection against viral infections which usually trigger wheezing episodes in the first year of life. There was an association between sensitivity to house-dust mite and formula feeding. No firm conclusions could be drawn on the relationship to skin test sensitivity as the only infants who were skin tested were those who showed symptoms and who had been referred to the clinic by health visitors. This possible association needs confirmation.

Passive smoking is known to increase bronchial responsiveness and symptoms in children with asthma[132]. Parental smoking, particularly maternal smoking, increases the risk of respiratory illness during the first year of life[126,127,130]. We found a significant effect of maternal smoking on wheezing in infants. Paternal smoking was not a risk factor presumably because the father does not usually smoke in the vicinity of the infant for sufficiently long periods. It is unlikely that parents misled us about their smoking habits as information on smoking, as well as the presence of pets, was checked by the health visitors who visit homes frequently after the birth of the baby. Recently Murray and Morrison[133] reported that children with atopic dermatitis are at a greater risk of developing asthma if the mother smokes. It was thought that atopic dermatitis signified a predisposition to atopy while smoking acts as an adjuvant factor. In the present study infants exposed to maternal smoking did show a non-significant trend to develop sensitivity to HDM. The effect of maternal smoking was independent of hereditary factors such as maternal asthma or sex of the infant and skin test sensitivity of the infant. Maternal smoking did not affect the development of definite allergy in infants. The evidence suggest that exposure to passive smoking in early life increases bronchial hyper-reactivity and may contribute to sensitisation to some allergens.

The data for social classes 1, 2 and 3 were combined as the number for classes 1 and 2 was small and they behaved in a similar manner. Classes 4 and 5 were also combined for the same reason. There was an independent effect of social class on asthmatic symptoms. It was probably an indirect effect through bad housing conditions, such as dampness and crowding with increase risk of transmissible respiratory infections. Other studies have found a relationship between social class and wheezing during infancy[52,121].

There was a significant effect of season of birth on asthmatic symptoms. Infants born in the summer developed asthma and definite allergy more often than those born in winter months. All six infants with positive SPT to grass pollen were born in spring or summer months. The numbers are too small to make any firm conclusions but the effect of exposure to birch pollen in early life on subsequent allergy to birch pollen was suggested by Bjorksten et al[136]. Morrison-Smith and Springett[140] found a higher risk of asthma and positive skin reactions to house-dust mite for infants born in summer months. The cause of this relationship remains unclear. Virus infections are more common in winter so this can not explain an increased incidence of respiratory symptoms. Further studies are needed to clarify the association between month of birth and allergic sensitisation.

Environmental factors play an important role in the prevalence of asthma but not other allergic disorders in the first year of life. Exposure to some environmental factors in early life may be important in increasing the risk of sensitisation. Unfortunately there seems to be a clustering of avoidable risk factors. Mothers who formula fed their babies tend to smoke and more often belong to lower socio-economic groups. It is important to educate mothers especially those belonging to lower socio-economic groups to breast feed and avoid smoking.

Chapter 9: **TWO YEAR FOLLOW-UP**

Results

At the second year follow-up information was available on 1261 (92%) of the 1370 infants originally studied at one year . One infant died during the second year, 8 parents refused follow-up and 24 moved from the Island. Questionnaires were completed by parents of 1228 infants. Four hundred and ninety parents indicated that there might be some problem in the child related to allergy. These parents were asked to attend a hospital clinic. Fifty four children did not attend the clinic despite two or three reminders. Four hundred and thirty six children were assessed and skin tested in the hospital clinic. Two hundred and seventy five of these were confirmed to have allergic disorder. Fig. 9.1 shows the prevalence of various allergic disorders with positive (definite allergy) and negative skin tests (probable allergy). The prevalence varied from 3.2% for rhinitis to 10.9% for asthma. Fifty three children were intolerant of 79 foods. The most frequent was cow's milk in 21 children, followed by egg (19), wheat (6) and other foods such as orange, fish, strawberry and peanut (15).
Table 9.1 shows percentages of children with allergic disorders in relation to various hereditary and environmental factors.
Sixty children (of 436 tested) reacted positively on skin prick test (SPT) to 89 allergens. House-dust mite was the commonest reaction in 37 children followed by egg (17), cat (13), grass-pollen (7), dog (5) and Other reaction (10) which included cow's milk, budgerigar, fish, strawberry and peanut. Even at this early age inhalant allergens were more than twice as frequent (64 vs. 25) as food allergens. Table 9.2 outlines percentages of positive SPT to inhalant and food allergens with respect to various possible risk factors. Logistic regression was used to obtain independent effect of the factors on skin test reactivity to these allergens.

Table 9.1: Genetic and environmental factors and development of allergic disorders at two year (n=1174).

	Total n=275	Definite n=60	Asthma n=128	Eczema n=126	Rhinitis n=38	Food Int. n=53
Family History						
Positive:(n=661)	27.7***	5.5	12.6*	14.4***	4.1	5.3
Negative:(n=511)	17.8	4.7	8.6	6.1	2.2	3.5
Cord Ige (ku/l)						
> 0.5:(n=109)	25.7	8.3	9.2	16.5*	1.8	5.5
< 0.5:(n=911)	22.9	4.5	10.9	9.9	3.5	4.4
Sex						
Male:(n=594)	24.8	6.1	13.0*	9.9	2.7	5.1
Female:(n=580)	22.1	4.1	8.8	11.6	3.8	4.0
Birth Weight (kg)						
< 2.5:(n=38)	36.8*	15.8**	26.3**	7.9	2.6	7.9
> 2.5:(n=1105)	22.9	4.8	10.5	10.7	3.4	4.4
Method of Feeding						
Formula:(n=733)	23.7	5.7	12.1	10.0	3.1	4.5
Breast:(n=436)	22.9	4.1	9.0	12.2	3.2	4.6
Maternal Smoking						
Smoking:(n=257)	27.6	4.7	17.9***	8.2	3.1	4.7
No Smoking:(n=915)	22.2	5.3	8.9	11.5	3.3	4.5
Season of Birth						
Summer:(n=273)	23.8	4.0	12.1*	8.8	3.7	5.1
Autumn:(n=262)	25.2	6.1	12.6*	12.2	5.0	3.8
Spring:(n=272)	25.0	4.4	12.9*	10.3	2.6	6.3
Winter:(n=367)	20.7	5.7	7.4	11.4	2.2	3.3
S-E Group						
Lower:(n=367)	25.6	6.3	14.2*	9.3	5.5	5.5
Higher:(n=318)	23.6	4.1	8.5	12.6	2.5	5.4
Cats and/or Dogs						
Yes:(n=700)	22.4	4.1	11.0	10.9	3.1	2.7
No:(n=474)	24.9	6.5	10.8	10.6	3.4	7.2***

*p<0.05, **p<0.01, ***p<0.001 (X^2 test with Yates's correction)
All figures are percentages.

Table 9.2: Genetic and environmental factors and skin test reactivity to common inhalant and food allergens.

	GP (n=7)	HDM (n=37)	Cat (n=13)	Dog (n=5)	Egg (n=17)	Others (n=10)
Family History						
Positive: (n=271)	1.5	10.0	3.0	1.5	3.3	2.2
Negative: (n=164)	1.8	6.1	3.1	0.6	4.9	2.4
Cord Ige (ku/l)						
> 0.5: (n=43)	0	9.3	2.3	0	14.0**	2.3
< 0.5: (n=334)	1.8	7.5	3.3	1.2	2.4	2.4
Sex						
Male: (n=234)	2.1	8.1	3.4	0.9	4.7	3.0
Female: (n=202)	1.0	8.9	2.5	1.5	3.0	1.5
Birth Weight (kg)						
< 2.5: (n=19)	5.3	21.1*	0	5.3	0	0
> 2.5: (n=401)	1.5	8.0	3.2	0.8	4.0	2.2
Method of Feeding						
Formula: (n=279)	2.2	9.0	3.2	1.4	4.3	2.5
Breast: (n=156)	0.6	7.7	2.6	0.6	3.2	1.9
Maternal Smoking						
Smoking: (n=99)	0	6.1	4.0	3.0	4.0	4.0
No Smoking: (n=336)	2.1	9.2	2.7	0.6	3.9	1.8
Season of Birth						
Summer: (n=96)	2.1	9.4	1.0	0	0	1.0
Autumn: (n=103)	1.0	6.8	4.9	1.9	9.7	2.9
Spring: (n=107)	1.9	5.6	2.8	0.9	3.7	2.8
Winter: (n=130)	1.5	11.5	3.1	1.5	2.3	2.3
S-E Group						
Lower: (n=142)	2.1	9.2	2.1	2.1	5.6	4.2
Higher: (n=124)	0.8	6.5	3.2	0	3.2	0.8
Cats and/or Dogs						
Yes: (n=258)	0.8	7.8	3.1	1.6	2.7	1.9
No: (n=178)	2.8	9.6	2.8	0.6	5.6	2.8

GP=Grass Pollen, HDM=House-dust mite
'Others' include Tree pollen, Budgie, Cow's milk, Wheat, Peanut, Strawberry and Fish. All figures are percentages

Some of these risk factors are inter-related. Cord IgE levels are higher in male infants and those with a family history of atopy. Formula fed infants more often belong to a lower socio-economic group and more frequently exposed to passive smoking than are breast fed infants. To obtain independent effect of these factors multivariate logistic regression analysis was used which gives adjusted odds ratios for each factor. The baseline for each factor was defined as:

Family History:	Negative family history
Cord IgE:	Low IgE (< 0.5 ku/l)
Sex:	Female
Birth Weight:	Normal birth weight (> 2.5 kg)
Method of Feeding:	Breast feeding
Smoking:	No maternal smoking
Season of Birth:	Winter births
S-E Groups:	Higher S-E group
Pets:	No cats or dogs

Total and definite allergy and various allergic disorders were analysed (as dependent variables) to all risk factors of interest (as independent variables). Odds ratio and 95% confidence limits for total allergy and definite allergy are shown in table 9.3 and for various allergic disorders in table 9.4.

Family History: Positive family history was a significant risk factor for total allergy, asthma, eczema and rhinitis (Tables 9.3 & 9.4). Data were analysed separately for maternal, paternal and sibling allergy. Maternal and sibling allergy were significant for total allergy and eczema. Paternal allergy was not a significant risk for any of the allergic disorders.

Cord IgE: A significantly higher percentage of children with high cord IgE (>0.5 ku/l) developed eczema (Table 9.1) but when adjusted for other variables this became non-significant. The only other effect of high cord IgE was seen on skin test reactivity to egg (Table 9.2). The adjusted odds ratio (CL) were 6.51 (1.8-22.9).

Cord IgE has been claimed to predict the development of allergy. The sensitivity, specificity and predictive value

of cord IgE at various levels of cut-off was estimated (Table 9.5). When detectable IgE (0.2 ku/l) was considered as high IgE, the sensitivity and specificity were both low and predictive value was only 23%. As the cut-off level is increased specificity improves but sensitivity becomes too low to be of any value.

Table 9.5: Sensitivity, Specificity and Predictive value (in percent) with 95% confidence limits, of cord IgE (ku/l) at various cut-off levels in relation to development of allergy.

Cord IgE	Sensitivity	Specificity	Predictive Value
> 0.2:	41.8 (38.7-44.9)	58.6 (55.5-61.7)	23.4 (20.7-26.1)
> 0.5:	11.8 (9.8-13.8)	89.7 (87.8-91.6)	25.7 (22.9-28.4)
> 0.8:	5.5 (4.1-6.9)	94.6 (93.2-96.1)	23.6 (21.0-26.3)
> 1.0:	4.6 (3.3-6.0)	96.9 (95.9-98.0)	31.4 (28.5-34.3)

Sex: Male children developed asthma and definite allergy more often than female (Table 9.3 & 9.4).

Birth Weight: Low birth weight (<2.5 kg) infants developed total and definite allergy, asthma and food intolerance significantly more often than those with normal birth weight (Tables 9.3 & 9.4). Low birth weight was also a significant risk for skin test reactivity to house-dust mite: 3.38 (1.0-11.7)

Method of Feeding: No significant relationship could be detected between method of feeding and various allergic manifestations in the second year of life. A similar conclusion was reached for the time of introduction of solid foods (data not shown).

Maternal Smoking: Asthma was significantly related to maternal smoking (Table 9.4) but no effect of paternal smoking was observed. Maternal smoking was also a significant risk factor for skin test reactivity to dog dander (OR: 14.26; CL: 1.1-197.6; p=0.04).

Season of Birth: The main effect of season of birth was on the development of asthma and rhinitis. Children born during

winter months had significantly less asthma compared to other months. A higher percentage of children born during autumn months developed rhinitis. When adjusted for other variables this was significant (Table 9.4).

Socio-Economic Group: The percentage of children who developed asthma and rhinitis was higher among lower S-E group (Table 9.1). When adjusted for other variables the risk was significant for rhinitis (OR:2.63; CL: 1.1-6.29; p=0.03) but non-significant for asthma.

Pets: There was no effect of presence of furry pets (cat and/or dogs) on the development of allergy. A curious relationship was observed with significantly less food intolerance in children with furry pets inside the house (Table 9.4).

Table 9.3: Effect of various risk factors on prevalence of total and definite allergy at two year evaluated with logistic regression analysis (n=1136).

Risk Factors	Total Allergy	Definite Allergy
Positive F.H.:	1.8 (1.3-2.4)***	1.0 (0.5-1.7)
Maternal Allergy:	1.4 (1.1-1.9)*	0.6 (0.3-1.2)
Sibling Allergy:	1.9 (1.3-2.7)***	1.4 (0.7-2.7)
Male Sex:	1.2 (0.9-1.6)	1.8 (1.0-3.2)*
Low Birth Weight:	2.0 (1.0-4.0)*	3.5 (1.1-10.7)*
Maternal Smoking:	1.3 (1.0-1.9)	1.0 (0.5-2.1)
Season of Birth:		
Spring:	1.3 (0.9-2.0)	0.8 (0.3-1.7)
Autumn:	1.3 (0.9-1.9)	1.1 (0.5-2.2)
Summer:	1.2 (0.8-1.7)	0.5 (0.2-1.2)
Cats and/or dogs:	0.9 (0.7-1.1)	0.7 (0.4-1.2)

Odds ratio (95% confidence limits) are given.
Other factors evaluated but found to be non-significant were paternal allergy, formula feeding and high cord IgE.

Table 9.4: Effect of various risk factors on prevalence of allergic disorders at two year evaluated with logistic regression analysis (n=1136).

Risk Factors	Asthma	Eczema	Rhinitis	Food Int.
Positive F.H.:	1.7 (1.1-2.5)**	2.5 (1.6-3.8)***	1.9 (1.0-4.0)*	-
Maternal Allergy:	-	1.7 (1.2-2.4)**	-	-
Sibling Allergy:	-	3.1 (1.9-5.0)**	-	-
Male Sex:	1.6 (1.1-2.3)*	-	-	-
Low Birth Weight:	3.0 (1.4-6.5)**	-	-	5.2 (1.3-21.1)*
Maternal Smoking:	2.2 (1.5-3.4)***	-	-	-
Season of Birth:				
Spring:	2.0 (1.2-3.5)**	-	-	-
Autumn:	1.9 (1.1-3.3)*	-	2.9 (1.1-7.4)*	-
Summer:	1.7 (1.0-2.9)*	-	-	-
Cats and/or dogs:	-	-	-	0.6 (0.4-0.9)***

Odds ratio (95% confidence limits) are given.
Other factors evaluated but found to be non-significant were paternal allergy, formula feeding and high cord IgE.

Discussion

Two hundred and seventy five of 1174 (23%) children were confirmed to have allergic disorder following assessment in the clinic. A subgroup of children was defined whose symptoms were backed by relevant positive skin tests. These were classified as definite allergy. Among those children who were seen in the clinic but did not fulfil the criteria, very few (5 of 161) had positive skin tests. Although they might develop clinical disorder in future, for the present analysis they were considered non-allergic. Skin tests were done after the diagnosis was made so there was no bias in the reporting of clinical diagnosis. Possible bias can not be excluded however for the information that was available at the time of assessment. This includes sex, method of feeding, parental smoking, month of birth and presence of pets.

As expected family history of atopy was the main risk factor for most allergic disorders. When analysed separately for allergy in the individual family members maternal and sibling allergy were significant but paternal allergy was not a significant risk factor for any of the disorders. In most instances the history was taken from the mother and this lack of effect might be due to inaccurate information about fathers' allergy. Other investigators have reached similar conclusions. Michel et al[31] found that allergy in the infant doubled if mother was allergic but no relationship to fathers' allergy was detected. Happle and Schnyder[70] evaluated the children of men and women with atopic asthma. In children of atopic fathers, 32 of 98 (33%) were effected by some form of atopic disease compared to 45 of 93 (48%) of children of atopic mothers. They believed that this deference in hereditary risk was due to the Carter effect[71] providing evidence for polygenic inheritance in allergy. Another interesting finding was the relationship to sibling allergy. Among the immediate relatives the effect of a sibling with an allergic disorder was most closely related to child's allergy. A similar observation was reported by Lebowitz[18] for serum IgE in children which was related to sibling, maternal and paternal IgE in that order of significance.

Several studies have found cord IgE to be useful in predicting atopy in children[31-35,41-51]. In the present study the two year follow-up confirms the first year results that cord IgE is a poor predictor of allergic disease. The predictive value ranged from 23% to 43% (Table 9.5). The only significant relationship of high cord IgE levels was with sensitivity to egg.

In line with previous studies[74-77] male children were found to be at a higher risk for developing asthma. Although serum IgE levels are usually found to be higher in males compared to females[21,22] there is no such consensus for allergic disorder apart from asthma. Some studies have found males to be at a higher[33,34,59,61] risk but others could not find a difference in the incidence of allergy between sexes[58,60,72]. We were unable to find a difference between sexes with eczema, rhinitis or food intolerance or when any allergy was considered. There is though evidence that male children are more prone to allergic sensitisation. The risk for developing definite allergy was double in males to that found in females.

Low birth weight was found to be a highly significant risk factor for allergic disorders. The effect was stronger than that of family history of atopy. These children were also at risk to develop sensitivity to house-dust mite by the age of two year. Low birth weight infants are known to develop some respiratory problems[179] later in life. A recent report[180] suggests that these infants are at a higher risk of developing asthma at the age of 17. There is evidence to suggest that an immunological immaturity exists in the early months of life and exposure to allergens at this vulnerable period leads to development of allergy later in life[138]. It is possible that low birth weight infants are particularly at risk because of the immaturity of their immune system and are liable to be sensitised to various allergens. No relationship of allergic disorders or positive skin tests could be found to the prematurity when calculated from week of gestation but information on week of gestation is not always accurate.

Breast feeding did not protect against allergic disorders or sensitisation to allergens. Data were repeatedly analysed with various definition of breast feeding (breast feeding initiated, exclusive breast feeding for 3 months, 6 months and 9 months) but the outcome was not different. As Sauls[117]

points out mothers who chose to breast feed and formula feed their infants differ in several variables which effect the development of allergic disease. Some of the studies[78-80] claiming benefit from breast feeding have failed to adjust for these confounding variables. In this study the two feeding groups did not differ regarding family history of atopy, cord IgE levels, sex of the infant, age of introduction of solid foods, birth weight and presence of pets but there were significant differences in parental smoking habits and social class. When analysing the effect of method of feeding all variables were taken into account.
Maternal smoking has been associated with increased cord IgE levels[39] and exposure to passive smoking may increase serum IgE level in children[27]. We found a highly significant effect of maternal smoking on the development of asthma. This may be due to an irritant effect on the bronchial mucosa of the infants and may not lead to permanent bronchial hyperreactivity. It is known that children of smoking parents suffer from more frequent wheeze and lower FEV_1 than those of non-smoking parents[98,132]. Passive smoking also predisposes to respiratory illness during infancy[126,128] which itself is a risk factor for asthma later in life[149-152]. A longer follow-up is required to clarify this effect. Paternal smoking was not a risk factor for any of the allergic disorders. The likely explanation would be that fathers do not frequently smoke in the vicinity of the child.
Asthma occurred least frequently in children born in winter months. It can be hypothesised that this effect is due to the high concentration of tree and grass pollens during spring and summer respectively and house-dust mite during autumn months. Although at this age there was no evidence of increased sensitivity to these allergens for the appropriate months other workers have found this relationship[135-137] at a later age.
Soothill[140] followed 58 infants up to the age of 12 months and found a significantly higher positive skin test to any allergen for infants born in September and October. We found a significant risk (odds ratio: 2.9) of rhinitis for children born in autumn months.
Low socio-economic group was a risk factor for rhinitis and a non-significant trend was seen for asthma. This is likely to be an effect of overcrowding, poor housing conditions and

increased exposure to moulds. Presence of pets was not related to the development of allergy. At the age of two the presence of a cat or dog in the house did not cause increased sensitisation to cat or dog dander.

Chapter 10: **CUMULATIVE PREVALENCE**

Results

The cumulative prevalence of allergic disorders was estimated for 1174 infants in whom information was available for both years (Fig. 10.1). Table 10.1 shows percentages of children with allergic disorders in relation to heredity and environmental factors. Total and definite allergy and various allergic disorders were analysed using logistic regression to all risk factors of interest. Odds ratio and 95% confidence limits for total allergy and asthma are shown in tables 10.2 and 10.3.

Table 10.4 outlines the percentages of positive SPT to inhalant and food allergens with respect to various possible risk factors. Logistic regression was used to obtain the independent effect of factors on skin test reactivity to these allergens.

Family History: Positive family history was a significant risk factor for the cumulative prevalence of total allergy, asthma, eczema, rhinitis and food intolerance (Tables 10.2 & 10.3). Maternal allergy was a significant risk factor for total allergy, eczema and rhinitis. Sibling allergy was a risk factor for total allergy and eczema. Paternal allergy was not a significant risk for any of the allergic disorders.

Cord IgE: Cord IgE was not related to allergic disorders during the first two years of life. The only effect of high cord IgE was seen on skin test reactivity to egg (Table 10.4). The adjusted odds ratio (CL) were 3.13 (1.24-7.85).

Sex: Male children developed asthma more often than female. When adjusted for other factors male sex was a significant risk for definite allergy (Table 10.2).

Birth-Weight: Cumulative prevalence of definite allergy and asthma was significantly higher in low birth weight children. Low birth weight was a significant risk for skin test reactivity to house-dust mite (OR: 3.20, CL: 1.07-9.62, p=0.04) and dog (OR: 11.85; CL: 1.79-78.49; p=0.01).

Method of Feeding: Breast feeding seemed to offer some protection against asthma (Table 10.1) but when adjusted for other variables no significant difference was found between the two feeding groups. When skin test reactivity to egg was evaluated against possible risk factors in the logistic regression model, formula feeding was found to be significant (OR: 2.37, CL: 1.00-5.69, p= 0.05). Age of introduction of solid food was not related to development of allergic disorder or skin test reactivity.

Maternal Smoking: Cumulative prevalence of asthma was significantly related to maternal smoking.

Season of Birth: The main effect of season of birth was on the development of asthma and rhinitis. Children born during winter months had significantly less asthma compared to other months (Table 10.3). Children born during summer and autumn months were at a higher risk of developing rhinitis (Table 10.3).

Socio-Economic (S-E) Group: The percentage of children who developed asthma was higher among lower S-E group (Table 10.1). When lower S-E group was added to the regression model it was a significant factor for asthma (OR: 1.89, CL: 1.17-3.07, p=0.01).

Pets: There was no effect of the presence of furry pets (cat and/or dogs) on the development of allergy during the first two years. The relationship observed at two year follow-up between food intolerance and absence of furry pets inside the house remains significant (Table 10.3). There also seemed to be a difference in skin test reactions to HDM in favour of children with furry pets (Table 10.4) but this effect was not observed when other factors were taken into account (OR: 0.76, CL: 0.41-1.41, p=0.4).

Table 10.1: Genetic and environmental factors and cumulative prevalence of allergic disorders during the first two years of life.

	Total n=357	Definite n=105	Asthma n=167	Eczema n=174	Rhinitis n=67	Food Int n=98
Family History						
Positive:(n=661)	36.5***	10.1	15.6	19.8***	7.3*	10.1*
Negative:(n=511)	22.5	7.4	12.3	8.4	3.7	6.1
Cord Ige (ku/l)						
> 0.5:(n=109)	32.1	12.8	11.0	18.4	2.8	11.0
< 0.5:(n=911)	29.1	7.8	14.4	14.1	6.3	7.6
Sex						
Male:(n=594)	31.5	10.4	16.5*	14.1	5.2	8.8
Female:(n=580)	29.3	7.4	11.9	15.5	6.2	7.9
Birth Weight (kg)						
< 2.5:(n=38)	42.1	21.1**	31.6**	13.2	2.3	10.5
> 2.5:(n=1105)	29.9	8.5	13.8	14.8	5.9	8.0
Method of Feeding						
Formula:(n=733)	30.6	9.8	16.2*	13.4	5.7	8.2
Breast:(n=436)	30.3	7.6	11.0	17.4	5.5	8.7
Maternal Smoking						
Smoking:(n=257)	35.0	9.3	23.7***	13.2	5.5	10.1
No Smoking:(n=915)	29.1	8.9	11.5	15.3	5.8	7.9
Season of Birth						
Summer:(n=273)	33.7	10.3	18.3**	16.1	7.7*	9.9
Autumn:(n=262)	30.9	9.9	15.3*	16.4	8.0*	6.9
Spring:(n=272)	32.0	9.2	15.4*	12.5	4.4	11.0
Winter:(n=367)	26.4	7.1	9.5	14.4	3.5	6.3
S-E Group						
Lower:(n=367)	32.2	10.1	18.3**	12.8	7.6	9.8
Higher:(n=318)	31.5	10.7	10.1	17.9	6.0	9.1
Cats and/or Dogs						
Yes:(n=700)	30.0	7.9	14.4	14.6	5.7	6.7
No:(n=474)	31.0	10.6	13.9	15.2	5.7	10.8*

*p<0.05, **p<0.01, ***p<0.001 (X^2 test with Yates's correction)
All figures are percentages.

Table 10.2: Effect of various risk factors on cumulative prevalence of total and definite allergy evaluated with logistic regression analysis (n=1136).

Risk Factors	Total Allergy	Definite Allergy
Positive F.H.:	2.0 (1.5-2.6)***	1.4 (0.9-2.1)
Maternal Allergy:	1.6 (1.2-2.0)***	1.1 (0.7-1.6)
Sibling Allergy:	1.9 (1.3-2.6)***	1.2 (0.7-2.1)
Male Sex:	1.1 (0.9-1.4)	1.6 (1.0-2.4)*
Low Birth Weight:	1.7 (0.8-3.2)	2.9 (1.3-6.7)**
Maternal Smoking:	1.4 (1.0-1.8)	1.0 (0.6-1.7)
Season of Birth:		
Spring:	1.3 (0.9-1.9)	1.5 (0.8-2.6)
Autumn:	1.3 (0.9-1.9)	1.5 (0.8-2.6)
Summer:	1.4 (1.0-2.0)*	1.4 (0.8-2.5)
Cats and/or dogs:	0.9 (0.7-1.2)	0.7 (0.5-1.10

Odds ratio (95% confidence limits) are given.
Other factors evaluated but found to be non-significant were paternal allergy, formula feeding and high cord IgE.

Table 10.3: Effect of various risk factors on cumulative prevalence of allergic disorders evaluated with logistic regression analysis (n=1136).

Risk Factors	Asthma	Eczema	Rhinitis	Food Int.
Positive F.H.:	1.4 (1.0-2.0)	2.6 (1.8-3.7)**	2.1 (1.2-3.6)*	1.7 (1.1-2.6)
Maternal Allergy:	-	1.8 (1.3-2.5)**	1.7 (1.0-2.7)	-
Sibling Allergy:	-	2.5 (1.6-3.8)**	-	1.7 (1.0-2.9)
Male Sex:	1.5 (1.1-2.1)	-	-	-
Low Birth Weight:	2.6 (1.2-5.5)*	-	-	-
Maternal Smoking:	2.3 (1.6-3.4)**	-	-	-
Season of Birth:				
Spring:	1.8 (1.1-3.0)	-	-	-
Autumn:	1.8 (1.1-3.0)	-	2.8 (1.4-5.9)*	-
Summer:	2.1 (1.3-3.4)*	-	2.5 (1.2-5.2)*	-
Cats and/or dogs:	-	-	-	0.6 (0.4-0.9)*

Odds ratio (95% confidence limits) are given.
Other factors evaluated but found to be non-significant were paternal allergy, formula feeding and high cord IgE.
Non-significant relative risk are not listed for clarity.

Table 10.4: Genetic and environmental factors and skin test reactivity to common inhalant and food allergens during first two years.

	GP n=12	HDM n=49	Cat n=22	Dog n=9	Egg n=38	Others n=28
Family History						
Positive:(n=340)	1.8	10.6	4.4	1.5	7.1	5.3
Negative:(n=197)	3.1	6.6	3.6	2.0	7.1	5.1
Cord Ige (ku/l)						
> 0.5:(n=49)	0	8.2	4.1	4.1	16.3**	6.1
< 0.5:(n=418)	2.6	7.2	4.1	1.2	5.7	4.8
Sex						
Male:(n=288)	2.8	8.7	4.9	1.7	7.3	5.9
Female:(n=250)	1.6	9.6	3.2	1.6	6.8	4.4
Birth Weight (kg)						
< 2.5:(n=22)	4.6	22.7*	0	9.1*	4.6	0
> 2.5:(n=496)	2.2	8.5	4.4	1.2	7.1	5.2
Method of Feeding						
Formula:(n=338)	2.4	10.9	4.7	1.8	8.0	5.0
Breast:(n=199)	2.0	6.0	3.0	1.5	5.5	5.5
Maternal Smoking						
Smoking:(n=115)	0.9	11.3	7.0	3.5	5.2	7.0
No Smoking:(n=422)	2.6	8.5	3.3	1.2	7.6	4.7
Season of Birth						
Summer:(n=133)	3.0	9.8	3.0	0.8	5.3	6.0
Autumn:(n=122)	0.8	8.2	6.6	1.6	10.7	8.2
Spring:(n=129)	3.9	6.2	3.1	3.1	9.3	3.1
Winter:(n=154)	1.3	11.7	3.9	1.3	3.9	3.9
S-E Group						
Lower:(n=172)	2.3	9.3	2.9	2.9	9.3	6.4
Higher:(n=155)	3.2	7.1	6.5	0.7	9.0	5.2
Cats and/or Dogs						
Yes:(n=316)	1.0	8.5	4.4	1.9	6.0	4.8
No:(n=222)	4.1*	9.9	3.6	1.4	8.6	5.9

GP=Grass Pollen, HDM=House-dust mite
'Others' include Tree pollen, Budgie, Cow's milk, Wheat, Peanut, Strawberry and Fish. All figures are percentages

Discussion

In general the effects of risk factors at the end of the second year of follow-up mirror the first year results. When discussing the cumulative prevalence only the differences will be emphasised.

For cumulative prevalence a positive family history of atopy was a risk factor for all allergic disorders except for the sub-group classified as having definite allergy. Paternal allergy remains non-significant as at two year follow-up. In males low birth-weight was a significant risk factor for asthma and definite allergy. Low birth weight children also developed skin test reactivity to house-dust mite and dogs significantly more often than those with normal birth weight during the first two years of life.

Breast fed infants appeared to have less asthma (Table 10.1) but when adjusted for other factors in the regression analysis the significance disappears. There may be some protective effect of breast feeding on respiratory infections through the transfer of IgA and IgG to the infant. This may reduce some of the wheezing associated with infections but the effect was not strong. The only other effect of method of feeding was an increased risk of positive skin test reaction to egg in formula fed infants. This was more prominent at one year but disappeared by the age of two. Children born in summer and autumn months developed rhinitis significantly more often than those in spring and winter. Low socio-economic group was a risk factor for the cumulative prevalence of both asthma and rhinitis.

Chapter 11: **PREVENTION OF ALLERGY**

Introduction

A positive family history of atopy is the most important indicator of infants at risk of developing disorders such as allergic asthma and atopic eczema. Some argue that elevated cord serum IgE level is also useful in predicting atopy[44]. There is evidence to suggest that immediate hypersensitivity in later life depends on allergenic factors encountered in infancy[138,181]. A recent study[148] supports a direct correlation between exposure to house-dust mite antigen in infancy and the development of sensitivity to house-dust mite and the symptoms and severity of asthma in later childhood. Increased sensitisation to cat dander was shown in infants exposed to cats from birth[138]. Parental smoking and overcrowding in the house could also be contributing factors[38,121].

Glaser and Johnstone[159] observed frequent intolerance to egg yolk in infants when the food was introduced at the age of three months and rare intolerance when introduced at the age of nine months or later. In 1936 Grulee and Sanford[78] demonstrated a seven fold increase in eczema in babies fed cow's milk. Subsequent studies have questioned these findings and the subject remains controversial[173]. In the last decade it has been shown that small amounts of protein ingested by the mother are secreted unaltered into breast milk[110,112]. In this way potentially allergenic food taken by the mother can be transferred to the infant and cause sensitisation. Thus maternal dietary restriction during lactation seems to be of crucial importance[164,165,167].

In this study we have tried to determine whether the combination of food and house-dust mite allergen avoidance in early life protects against the development of allergic manifestations in at risk infants.

Subjects and Methods

Design

A prospective, prenatally randomised, controlled study of infants at high risk of allergy with blind assessment at one year.
Approval for the study was given by the ethical committee of the Isle of Wight Health Authority.

Inclusion Criteria:

Infants at high risk of developing allergic disease were defined as:
I. Infants with dual heredity i.e. history of allergic disease in both parents, or one parent and one sibling, or two siblings. Although cord blood IgE was measured in these infants it did not affect their eligibility to the study.
II. Infants with single heredity (one parent or one sibling affected) with cord blood IgE level > 0.5 ku/l.
As cord IgE result could not be available for a few days after birth, infants with single heredity for allergic disease were provisionally included in the study. If cord IgE proved to be less than 0.5 ku/l the infant did not continue with the study. This was explained to the parents at the first consultation.

Exclusion Criteria:

Prematurity - Infants with less than 37 week gestation and/or birth weight of less than 2.5 Kg.

Recruitment:

In a twelve month period between March 1990 and February 1991 all pregnant women (n=1116) in their third trimester attending ante-natal clinic in a district hospital (St. Mary's Hospital, Newport) were seen and the study was explained. Information on the family history of allergic disorders was obtained using a questionnaire (Appendix V). Five hundred and four (45%) had a history of allergic disease (previously diagnosed asthma, atopic eczema, allergic rhinitis or food allergy) in the parents themselves or their children. Three hundred and one (60%) agreed to take part and signed an informed consent (Appendix VI). 143 mothers were randomly allocated to prophylactic group (P) and 158 to control group (C) with an intended 50:50 randomisation, based on a computer generated list of random

numbers. Odd numbers were assigned to the control group and even numbers to the prophylactic group.
Total IgE was measured on cord blood of all infants using enzyme linked immunoassay EIA ULTRA^{R} (method described earlier, page). Serum IgA was measured (method described on page) on all samples to screen for contamination of cord blood with maternal blood during sampling. If the cord IgA was found to be high (> 10 mg/l) the respective IgE result was disregarded.
To fulfil the inclusion criteria infants with single heredity (194 of 301) were required to have a high (> 0.5 ku/l) cord IgE level. The diet of mothers and infants in the prophylactic group was restricted (as outlined below) until the cord IgE result was available. One hundred and sixty two infants with single heredity (P: 72, C: 90) were excluded as their cord IgE was less than 0.5 ku/l (including 8 infants where cord IgA was high indicating possible contamination). Infants with dual heredity (n=107) in both groups continued irrespective of the level of cord IgE.
Three premature infants (P: 2, C: 1) were also excluded because of their special dietary requirements. Out of 301 mothers/infants initially randomised, 136 fulfilled the inclusion criteria and entered the study (P: 69, C: 67).

Parental Smoking: Information was obtained on the parental smoking habits separately for mother and father. A note was made if any other person was likely to smoke inside the house. Those who smoked regularly (one or more cigarettes a day) were regarded as smokers. Occasional smoking (less than one cigarette per day) was disregarded.
Pets: Information was also obtained on the presence or otherwise of pets in the house. Pets living primarily outside the house or in the garden such as rabbits, were not included as infants are unlikely to be exposed to them.

Prophylactic Group

Dietary Measures
Mothers were instructed to follow prophylactic dietary measures as outlined below for themselves while they were breast feeding, and for their infants up to the age of one year. Up to nine months breast feeding was supplemented, if

required with hypoallergenic formula milk, Aptamil HA; a soya and collagen based protein hydrolysate (Milupa, U.K.).
Mothers: Breast feeding mothers avoided completely dairy products, egg, fish and nuts in their diet up to nine months or the duration of lactation if shorter. If they continued to breast feed after nine months they were allowed cow's milk and its products from 9 months, egg from 11 months and fish and nuts from 12 months.
Infants: Infants who were not breast fed were given Aptamil HA from birth up to the age of nine months. Solid foods were introduced between three to five months. Each new food was introduced at monthly intervals.
3 - 5 months: Rice with water or Aptamil HA
4 - 6 months: Pureed vegetables
5 - 7 months: Pureed fruits (not orange)
6 - 8 months: Pureed meats
9 months: Cow's milk and other dairy products
10 months: Orange and wheat and other cereals
11 months: Egg
12 months: Fish and nuts could be introduced at this stage. There was no restriction in diet from 12 months onwards.
A dietitian saw all mothers when the infant was born and explained the dietary restrictions in detail. Written instructions were also provided with a list of foods to take and avoid at various stages for both mother and infant. All lactating mothers were provided with calcium (1000 mg/ day) and multi-vitamin supplements. The dietitian was available through the hospital switchboard during working hours to answer any queries. It was the dietitian's responsibility to make sure that the diet of the mother and the infant was nutritionally adequate.
All the midwives of the maternity department and health visitors of the community medical department were fully informed about the nature and purpose of the study and the special dietary requirements of the mothers and infants in the prophylactic group. Arrangements were made with the hospital catering department to provided special menus (colour coded) for mothers on dietary restriction during their stay in the hospital.
A sticker system was designed to help midwives in the labour and maternity wards to know which mothers and infants were on the study and to which group they had been allocated so they were aware of possible restrictions to mothers and/or

infants' diet. These stickers were placed on mother's maternity notes at the last ante-natal visit and mother's beds and infant's cots after delivery.

Red: Prophylactic group (Breast feeding)
Blue: Prophylactic group (Formula feeding)
Yellow: Control group (Breast feeding)
Green: Control group (Formula feeding)

Environmental Measures:

Infant's bedrooms were kept as free as possible of dust and moulds. Special measures were taken to reduce house dust mite antigen load in the infants bedroom and living room.

House-dust mite

Introduction: Parents were offered advise and practical help to apply measures to reduce house dust mites and their antigen level. The extermination of house dust mites and removal of their excreta with subsequent reduction of antigen level was achieved using Acarosan^{R} foam and moist powder (Crawford Chemicals, U.K.). Areas treated were those where the infant was likely to spend most of his/her time i.e. living room and infant's bed room.

Acarosan foam consist of benzyl benzoate in a polymerised form (6.6 %), anionic tensides (1.5 %), and water to 100 %. Acarosan powder contain 10 % benzyl benzoate in a polymerised form, absorbent powder 42 %, isoparaffin 8 %, and water to 100 %. Acarosan foam and powder is not irritant to skin and mucous membrane. It has been regarded harmless toxicologically and dermatologically. In a recent study[182] only 2 (of 49) patients complained of minor side effects.

Methods:

1) All infants used polyvinyl covered mattresses, some with vented head area, available from various manufacturers.

2) Infants' home were visited within 72 hours of the delivery by the research nurses. Dust samples were collected from infants' bedroom carpet, living room carpet, upholstered furniture, rugs and any other area likely to contain significant numbers of house-dust mites. Significant amounts of dust were not obtained from covered mattresses.
3) Dust samples were collected with a hand held, mains operated (500 watts) vacuum cleaner (Hoover, U.K.), using

special dust filters available from ALK, Denmark. An area of $1m^{183}$ on the carpet was chosen usually in the centre of the room and the vacuum cleaner was operated for approximately 1 minute. In most instances though, sufficient dust was not obtained and the area and the time of suction was increased. From upholstery dust was collected for 1 minute or longer if required.

4) All the samples were halved. One half was used for the Acarex test and other half was saved for later determination of major house-dust mite antigen [antigen p_1 of Dermatophagoides Pteronyssinus (*Der p* I)] level.

5) Anti dust-mite treatment with Acarosan foam (for soft furnishings) and powder (for carpets in the living room and bedroom) was applied by the nurses according to the manufacturers instructions. Mothers were advised to vacuum carpet after 24 hours and upholstery after one week. The nurses visited the infants' homes for vacuum cleaning following treatments if mothers were unable or unwilling to do it themselves.

6) The procedure of dust collection and anti-housedust mite treatment was repeated at 3 monthly intervals up to 9 months.

7) Other advice offered included regular (at least twice a week) vacuum cleaning and once a week washing of the infants' bedding and soft toys. Unwashable soft toys were treated with Acarosan foam.

Acarex test: The Acarex test measures guanine content of the house-dust and gives a semiquantitative estimation of the antigenicity of the dust. It has been said to correlates well with *Der p* I levels [2]. Acarex test is supplied in a package. Each package contains:

10 aluminium bags containing test fluid
1 vial containing 10 test strips
1 measuring spoon
1 colour scale
1 test cavity in the package lid

Coarse dust, hair and fluffy material is separated from the fine dust. A heaped spoonful of fine dust is placed in the

test cavity of the package lid. The test fluid from the aluminium bag is poured on to the fine dust and mixed lightly using the handle of the spoon. The test strip is dipped into the paste thus formed and removed immediately. Any remaining paste is scraped off and the colour of the strip is matched to the colour scale supplied with the package. Four classes of guanine content are identified from zero to three. Class 0 indicating little or no dust mite antigen to class 3 indicating high levels.

Measurement of *Der p* I: Dust samples were analysed by a sandwich-type ELISA using monoclonal antibody labelled microtitre plates (ALK, Denmark) for major house-dust mite antigen *Der p* I.

Material:
Sieve: 300 micron mesh - to filter dust prior to extraction.
Dust extraction buffer: 0.125 M ammoniumhydrogencarbonate containing 0.1% sodium azide.
Reference Mite antigen extract, 4 ug/ml (ALK)
Phosphate Buffered Saline, PH 7.2 (PBS): NaH_2Po_4, H_2O 3.45 g, Na_2HPo_4, $12H_2O$ 26.8 g, NaCl 84.74 g, distilled water to 10 L.
Washing buffer: 0.05% Tween 20 (Polyethylenesorbitanmono-laurate) in PBS.
Dilution buffer: 0.5% bovine serum albumin (BSA) in PBS.
House-dust mite control solution (ALK)
Plastic microtitre plates with 96 wells coated with monoclonal anti-Der p I antibodies (ALK).
HRP*-labelled antibody (ALK).
Substrate solution: 4 OPD (Orthophenyloxene Diamine) tablets (ALK) dissolved in 12 ml citrate/phosphate buffer + 5 ul 30% Hydrogenperoxide.
Citrate/phosphate buffer 0.1 M, PH 5: Citric acid, H_2O 7.65 g, Na_2HPo_4, $12H_2O$ 23.88 g, distilled water to 1 L.
Sulphuric acid, 1 M
Dynatek microtitre plate reader.
(*=Horse Ransh Peroxide)

Method: The sample dust from the dust-filter was sieved to obtain fine dust. 200 mg of fine dust was mixed with 2 ml of extraction buffer and left at room temperature for 2 hours.

The suspension was then filtered through a 0.22 um membrane filter and stored at -20^{o} C until analysed.
A dilution series of reference antigen was prepared from mite extract by 2 fold dilutions with dilution buffer (Range: 0.4 ug/ml to 0.0015 ug/ml). Each sample extract was assayed undiluted and diluted 1:10 and 1:100 (V/V, in dilution buffer) to obtain readings within measurement limits. 0.1 ml sample extract dilutions (or reference antigen/specificity control solutions) were added to the wells in the microtitre plate and the plate was incubated overnight at 20^{o}. The wells were then washed three times with washing buffer (PBS-Tween). 0.1 ml of HRP-labelled antibody was added in each well and incubated for one hour at 20^{o} C. The wells were then washed 3 times with PBS-Tween. 0.1 ml substrate solution was added in each well and incubated at 20^{o} in darkness for 15 minutes. The reaction was then stopped by addition of 0.1 ml 1 M sulphuric acid and absorbance read half an hour later in each tube at 490 nano-meter on Dynatek plate reader.
Reliability: A calibration curve was constructed by plotting the absorbance for each standard measurement (Fig. 11.1). The upper limit of the range was 85% of the maximal response. The lower limit was set as twice the value of the blanks. The content of mite antigen in the unknown sample was read from the calibration curve. The dust concentration in ng/g dust was obtained by multiplying the sample extract-concentration (in ng/ml) by a factor of 10.
Reproducibility: Reproducibility of the assay is shown in table 11.1. Within and between batch variation was calculated for 'low' and 'high' quality control sera. Means of best and worst coefficient of variation are given.

Table 11.1: Reproducibility of ELISA *Der p* I.

	Number of assays	Mean (SD) (ng/ml)	CV
Within Batch:			
Low Control:	8	14.14 (1.14)	8.0%
High Control:	8	61.2 (5.26)	8.6%
Between Batch:			
Low Control:	12	13.75 (1.86)	8.0%
High Control:	12	65.0 (5.3)	8.2%

SD: Standard Deviation, CV: Coefficient of Variation

Control Group

Mothers and infants in the control group had a normal diet following the advice of their health visitors. Mothers who wished to breast feed their babies were encouraged to do so. A conventional cow's milk formula was used if a supplement was required. Mothers who did not wish to breast feed gave a cow's milk formula of their choice. Health visitors gave standard advice on the time of introduction of solid foods. No advice regarding environmental allergens was given to mothers in the control group. The homes of the control group infants were visited twice during the study to collect dust samples from infants' bedroom, living room and upholstered furniture for the Acarex test and *Der p* I levels. First visit was soon after birth and the second at the age of nine months.

Follow-up

Sixteen infants did not complete the follow-up (P: 11, C: 5). In the prophylactic group one infant was given cow's milk formula in the nursery by mistake and ten mothers found the diet too restrictive and gave-up within the first four weeks (usually within the first few days). As the avoidance was too short to have any possible effect these infants were not followed. In the control group 3 mothers declined to attend follow-up clinic and 2 moved from the area. These 16 infants are compared for some important variables to exclude

any possible bias occurring in the study groups (Table 11.2).

Table 11.2: Comparison of some variables in the two groups for 16 infants who were excluded.

Variables	Prophylactic n=11	Control n=5
Sex (M):	5 (45%)	1 (20%)
Dual History:	6 (54%)	3 (60%)
Maternal Allergy:	9 (82%)	4 (80%)
Paternal Allergy:	5 (45%)	3 (60%)
Sibling Allergy: (P=8, C=2)	6 (75%)	2 (100%)
Smoking:		
Mother:	1 (9%)	1 (20%)
Father:	3 (27%)	2 (40%)

Percentages are given in brackets.

Infants and mothers in both groups were seen in the hospital clinic at 3 and 6 months by the research fellow and the dietitian. Any symptoms and signs related to allergic disorder were recorded and appropriate advice given (Appendix III and IV). For infants in the prophylactic group any deviation from the recommended avoidance measures was noted (Appendix VII). Additional visits occurred if required to evaluate any allergy related problem. The dietitian assessed the nutritional adequacy of the diet for infants and mothers in the prophylactic group. Compliance with the prophylactic regimen was checked by questioning the mother and regular home visits by research nurses. Between 4 to 12 weeks 8 mothers gave up the diet. 3 infants were introduced to cow's milk and wheat between 24 and 32 weeks. These infants were included in the final analysis.

Between ten and twelve months a blind assessment was made by a paediatric allergist (Appendix IV and VIII).
Data on the presence of pets and parental smoking habits were up-dated at each visit. Birth weight was recorded and infants were weighed at each visit. Information was also obtained on social class and whether the infant was sharing a bedroom with the parent or other children (Appendix VIII).

Clinical criteria:
Asthma: Three or more separate episodes of cough and wheezing.
Eczema: Chronic or chronically relapsing dermatitis (lasting more than six weeks) with characteristic morphology (areas of scaly, erythematous, pruritic lesions) and distribution (face, post-auricular area, scalp, extensor surface of extremities and flexural creases).
Food intolerance: A history of vomiting, diarrhoea, colic or rash within four hours of ingestion of a recognised food allergen. The food was excluded from the diet for 4 weeks. The diagnosis of food intolerance was accepted if symptoms recurred on open challenge.

Skin prick test: All skin prick tests were performed by the same research nurse using allergen extracts (Soluprick ALK, Denmark) against house-dust mite, grass pollen, cat dander, cow's milk and egg and any other allergen relevant in a particular case. A mean wheal diameter (half the sum of largest diameter and its perpendicular) of more than 2 mm but at least half the size of histamine was considered positive.

Socio-Economic (S-E) Group: The social classes were defined according to the Registrar General's classification. To highlight the effect of socio-economic class, analysis was performed with classes 1, 2 and 3 grouped together as higher socio-economic (S-E) group (professional and skilled workers) and classes 4 and 5 (semi-skilled, unskilled and unemployed) grouped together as lower S-E group.

Results

Some inherited and environmental factors important in the development of allergic disorders are compared in table 11.3. Allergic diseases were more prevalent in the families of infants in the prophylactic group. By chance relatively more infants had dual heredity in the prophylactic group than the control group. There was a higher incidence of smoking mothers in the control group and this could effect the incidence of respiratory symptoms in infants.

Table 11.3: Comparison of some variables in the two groups.

Variables	Prophylactic n=58	Control n=62
Sex (M):	28 (48.3)	34 (54.8)
Dual History:	51 (87.9)	42 (67.7)
Single+IgE >0.5:	7 (12.1)	20 (32.3)
Maternal Allergy:	42 (72.4)	41 (66.1)
Paternal Allergy:	31 (53.5)	34 (54.8)
Sibling Allergy: (P=50, C=51)	36 (72.0)	31 (60.8)
Cord IgE (Percentiles, ku/l)		
25th:	0.34	0.49
50th:	0.65	0.66
75th:	0.85	0.81
90th:	0.95	0.93
Smoking:		
Mother:	8 (13.8)	16 (25.8)
Father:	21 (36.2)	22 (35.5)
Either:	21 (36.2)	27 (43.6)
Lower S-E Group:	28 (48.3)	29 (46.8)
Sharing Bedroom:	26 (44.8)	25 (40.3)
Pets:	36 (62.1)	38 (61.3)

Siblings in the Prophylactic group n=50, Control group n=51
Percentages are given in brackets.

The duration of breast feeding and the age of introduction of formula milk and solid foods was similar in the two groups (Table 11.4). Infants in both groups gained weight satisfactorily (Table 11.4). Growth pattern of infants fed Aptamil HA from birth was similar to the rest of the group (data not shown).

Table 11.4: Comparison of method of feeding and birth weight for infants in the two groups.

Variables	Prophylactic n=58	Control n=62
Duration of Breast Feeding		
Initiated:	71	77
3 Months:	43	48
6 Months:	28	31
9 Months:	17	15
Introduction of Formula Feeding		
1 Month:	50	44
3 Months:	78	71
6 Months:	88	84
Introduction of Solid Foods		
3 Months:	40	48
6 Months:	97	97
Weight (SD)		
At Birth:	5.62 (0.84)	5.73 (0.84)
9 Months:	9.18 (1.15)	9.56 (1.34)

House-dust mite: In the prophylactic group *Der p* I values in the upholstery, bedroom carpet and living room carpet were reduced gradually following treatments so that they became significantly lower than the control group at 9 months (Fig. 11.2). Mean *Der p* I of all areas for the two groups from birth to 9 months is shown in fig. 11.3. There were highly significant reductions in *Der p* I levels in the prophylactic group. At birth *Der p* I levels in the prophylactic group were non-significantly higher than the control group but at 9 months they became significantly lower.

Allergic manifestations: At one year 4 infants (6.9 %) in the prophylactic group compared with 12 (19.4 %) in the control group had asthmatic symptoms (Fig. 11.4). Eczema was diagnosed in 4 infants (6.9 %) in the prophylactic group compared with 12 (19.4 %) in the control group. In the control group 7 infants (11.3 %) were classified as having food intolerance, usually to cow's milk or egg, at one year. Only two infants (3.4 %) in the prophylactic group developed food intolerance. One infant developed a rash when egg was introduced at the age of 7 months, the other infant had asthma and wheezed following cow's milk ingestion when Aptamil HA was stopped at nine months. Skin prick tests were positive in 6 infants (9.7 %) in the control group to a range of allergens including house-dust mite, cow's milk, egg, wheat, cat and grass pollen. Two infants (3.4 %) in the prophylactic group showed positive skin prick tests, one to egg and the other to cat dander. Although follow-up at 3 and 6 months was not blind the pattern was similar.
At one year 25 (40.3 %) infants developed one or more allergic disorders in the control group compared to 8 (13.8 %) in the prophylactic group. At 3 and 6 months respective percentages were: C; 17.7%, P; 5.2% and C; 32.3%, P; 12.1%). In view of the higher incidence of maternal smoking in the control group, 12 month data were analysed further taking into consideration exposure to passive smoking. Parental smoking had a major effect on the prevalence of asthma and other allergic manifestations especially in the control group (Fig. 11.5).
Despite randomisation genetic and environmental factors may not affect the development of allergic disorders in exactly the same way in the two groups. To control for this and to

assess the independent influence of various risk factors multivariate logistic regression analysis was used to obtain the adjusted odds ratios for each factor. The baseline for each factor was defined as:

Groups:	Prophylactic group
Smoking:	Both parents non-smoker
Maternal Allergy:	No history of allergic disease in mother
Father Allergy:	No history of allergic disease in father
Sibling Allergy:	No history of allergic disease in sibling
Sex:	Female
S-E Groups:	Higher S-E group
Pets:	No pets

Logistic regression was performed with the presence of any allergic disorder at one year as the dependent variable including all risk factors of interest as independent variables (Table 11.5). The process was repeated for any allergic disorder at 3 and 6 months (Table 11.5)

Table 11.5: Effect of risk factors on the prevalence of total allergy at 3, 6, and 12 months.

Risk Factors	3 Months	6 Months	12 Months
Control Group:	5.6 (1.3-24.2)*	4.0 (1.4-11.5)**	6.3 (2.0-20.1)***
Parental Smoking:			
Either Parent:	1.3 (0.2-6.4)	3.4 (1.1-10.7)*	3.97 (1.2-13.6)*
Both Parent:	5.1 (1.2-22.5)*	1.8 (0.5-6.9)	4.72 (1.2-18.2)*
Maternal Allergy:	2.4 (0.5-11.8)	3.2 (0.9-11.9)	5.92 (1.5-23.0)**
Sibling Allergy:	1.7 (0.4-6.9)	1.4 (0.5-4.0)	4.59 (1.3-15.8)*
Male Sex:	4.2 (1.0-18.3)*	1.9 (0.7-5.5)	1.44 (0.5-4.2)
Lower S-E group:	1.4 (0.4-5.4)	1.4 (0.5-4.0)	3.30 (1.1-10.2)*

(Odds Ratios and 95% confidence limits are given)
Factors tested but were non-significant and did not alter odds ratios for other variable were Paternal allergy, and presence of pets.
* $p<0.05$, ** $p<0.01$, *** $p<0.005$

Odds ratios (95% confidence intervals) were calculated for individual allergic disorders (asthma, eczema and food intolerance) as the dependent variables including all risk factors of interest as independent variables (Table 11.6). After adjusting for other confounding variables the control group was a significant risk for total allergy at each follow-up and for asthma and eczema at one year. Parental smoking proved to be the other important risk factor when either and both parents smoked in the house. Maternal smoking was not used as a separate variable as only five mothers smoked while their partners did not. As expected maternal allergy, sibling allergy and male sex were other significant risk factors for total allergy. The prevalence of allergy at one year in infants belonging to the lower S-E group was higher than those from the higher S-E group.

Table 11.6: Effect of risk factors on the prevalence of allergic manifestations at 12 month.

Risk Factors	Asthma	Eczema	Food Intolerance
Control Group:	4.1 (1.1-15.5)*	3.6 (1.0-12.5)*	3.3 (0.6-17.3)
Parental Smoking:			
Either Parent:	3.3 (0.8-14.6)	2.4 (0.7-7.9)	1.5 (0.2-9.7)
Both Parent:	11.0 (2.5-48.2)***	0.9 (0.2-5.6)	5.7 (1.1-29.5)*
Sibling Allergy:	5.7 (1.3-24.5)*	1.4 (0.4-4.5)	0.7 (0.2-3.7)

Factors tested but were non-significant and did not alter odds ratios for other variable were Male sex, Maternal and Paternal allergy, S-E group and presence of pets.

Discussion

As seasonal factors may influence the development of allergic disorders[136,140] the recruitment lasted throughout a 12 month period. A study comparing the effect of treatment should ideally be double blind but the nature of intervention was such that this was not possible. The blindness of the assessor was absolute as mothers were always briefed before entering the consulting room not to disclose whether they were in the prophylactic or the control group.

At present allergen avoidance is recommended for treatment but not for prophylaxis. Given the high motivation and time required for prophylaxis only infants at high risk of atopy are suitable for this kind of intervention. Exposure to highly allergenic food and inhalant antigenic protein could prime the immune system of genetically predisposed infants. IgE mediated food allergy often occurs to cows' milk, egg, fish and nuts. Important inhalant allergens are house-dust mite, grass and tree pollen and cat and dog dander.

Transplacental sensitisation is uncommon as RAST for specific IgE is rarely found in cord blood[31,47]. Two recent studies[162,164] have shown no benefit from food avoidance during the third trimester of pregnancy. Moreover it adversely affected weight gain during the third trimester and resulted in a small decrease in the term infants' birth weight. Therefore food avoidance during pregnancy is not advisable.

Previous studies[164,165,167] have shown reduction in eczema and food reactions when maternal dietary restrictions were employed during lactation in high risk infants. The duration of these restrictions varied from 3 months[165] to 12 months[164]. In only one study[164] was the infants' diet restricted. In the present study exposure to allergenic foods, either directly or through mothers' milk, was avoided up to 12 months of age. It is possible that a shorter duration of exclusion is sufficient. When 11 infants were excluded from the analysis because of diet violation the outcome was not improved. Mistakes are made in the nursery when cows' milk formula is given inadvertently to some infants. Generally this was avoided by close co-operation with mid-wives, warning stickers on infants' cots and

mother's beds, and educating mothers to be very vigilant. Occasional mistakes were reported by 16 mothers during the study who for example took a cup of tea with milk or gave the infant a jar food with soya or casein as an ingredient. Overall the compliance was remarkable for a very difficult diet. For mothers who did not wish to breast feed or who wanted to supplement breast milk, Aptamil HA provided a useful alternative. It is a soya and collagen based, extensively hydrolysed milk. The molecular weight of 99% of the molecules is <10,000 Dalton. Similar hydrolysed milk is used for the treatment of cow's milk allergy and in studies[164,165,167] has been shown to reduce the incidence of eczema and food reactions.

Previous studies[164,165] have concentrated on food-allergen avoidance and reported a reduction in eczema and food reactions but not in respiratory symptoms. Inhalant allergens and adjuvants are equally important[136,138] although more difficult to control. In the United Kingdom house-dust mite is the most common allergen in patients with extrinsic asthma and atopic eczema. Recently some chemicals have been available which not only kill dust-mites (acaricidal effect) but also help to reduce the antigen level already present in the carpet[184]. One such acaricide (Acarosan) was used in this study. All treatments were carried out by the same two research nurses throughout the study. To standardise treatment applications were repeated every three months irrespective of *Der p* I levels. Antigen levels were brought down only gradually with repeat treatments (Fig. 5) . For prophylaxis, treatment with an acaricide perhaps should be started a few months before the infant is born. The design of the present study was such that the effect of food and house-dust mite avoidance can not be separated.

Ideally one should ask parents in the prophylactic group to give up smoking and remove furry pets from the house. This was not attempted for fear of non-compliance and for the purpose of the study no advice was offered to either group regarding pets and smoking in the house.

In infancy bronchial hyper-reactivity reveals itself by recurrent cough and wheeze, usually following viral respiratory tract infections. It has been termed recurrent wheezing, wheezy bronchitis, infantile wheezing and asthma. We preferred the latter term as it has been shown in

longitudinal studies that many of these infants belong to the atopic/ asthmatic group[75] particularly in genetically predisposed infants[153], though some disagree with this approach[176]. Sporik et al[148] have shown that onset of wheezing was earlier in atopic children exposed to high levels of dust-mite in infancy. It can be hypothesised that in genetically predisposed infants exposed to high levels of allergen, viral respiratory infections and smoking act as adjuvants and lead to persistent bronchial hyper-reactivity and asthma.

For practical reasons open challenge was relied upon for the diagnosis of food intolerance. A double blind challenge would have been more definitive. However management of any adverse food reactions reported by the mother was the same in both groups and blind assessment should have kept any possible bias to a minimum. We avoided the term food allergy as evidence for IgE mediated food allergy (positive skin prick test to the relevant food antigen) was available in only 30% of cases. Positive skin prick test without symptoms (in one infant in the prophylactic group, to egg) was not regarded as an allergic manifestation. Prolonged 'cold' and rhinorrhea in the infants was reported by 17 mothers (prophylactic:7, control:10) at 12 months' follow-up. It was not included in the analysis as it is difficult to establish the aetiology or significance of this symptom. None had a positive skin prick test to inhalant allergens.

Individual disorders were two to three times more common in the control group and a higher prevalence for asthma and eczema was noted at 12 months. Data were analysed using logistic regression to adjust for other confounding variables. Prevalence of asthma and eczema at one year was significantly higher in the control group. When children manifesting any disorder were considered statistically significant differences were found at each of the three assessments. Parental smoking had a profound effect on the prevalence of asthma and total allergy. At one year bi-parental smoking was highly significant for asthma. In some of these infants it may represent transient bronchial hyper-reactivity but one can not exclude the possibility of continued wheezing in a significant proportion of these genetically predisposed infants. Advice regarding smoking should be included in any prophylactic regime to reduce allergic disorders.

We conclude that reduction in food and house dust mite allergen exposure in high risk infants significantly reduces the allergic manifestations in infancy. Parental smoking contributes significantly to the allergic manifestations during infancy and should be avoided specially in genetically predisposed individuals. It is possible that allergen avoidance merely delays and does not prevent the development of allergic disorders. This was said to be the case for fish and citrus allergy in a study by Saarinen and Kajosaari[158]. In the present study foods were introduced between 9 and 12 months in the prophylactic group but only one infant reacted to cows' milk. A longer follow up is required, at least into later childhood, to determine if the reduction in allergic manifestations is maintained. Because of their high prevalence, allergic disorders are a huge burden to personal and family life and a considerable expense to the provision of health care. If the benefit shown in this study is maintained then this is likely to out-weigh the cost of dietary supervision, hypoallergenic formulae and antidust-mite measures.

Chapter 12: **DISCUSSION**

Allergic disorders include asthma, atopic eczema, allergic rhinitis and some forms of food allergy. There are several problems in the epidemiological study of these disorders. Allergy is a common word and people tend to attribute a wide variety of symptoms and ailments to allergy. The cause and effect relationship is often difficult to establish. There is no test which completely rule out allergy as the cause of a problem in an index patient. As these disorders are common the proportion of subjects from the population who needs to be assessed is large. Even when a correct diagnosis of asthma or eczema is made it does not always represent type I IgE mediated allergy.

A history of allergic disorders in the immediate family was established by questioning the mother. In a quarter of cases both parents were available for interview. For asthma and rhinitis simple questions were asked such as "Have you ever suffered from asthma?". According to IVATLD bronchial symptom questionnaire[185] this is remarkably specific although it might underestimate the prevalence. In most cases of asthma and rhinitis diagnosis has already been made by a doctor. In some, although the label of asthma or rhinitis had not been applied, the historical evidence was adequate, for example an attack of wheezing when exposed to animal dander or hay-fever symptoms in summer. In a small study to assess the efficacy of a new anti-histamine (cetirizine) 39 of 40 patients claiming to be hay-fever sufferers reacted to grass pollen on skin test (unpublished data).

The diagnosis of eczema is rather more difficult to validate. Various kinds of rashes and skin diseases are often labelled as eczema. Even when eczema is diagnosed correctly, no single characteristic differentiates non-atopic from atopic eczema. With careful questioning regarding chronicity, age of onset and associated symptoms the diagnosis of atopic eczema was established in the immediate family. Food allergy/intolerance was the most difficult area. Overestimation of this diagnosis is easy.

Only those foods were considered which typically cause food allergy such as egg, fish and nuts. Moreover symptoms such as rash and wheezing were thought to be more likely to represent IgE mediated food allergy than non-specific symptoms as diarrhoea and colic. Eczema and food allergy appearing for the first time in adult life were not considered.

There was less allergy in fathers (24%) compared with mothers (33%). The percentages were similar for asthma (Fathers: 10%, Mothers: 10.7%) but eczema and rhinitis were significantly less in fathers compared to mothers (6% vs 12% and 14% vs 20% respectively). Food allergy was 1% in both sexes. It is possible that as the history was obtained from the mother in most cases there may have been an underestimation of fathers' allergy. Some mothers may not be aware of childhood eczema or asthma which has been in remission for years, in their spouse. Unfortunately we did not identify on the questionnaire which parent provided the information. Therefore we are unable to check reterospectively if the prevalence of paternal allergy was different in maternal or maternal/paternal completed questionnaire. Ideally both parents should have been interviewed and skin tested to confirm their atopic status. However constraint on resources did not allow this.

IgE unlike IgG does not cross the placenta. The evidence is many fold. The foetus is able to synthesis IgE since 11th week of gestation[6]. The concentration of IgE found in the cord blood is very small[14] and generally bears little relation to maternal IgE levels[32]. Specific IgE is rarely found in cord blood[31]. Very occasionally when specific IgE to an antigen is present in the cord blood it may not be found in maternal blood pointing towards intra-uterine sensitisation[31].

Commercially available radioimmunoassay, PRIST^{R} is not sensitive to measure small amounts of IgE present in the cord blood. We tested a new commercially available enzyme-linked immunoassay, EIA ULTRA^{R} to measure total IgE in the cord blood in a large number of infants. The method proved to be easy to use and reliable. The lower detection limit was said to be 0.05 ku/l but we found that below 0.2 ku/l the standard curve was nearly horizontal and the overlap of absorbance units was considerable. We therefore regarded all values below 0.2 ku/l as undetectable. This is

still more sensitive than PRIST which has a detection limit of 0.5 ku/l. There was a good correlation between EIA ULTRA and PRIST (r= 0.99) up to the detection limit of PRIST.
The distribution of cord IgE was highly skewed. The majority of samples (59%) had IgE level below the detection limit of assay. Very few samples (2.6%) had IgE more than 1.0 ku/l. It is likely that the synthesis of IgE by the foetus is primarily under genetic control. The positive relationship between family history of atopy and male sex on cord IgE found in this and earlier studies[33-35] is in keeping with this hypothesis.
We found a weak though statistically significant correlation (r= 0.3; $p<0.001$) between maternal and cord IgE. This probably represents an effect of maternal allergy on the synthesis of foetal IgE rather than a passive transfer of IgE through the placenta. There was a significant relationship between maternal allergy and cord IgE ($p< 0.0002$). A very weak relationship of cord IgE was found with paternal allergy ($p=0.08$) and none with sibling allergy ($p=0.9$).
Environmental factors could theoretically effect cord IgE levels by acting through the placenta. We were unable to detect any effect of gestational age or birth weight on IgE levels. Maternal smoking has been said to increase cord IgE levels but we did not find any relationship between maternal smoking and IgE levels at birth. Month of birth was said to be a factor in determining cord IgE level. In this population, although mean IgE levels and the percentage of high (> 0.5 ku/l) values varied from one month to the other, no definite pattern or cyclical trend could be found. Our data points towards a predominantly genetic control of IgE synthesis by the foetus.
The difficulties encountered in the assessment of allergic disorders are worse in early childhood. Investigations are not easy especially in a population based study on a large number of children. Some investigations, such as pulmonary function tests, require active participation of the subject and are therefore not practical at this age.
We were able to get the co-operation of the health visitors for the first year follow-up. They screened the infants for symptoms which may be related to allergy and referred them to the hospital clinic. At one year follow-up we saw 366 of 1342 (27.3%) in the clinic. At two year follow-up we asked

the mothers to complete a questionnaire. Four hundred and thirty six of 1174 (37%) infants were said to have one or more symptoms related to allergic disorders and they were then assessed in the clinic and skin tested. The history taken from the mother by the health visitors or by the nurses may be coloured by her own or her other children's illnesses. This might introduce bias with somewhat selective referral from the mother. This would have an effect of augmenting the influence of heredity. Asymptomatic children were not skin tested. It is known that a proportion of the population is sensitised (positive skin test or RAST) but do not show any symptoms of allergy. It is appreciated that an unknown number of asymptomatic children in the study population may have been sensitised. Our data on skin test is relevant to children who showed symptoms related to allergy and were assessed in the clinic (27% in the first year and 37% in the second year.

The diagnosis of allergic disorders was made with strict diagnostic criteria within the limitations described above. The prevalence of allergy at each follow-up was not different (21% and 23%). Standard method[186] and reagents (ALK, Denmark) were used for skin testing. To avoid operator bias, the same nurse did skin tests throughout the study period. Egg was the most common allergen (n=32) at one year but even at this early age 18 infants reacted to house-dust mite (HDM). At two year HDM was the commonest, (n=37) followed by egg (n=17).

To identify infants at risk of atopy so that preventive measures can be applied, a screening test is needed. Any useful screening test ought to have low false positive and false negative values. Too many false negatives will give false assurance to those who will develop the disease. If there are too many false positives preventive measures would be directed toward those who do not need them which means a waste of resources. A good screening test should have both sensitivity and specificity above 95%. Family history of atopy falls short of that target as its sensitivity and specificity is generally around 50-80%. Efforts have therefore been directed towards finding a better test which can correctly identify infants at risk of developing allergy. A number of studies concluded that cord serum total IgE is a useful predictor of atopy and is better than family history of atopy[33,41-50]. We were unable to confirm this

conclusion. In our study there was no difference in the IgE levels in infants who developed allergic disorders by the age of two years compared to their non-allergic counterparts. The positive predictive value of cord IgE was 21% at one year which is the same as the prevalence of allergy at that follow-up. In other words no advantage was gained by the knowledge of cord IgE value.

When data were analysed for individual family members the maternal and sibling allergy were significantly related to allergy in the infant but paternal allergy had no influence. It is possible that information of father's allergy was incorrect as the history was obtained from the mother in the majority of cases. Happle and Schnyder[70] arrived at the same conclusion and called this "Carter effect", evidence for the polygenic transmission of atopy. Sibling allergy was most closely related to infants' allergy and this should perhaps be considered when selecting infants at high risk of allergy for prophylactic measures.

Development of allergic disorder depends on both genetic and environmental factors. We studied the relationship of development of allergy to a number of environmental factors. Low birth weight infants were more likely to develop asthma and other allergic disorders. There was also evidence to suggest that these infants are at a higher risk for sensitisation to various allergens. Low birth weight would not be a significant risk for the total population as a small number of infants had a birth weight less than 2.5 kg. The majority of infants who develop the disease have normal birth weight. The finding is important in the pathogenesis of allergic sensitisation. It is said that exposure to allergens in the first few months of life is important in the development of sensitisation due to a physiological immaturity of the immune system. Possibly low birth weight infants are particularly vulnerable because they are exposed to allergens at an earlier period. It might be possible to reduce the development of allergic disorders by an allergen avoidance regimen.

We found a protective effect of breast feeding on asthma at one year follow-up but the significance was reduced once other confounding variables such as maternal smoking and socio-economic groups were taken into account. No effect of method of feeding was seen at the age of two years. It is generally agreed that breast feeding protects against viral

infection by the transfer of IgA through the breast milk. It is possible that the effect on asthma in infancy is primarily due to the reduced viral respiratory infections and thus reduced number of wheezing episodes. Even when data were analysed for prolonged and exclusive breast feeding (up to 9 months), no benefit of breast feeding on the development of allergic disorders was seen. Similarly the age of introduction of solid foods was unrelated to the prevalence of allergic disorders during the first two years of life.

The main effect of maternal smoking was on the development of asthma. There were highly significant differences in the prevalence of asthma at both follow-up examinations in infants whose mother smoked and those whose mothers did not smoke after controlling for other factors. It is possible that this represents just the irritation of airways by cigarette smoke. There was some evidence of increased sensitisation to dog dander and house-dust mite in infants exposed to passive smoking. There is evidence in animals of increased sensitisation when exposed to high levels of tobacco smoke[123]. Some investigators believe that smoking can cause increase IgE levels and sensitisation in man[122,124] but others do not[125]. Exposure to passive smoking increases bronchial hyperresponsiveness[132]. Murray and Morrison showed that infants with a history of eczema develop asthma if their mothers smoke. A history of eczema presumably represents genetic predisposition while smoking acts as an adjuvant. A longer term follow-up of infants in our study will reveal if the majority of these infants continue to wheeze and develop other signs of allergic sensitisation.

Infants born in spring and summer months were at a significant risk of developing asthma and those born in autumn months had a similar risk for rhinitis. A possible explanation would be that infants born in spring and summer months are exposed to a high concentration of pollens in the early vulnerable period. Similarly house-dust mite concentrations are said to be higher in autumn months. We were unable to show increased sensitisation to pollens or house-dust mite in these infants but such data exist for birch pollen allergy[136].

Infants belonging to the lower S-E group were at a higher risk of developing asthma during the first two years of

life. The effect was independent of the risk factors studied but it probably represents a combination of factors such as dampness in the house, presence of moulds and overcrowding with increase in respiratory infections which itself can be a risk for later childhood asthma. The risk factors are often combined. Mothers belonging to the lower S-E group tend to smoke and formula feed their babies more often than those belonging to the higher S-E group. The risk of asthma was six times greater in this sub-group of infants with all three factors present. It is particularly important to educate these mothers not to smoke and preferably to breast feed their babies.

Prevention of Allergy

We know that genetic factors are important in the development of allergy but environmental factors do play a significant part in the expression of clinical disorder. It has been shown that exposure to allergens and probably some adjuvants in the early months of life is crucial to sensitisation and disease later in life[138,148]. If allergen exposure is avoided in the first few months of life this should lead to a reduction in the prevalence of allergic disorders. Foods are important allergens in the early life therefore most of the preventive studies have concentrated on the avoidance of food allergen usually by advocating breast feeding. The rather disappointing results of breast feeding in several studies, and recent evidence suggesting that unaltered food proteins can pass through the breast milk to the infant, has focused attention on avoidance of allergenic foods by lactating mothers. Little efforts has been given to the avoidance of inhalant allergens although these can be equally important. Inhalant allergens such as pollens, animal dander and house-dust mite are difficult to avoid. Adjuvants such as cigarette smoke can also sensitise and potentiate the effect of allergens.

Family history of atopy is generally regarded as a useful indicator to select infants for prophylactic measures. This is however, not an ideal screening test as the sensitivity and specificity is not more than 70%. The search for a better test has led many to believe that cord IgE is a more useful predictor of atopy and the two may be combined to select infants at very high risk of atopy. The results of our observational study (which did not confirm earlier

reports on usefulness of cord IgE) were not available when infants were recruited to the prevention study. Still we were reluctant to use cord IgE as the sole criteria so it was used to further increase the risk in infants with single heredity. Infants with dual heredity were included irrespective of the level of cord IgE.

Infants avoided allergenic foods either directly or through the breast milk (maternal avoidance). Chandra et al[163] recommended maternal avoidance of allergenic foods during pregnancy to avoid intra-uterine sensitisation. We did not attempt this for several reasons. Intra-uterine sensitisation is rare as specific IgE is rarely found in cord blood[31]. Other workers have not found any benefit of maternal diet during pregnancy on subsequent development of allergy[161,162]. Moreover Zeiger et al[164] reported that maternal diet during third trimester of pregnancy could lead to a lower birth weight in infants and lower weight gain by the mother. There is thus concern over the nutrition of the infant and the mother.

The strict dietary regime that was recommended to the lactating mothers in our study was difficult to follow and several problems were encountered which needed the help of a trained dietitian. Dietary intervention should not be recommended without such skilled supervision. The recent availability of chemicals which can not only kill the house-dust mite but also reduce the level of antigen (*Der p* I)[184], enabled us to include house-dust mite avoidance in the prevention regimen. Ideally we should have asked the parents to stop smoking and exclude furry pets from the house. This was not attempted for fear of non-compliance. These confounding factors were considered during the analysis. Although the groups were randomised there were more dual heredity infants in the prophylactic group. A higher percentage of infants in the control group were exposed to maternal smoking. The effect of these and other factors was adjusted during analysis using multivariate logistic regression model.

The mean level of *Der p* I before treatment, was high (25.5 ug/g dust) in our study compared to some previous studies. This was reduced significantly in the prophylactic group (from 25.5 ug/g dust to 5.9 ug/g dust) but this reduction was achieved after 3 application from birth to 9 months of age. Some regard 10 ug/g as high and >2 ug/g as likely to be

significant for sensitisation[148,184]. Although reduction in house dust antigen was achieved it was slow and not optimal and further improvement in antidust-mite preparation are required. As the exposure during the first few months may be more important for sensitisation the anti-house-dust mite measures should perhaps be started before the birth of the infant.

These infants were followed-up at 3, 6 and 12 months of age. A significant reduction in various allergic manifestations was shown in the prophylactic group. The design of the study was such that both avoidance measures were applied at the same time in the prophylactic group and it is not possible to separate the effect of food avoidance from the house-dust mite antigen avoidance on the development of allergy. The other significant risk factor, especially for asthma, was maternal smoking and advise regarding smoking should be included in all prevention programs. A longer term follow-up will determine whether the manifestations of allergy in the prophylactic group are prevented or only postponed.

Conclusions

- Foetal IgE production is primarily under genetic control.

- Allergic disorders in early childhood are common. The cumulative prevalence for the first two years of life was 30%.

- Skin test reactions to inhalant allergens are common even during infancy. By two years of age house-dust mite was the commonest allergen on skin tests.

- Cord total IgE is an poor predictor of allergy in the early childhood. Family history of atopy is a better indicator of infants at risk.

- Maternal and sibling allergy have positive relationship with infants' allergy but paternal allergy is not relevant.

- Low birth weight infants are at a higher risk of developing allergic disorders by the age of two years.

- Breast feeding does not protect against the development of allergy.

- Maternal smoking is a strong risk factor for asthma in infancy and early childhood.

- Infants born in summer and spring months are at risk for developing asthma while infants born in autumn are at a higher risk for rhinitis.

- Infants belonging to lower S-E group develop more asthma than those belonging to higher S-E group.

- Household pets are not a risk factor for allergy in early childhood.

- Maternal dietary avoidance should not be attempted without the dietary supervision of a trained dietitian.

- The house-dust mite antigen (*Der p* I) can be reduced by repeated use of solidified benzyl benzoate (Acarosan^{R}).

- Allergy in the infant can be reduced by reducing the exposure to food and house-dust mite allergens.

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(Appendix I)

IgE STUDY

INTRODUCTION

We are undertaking a study on all babies born in Isle of Wight to find out those babies who are likely to develop allergic diseases such as eczema, asthma and hay fever. This will involve taking a blood sample at the time of birth, not from the baby but from the blood vessel in the cord which connects baby to the mother. This sample will be tested for a substance called IgE which is an antibody found in greater amounts in most people with allergic disease. We would also like to know if there is any history of allergic disease in the family and would be grateful if you could complete the enclosed questionnaire. We will follow all the babies at intervals for at least two years to check their health with regard to these diseases. Most of the follow-up will be done at the health visitors clinic to minimise any inconvenience to you. If we succeed in predicting accurately those babies who are at high risk of developing allergic disease then in future preventive measures can be applied from birth for this group of children. We hope that this will reduce the number of children suffering from asthma, eczema and food allergy.
If you agree with the objectives of this study and willing to take part in it, kindly sign the consent below and fill in the questionnaire.

CONSENT:

I _ _ _ _ _ _ _ _ _ _ _ _ _ have been informed of the nature and objectives of this study.

I am willing to take part in this study.

Signature_ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _
Date _

I confirm that I have informed the above named subject of the nature and procedure involved in this study and have obtained her consent to participate.

Investigators Signature _ _ _ _ _ _ _ _ _ _ _ _
Date:_ _

(Appendix II)

QUESTIONNAIRE

Mother's name: Baby's Name:
Hosp. Ref. No. Hosp. Ref. No.
Address: Date of birth:

Tel: Follow-up clinic:

Do you, your Partner or your children suffer from or have suffered from:
(circle correct answers_ Y=Yes, N=No)

		Yourself		Partner		Children	
I.	*Asthma or Wheezing attack ?*	Y	N	Y	N	Y	N
	If so:						
	(a) Was it diagnosed by a doctor ?	Y	N	Y	N	Y	N
	(b) Has it ever been treated ?	Y	N	Y	N	Y	N
II.	*Eczema ?*	Y	N	Y	N	Y	N
	If so:						
	(a) Was it diagnosed by a doctor ?	Y	N	Y	N	Y	N
	(b) Approx. age of onset (select):						
	(i) Infancy (Before first birthday)	(i)		(i)		(i)	
	(ii) Childhood	(ii)		(ii)		(ii)	
	(iii) Adult life	(iii)		(iii)		(iii)	
III.	*Hay Fever / Allergic Rhinitis ?*	Y	N	Y	N	Y	N
	If so:						
	(a) Was it diagnosed by a doctor	Y	N	Y	N	Y	N
	(b) Time of the year: (select):						
	(i) Spring / Summer	(i)		(i)		(i)	
	(ii) Winter	(ii)		(ii)		(ii)	
	(iii) All the time	(iii)		(iii)		(iii)	
IV.	*Food Allergy ?*	Y	N	Y	N	Y	N
	If so:						
	Please write name/s of the food:	(a)		(b)		(c)	
	What happens if this food is taken (select):						
	(i) Vomiting	(i)		(i)		(i)	
	(ii) Diarrhoea	(ii)		(ii)		(ii)	
	(iii) Rash	(iii)		(iii)		(iii)	
	(iv) Wheezing	(iv)		(iv)		(iv)	
	(v) Anything else (please write):	(a)		(b)		(c)	
V.	*Does anyone smoke in the house ?*	Y	N	Y	N		
	If so: who does and how many per day?	No:		No:			
Vi.	*Any pets inside the house ?*						
	If so: Please write:_____						

(Appendix III)

FOLLOW UP (9 MONTHS) **STUDY NO:**

NAME: SEX: Male / Female

ADDRESS: D.O.B. / /

HOSP. REF. NO.

TEL: CLINIC:

HEIGHT: |__|__|.|__| Cm WEIGHT: |__|__|.|__||__| Kg

NUTRITION:

Mode of feeding at present: Breast / Formula / Combination

Duration of breast feeding: |__|__| Weeks

Age of first formula (or Cow's milk) feed: |__|__| Weeks

Age of introduction of cereals/solids: |__|__| Weeks

ATOPIC DISEASE

I. **ASTHMA:** Yes / No
If no,
any wheezing episodes ? Yes / No

Recurrent chest infections ? Yes / No

II. **ECZEMA :** Yes / No

III. **RECURRENT NASAL CONGESTION:** Yes / No

V. **FOOD INTOLERANCE:** Yes / No
If yes,

Please write name of the food: _ _ _ _ _ _ _ _ _

What happens if this food is taken ?
1. Vomiting 2. Diarrhoea
3. Colic 4. Wheezing
5. Rash 6. _ _ _ _

VI.SMOKING *inside the house:* (a) None (b) |__|__| / day

VII.PETS *inside the house:* _ _ _ _ _ _ _ _ _ _

COMMENTS:

Signature:_ _ _ _ _ _ _ _ _ _ _ _ **Date:** / /

(Appendix IV)

CLINIC FOLLOW-UP : |__|__| Months

NAME: **STUDY NO:** |__|__|__|

(Circle or write as appropriate)

I. *ASTHMA:* Age: |__|__| |__|__| Weeks (first symptom)

Wheezing episodes: 1. < 3 2. > 3 3. Frequent

Cough: 1. Never 2. Occasional 3. Frequent

Nocturnal Symptoms: 1. Never 2. Occasional 3. Frequent

Wheezing on examination: 1. No 2. Yes

Treatment: 1. None 2. _ _ _ _ _ _ _ _ _ _ _ _ _ _

II. *ATOPIC ECZEMA:* Age: |__|__| |__|__| Weeks (first symptom)

Examination: 1. None 2. Dry Skin 3. Eczema

Lesions: (underline those seen on examination)

1. Face 2. Scalp 3. Neck

4. Post auricular area 5. Trunk 6. Buttocks 7. Hands and feet

8. Extensor aspects of arms and legs 9. Creases

Duration: 1. < Six Weeks 2. > Six Weeks

Itchy: 1. No 2. Yes

III. *RHINITIS:* Age: |__|__| |__|__| Weeks (first symptom)

Episodes: 1. Once 2. Two to ten 3. Most days

Nasal Symptoms: 1. Blockage 2. Discharge 3. Sneezing

Eye Symptoms: 1. Itchy 2. Discharge 3. Redness

IV. *FOOD INTOLERANCE:* Age: |____| |____| Weeks (first episode)

Suspected food: _ _ _ _ _ _ _ _ _ _

Reaction: 1. Vomiting 2. Diarrhoea 3. Colic 4.Rash

5. Other (Specify) _ _ _ _ _ _ _ _

Episodes: 1. Once 2. Two to five 3. Frequent

Temporal Relation: 1. None 2. > 4 hrs. 3. < 4 hrs.

V. *URTICARIA:* Age: |____| |____| Weeks (first episode)

Episodes: 1. Once 2. Two to five 3. Frequent

Trigger factor: _ _ _ _ _ _ _ _ _ _

Temporal Relation: 1. None 2. > 1 hr. 3. < 1 hr.

SKIN TEST: (attach copy) 1. Not done 2. Negative 3. Positive

If positive: Allergen Reaction Size

1. _ _ _ _ _ _

2. _ _ _ _ _ _

3. _ _ _ _ _ _

CONCLUSION:

1. No Atopy
2. Probable Atopy
3. Definite Atopy

Signature:_ _ _ _ _ _ _ _ _ _ _

Date: _ _ _ _ _ _ _ _ _ _ _ _ _

QUESTIONNAIRE **Study No.__ __ __**

Mother's name:
D.O.B:
Hosp. ref. no.
Address:

Tel:

E.D.D.:
Group: 1. PBF 2. CBF
3. PFF 4. CFF
Baby's name:
Sex:
D.O.B:
Hosp. no.

Do any members of your family suffer from or have suffered from:

Asthma or Wheezing attacks ? 1. No 2. Yes
If yes, please state which member:
1.Mum 2.Dad 3.First Child 4.Second Child 5.Third child 6.Fourth child

Diagnosed by: 1: Hospital Doctor 2: G.P. 3: Health Visitor
4: Others_____

(Use same code for family members and diagnosis in the following questions)

Eczema ? 1. No 2. Yes
If yes, please state which member: 1 2 3 4 5 6
Diagnosed by : 1 2 3 4._____
Approximate age of onset: 1. Infancy 2. Childhood 3. Adult life

Hay Fever / Allergic Rhinitis ? 1. No 2. Yes
If yes, please state which member: 1 2 3 4 5 6
Diagnosed by : 1 2 3 4._____
Time of the year: 1. Spring / Summer 2. Winter 3. All the time

Food Allergy ? 1. No 2. Yes
If yes, please state which member: 1 2 3 4 5 6
Diagnosed by : 1 2 3 4._____
Please write name/s of the food: _____
What happens if this food is taken: 1. Vomiting 2. Diarrhoea 3.Rash
4. Wheezing 5. Colic 6. Any other symptom _____

Tobacco smoking inside the house ? No / yes: Mum:_____ / day
Dad:_____ / day

Any pets inside the house ? _____

Date:___ ___ ___ ___ ___ ___

Appendix VI

ALLERGIC DISEASE STUDY

INTRODUCTION:

We are undertaking a study to assess the value of allergen avoidance in the early months of life on subsequent development of allergic disorders like asthma, eczema, hay fever and food allergy.
At 36 week ante-natal clinic visit a doctor or a nurse carrying out this research will ask you few questions to determine the presence of allergic disorders in the immediate family. Parents of infants at high risk of developing allergic disease will be invited to take part in the study. What it entails for you will depend on whether you plan to breast or bottle feed the baby and whether you are allocated to intervention or control group. Full explanation of this will be given to you by the investigator when he/she sees you in the clinic.

CONSENT:

If you are willing to take part in this study, kindly sign the consent below.

I_ _ _ _ _ _ _ _ _ _ _ _ _ _ have been informed of the nature and objectives of this study and I am willing to take part in it.

Signature_ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _

Date:_ _

I confirm that I have informed the above named subject of the nature and procedures involved in this study and have obtained her consent to participate.

Investigators Signature _ _ _ _ _ _ _ _ _ _ _ _

Date: _

Appendix VII

ALLERGY PREVENTION STUDY **STUDY NO:** |___|___|___|

PROPHYLACTIC GROUP **FOLLOW UP:** |___|___| Months

NAME: SEX: Male / Female

ADDRESS: D.O.B. / /

HOSP. REF. NO.

TEL: GROUP: Breast / Formula

HEIGHT: |___||___|.|___| Cm WEIGHT: |___|___|.|___|___| Kg

NUTRITION:

Mode of feeding at present: Breast / Formula / Combination

Duration of breast feeding: |___|___| Weeks

Age of Formula :

1. ---------- |___|___| Weeks
2. ---------- |___|___| Weeks
3. ---------- |___|___| Weeks

Name of solids and age first taken:

1. ---------- |___|___| Weeks
2. ---------- |___|___| Weeks
3. ---------- |___|___| Weeks

NON-COMPLIANCE:

MOTHER:

INFANT:

ATOPIC DISEASE

I. **ASTHMA:** Yes / No

If no,

any wheezing episodes ? Yes / No

Recurrent chest infections ? Yes / No

II. **ECZEMA:** Yes / No

III. **RECURRENT NASAL CONGESTION:** Yes / No

V. **FOOD INTOLERANCE:** Yes / No

If yes,

Please write name of the food: _ _ _ _ _ _ _ _

What happens if this food is taken ?

1. Vomiting 2. Diarrhoea
3. Colic 4. Wheezing
5. Rash 6. _ _ _ _ _

VI. **SMOKING** *inside the house:* Yes / No

Mum: |__|__| / day

Dad: |__|__| / day

VII. **PETS** *inside the house:* _ _ _ _ _ _ _ _ _ _

COMMENTS:

BREAST MILK SAMPLE: Yes / No

Signature:_ _ _ _ _ _ _ _ _ _ _ Date: / /

ALLERGY PREVENTION STUDY **STUDY NO:** |___|___|___|

12 MONTHS FOLLOW-UP

NAME: SEX: Male / Female
ADDRESS: D.O.B. / /
HOSP. REF. NO.
TEL: CLINIC:

BIRTH WEIGHT : |___|___|.|___|___| Kg

WEIGHT: |___|___|.|___||___| Kg

HEIGHT: |___|___|.|___| Cm

ATOPIC DISEASE

I. **ASTHMA:** Yes / No
If no,
any wheezing episodes ? Yes / No

Recurrent chest infections ? Yes / No

II. **ECZEMA :** Yes / No

III. **RECURRENT NASAL CONGESTION:** Yes / No

V. **FOOD INTOLERANCE:** Yes / No
If yes,
Please write name of the food:
What happens if this food is taken ? __ __ __ __ __ __ __ ___
1. Vomiting 2. Diarrhoea
3. Colic 4. Wheezing
5. Rash 6. _ _ _ _

ENVIRONMENT

I. **SMOKING** *inside the house:* (a) None (b) |___|___| / day

II. **PETS** *inside the house:* __ __ __ __ __ __ __ ___

III. **FATHER'S OCCUPATION:** __ __ __ __ __ __ __ ___

IV. **BABY'S BEDROOM:**
1. Separate
2. With Other children
3. With Parents

COMMENTS:

Signature:_ _ _ _ _ _ _ _ _ _ _ _ Date: / /

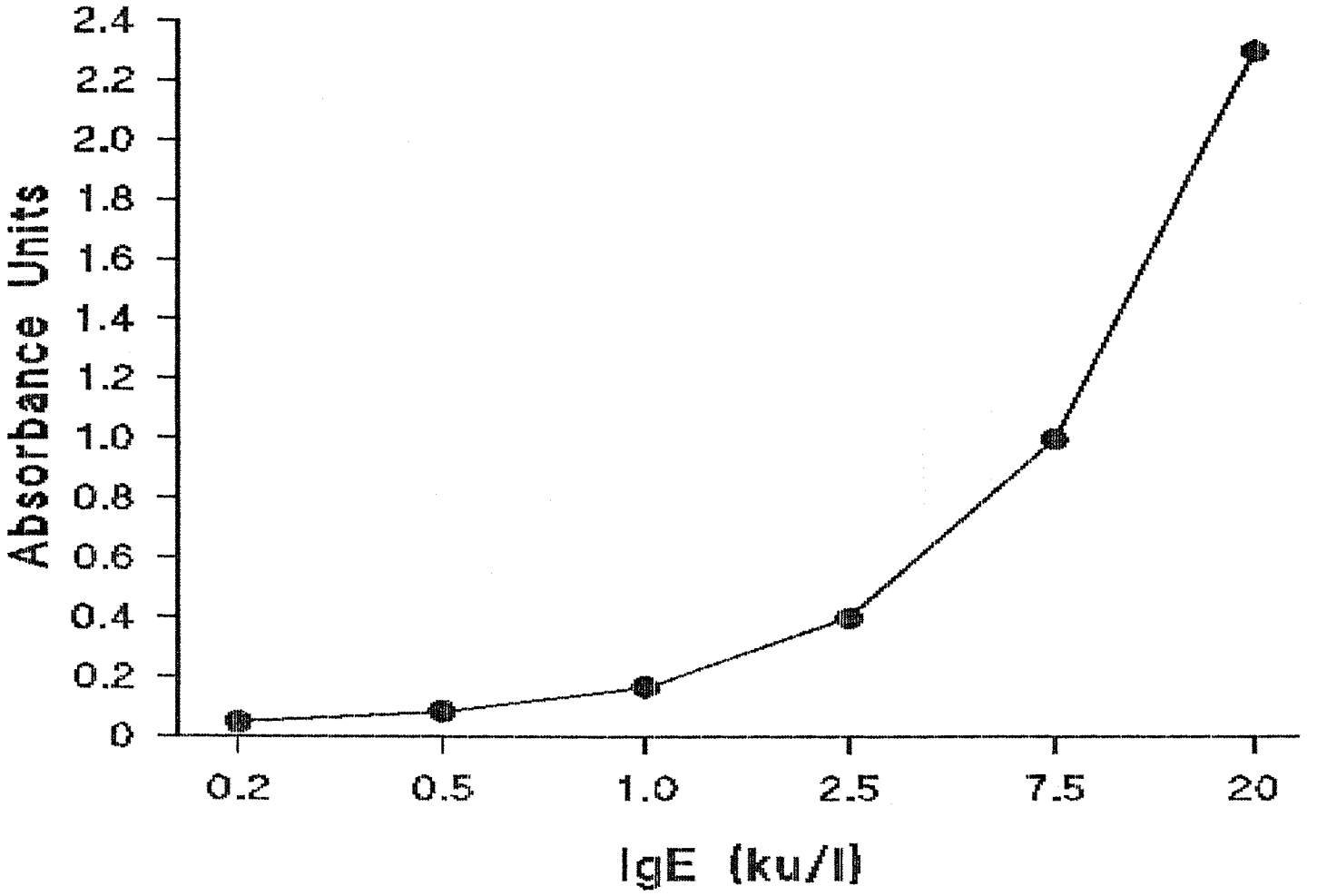

Fig. 4.1. Standard curve for IgE EIA ULTRA test.

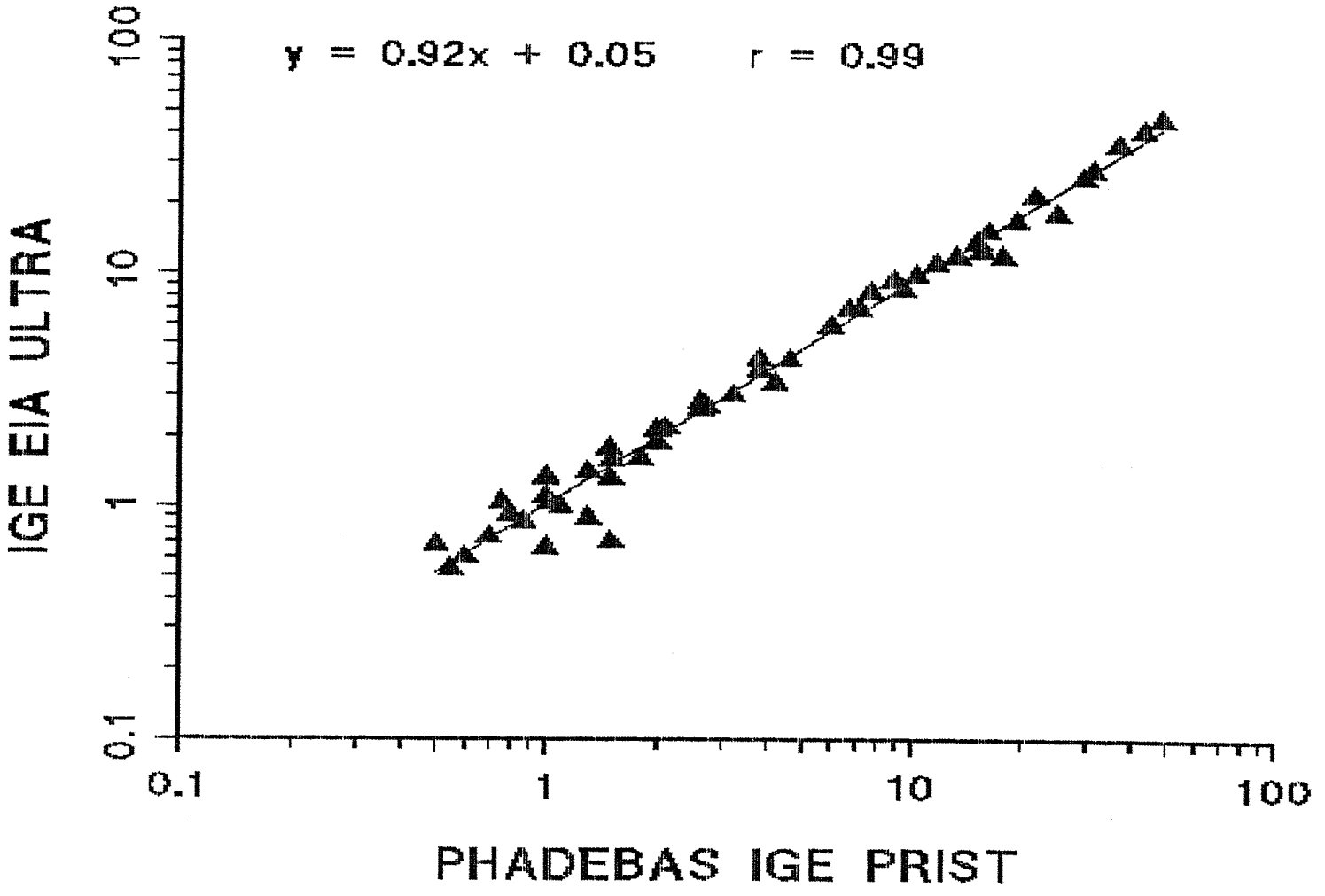

Fig 4.2: Correlation between IGE PRIST and IGE EIA ULTRA

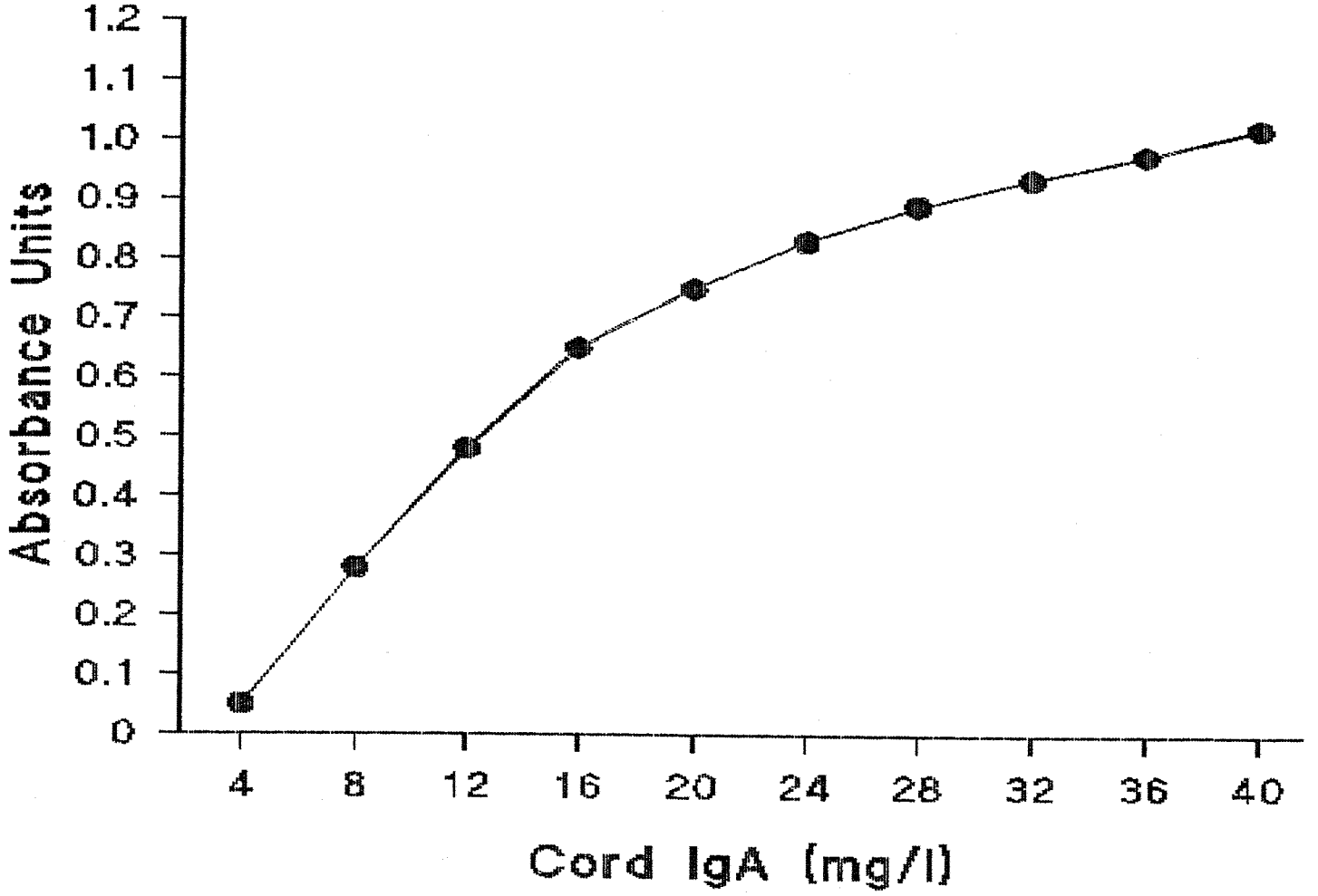

Fig. 4.3. Standard curve for ELISA IgA test.

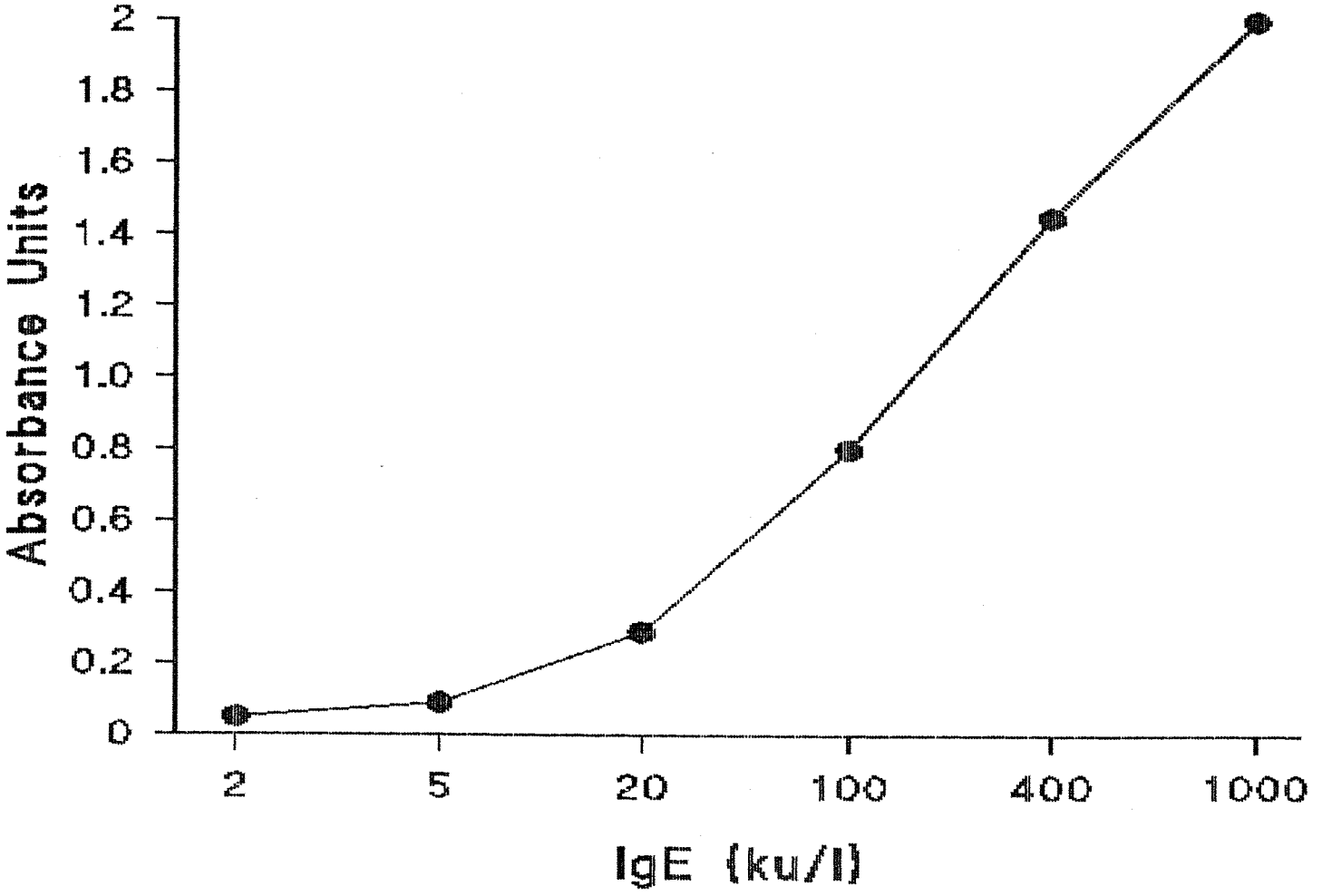

Fig. 4.4. Standard curve for IgE EIA 100 test.

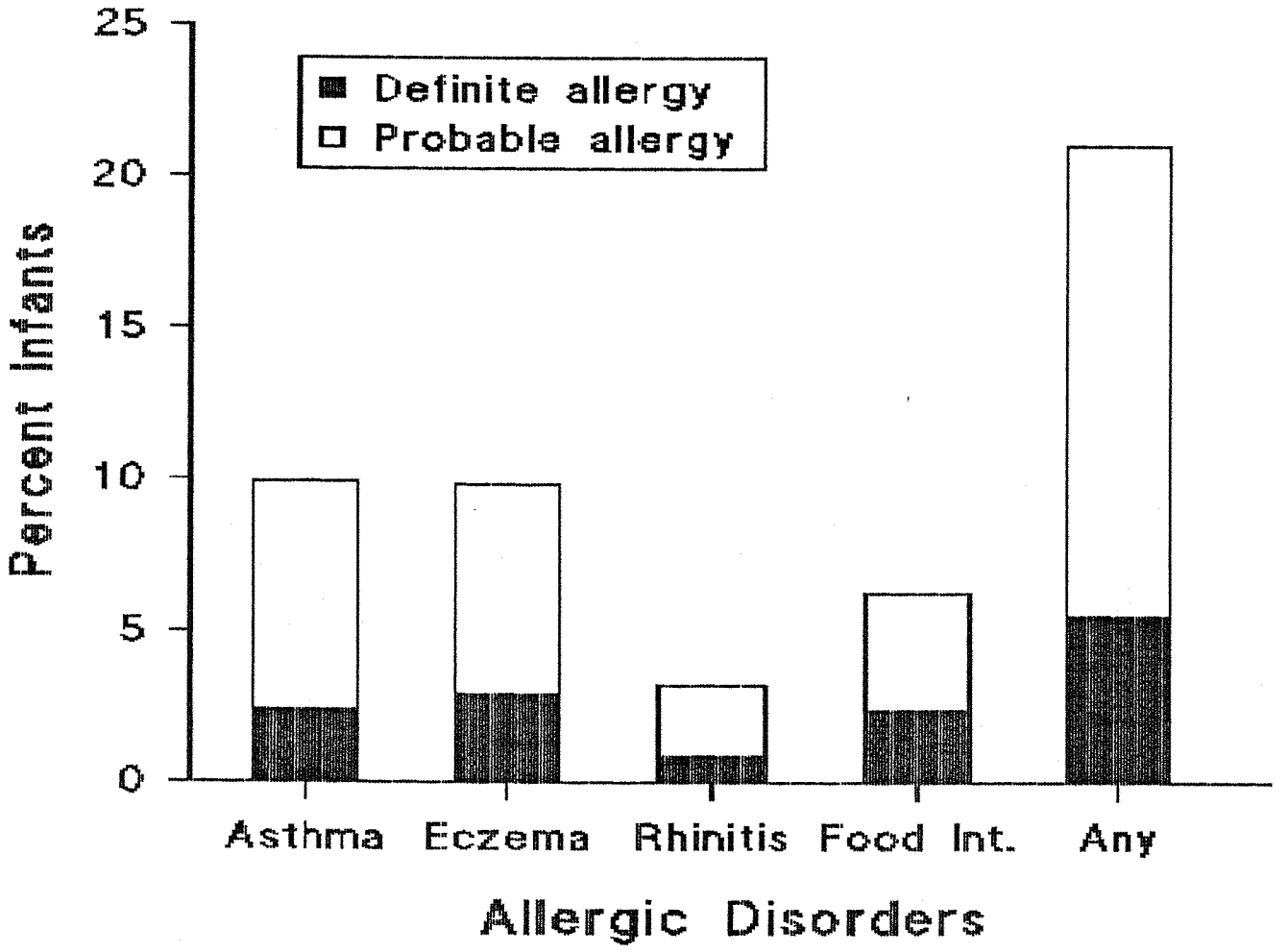

Fig. 5.1. Prevalence of allergic disorders during the first year of life. (Food Int.=Food Intolerance)

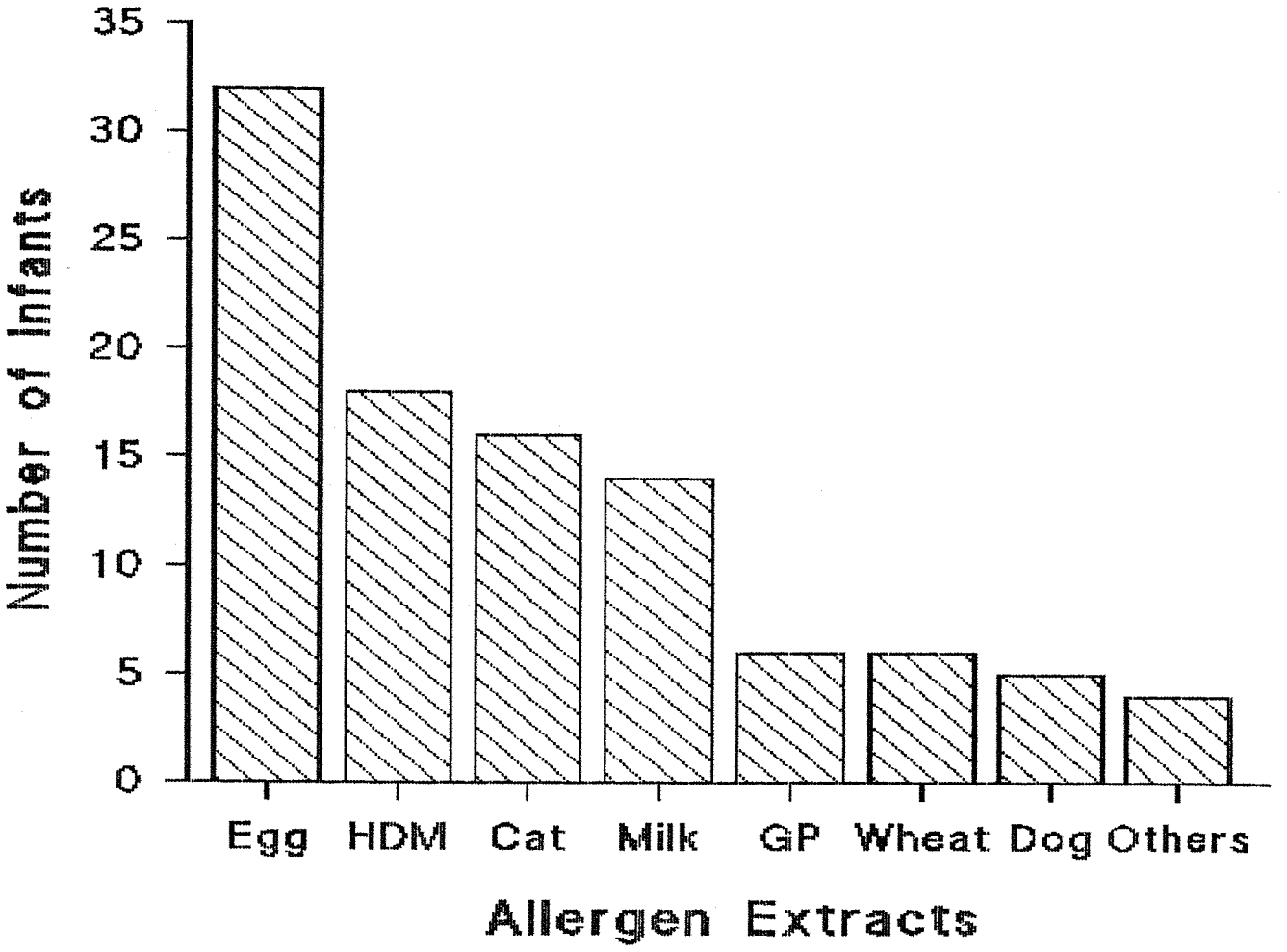

Fig. 5.2. Frequency distribution of positive skin prick tests. (HDM=House-dust Mite, GP=Grass Pollen, Others=Fish, Peanut, Budgie and Strawberry)

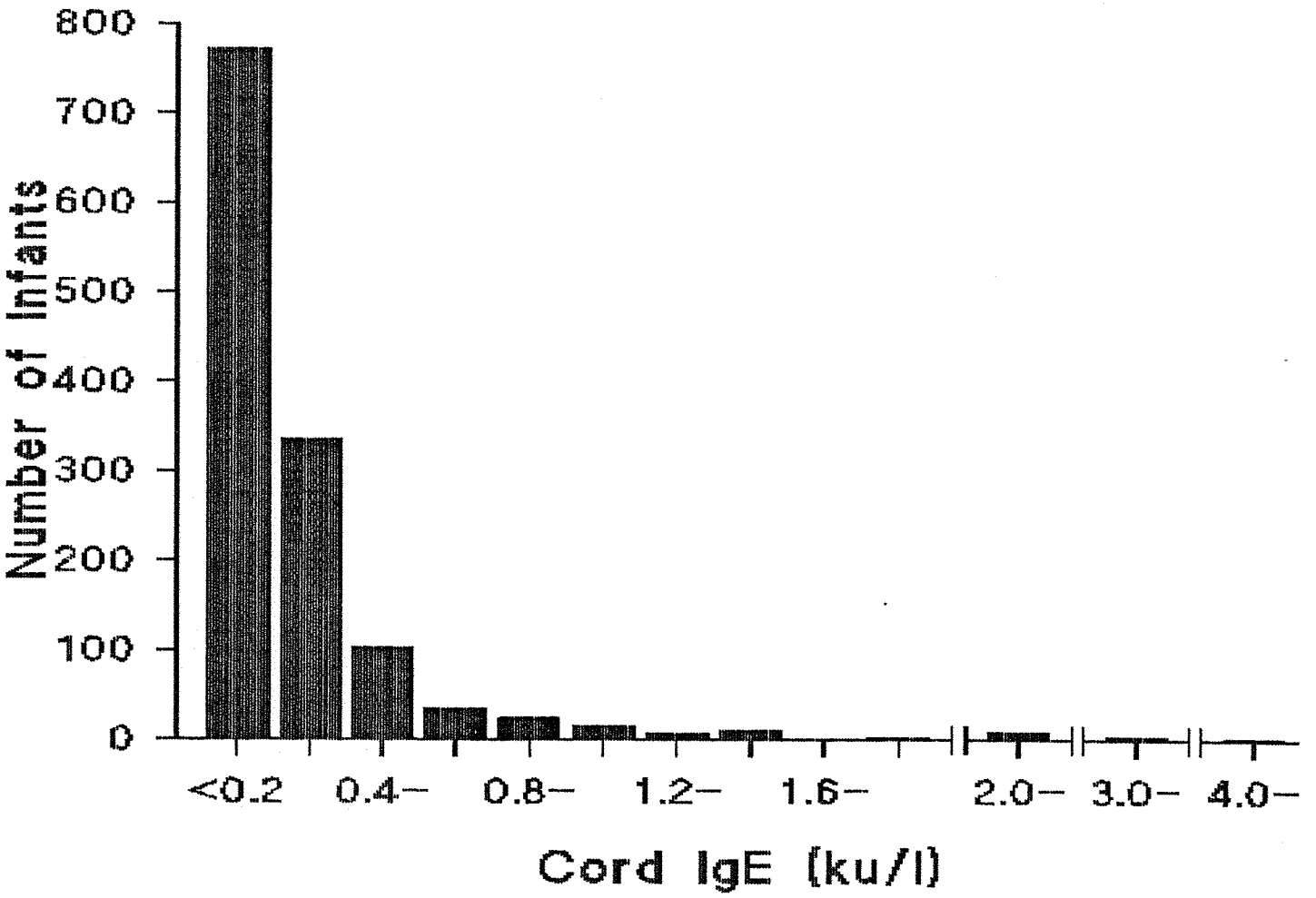

Fig. 6.1. Frequency distribution of Cord blood total IgE values in an infant population (n=1319).

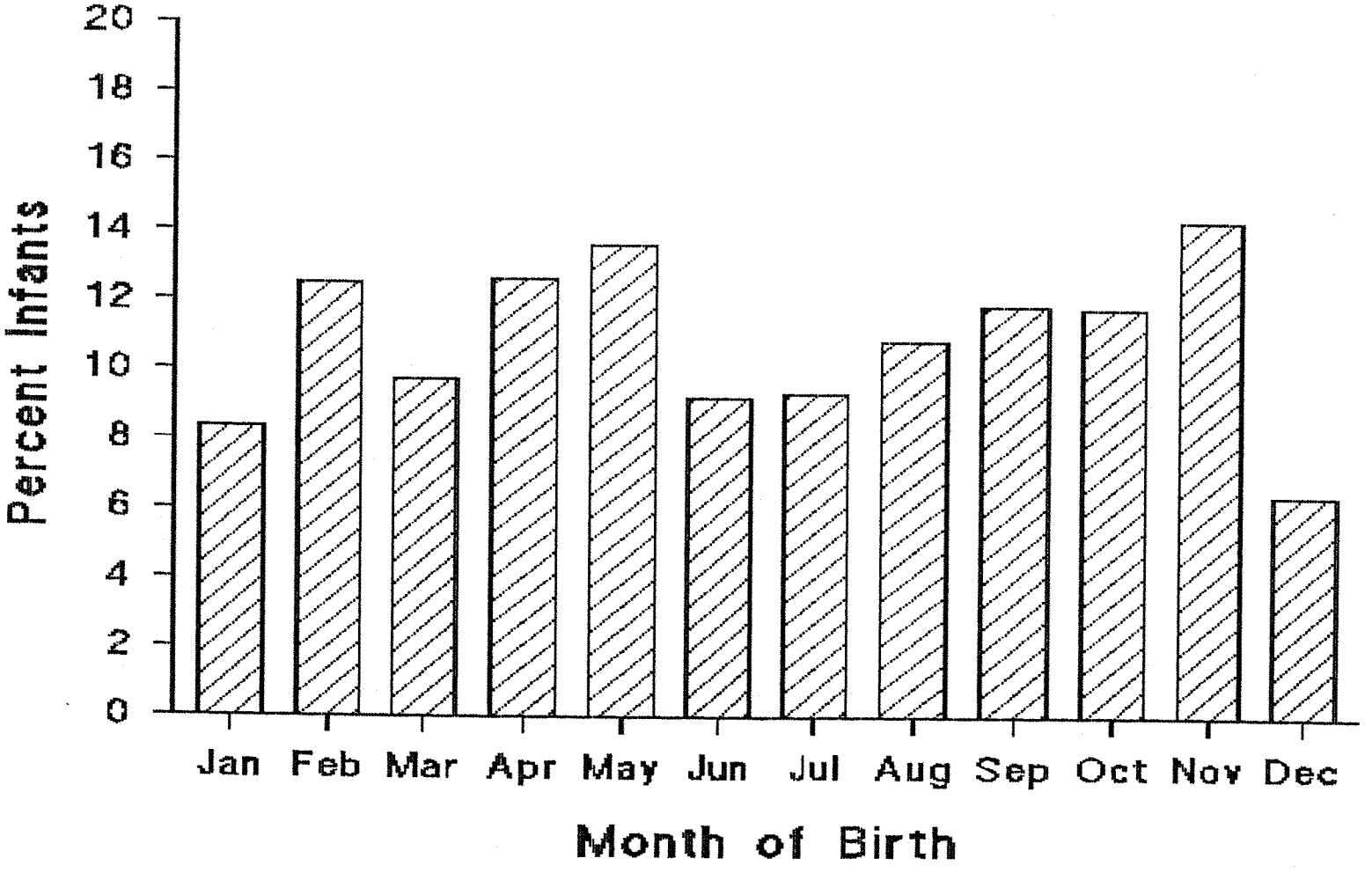

Fig. 6.2. Percent ihfants with high (>0.5 ku/l) cord lgE born in each calendar month.

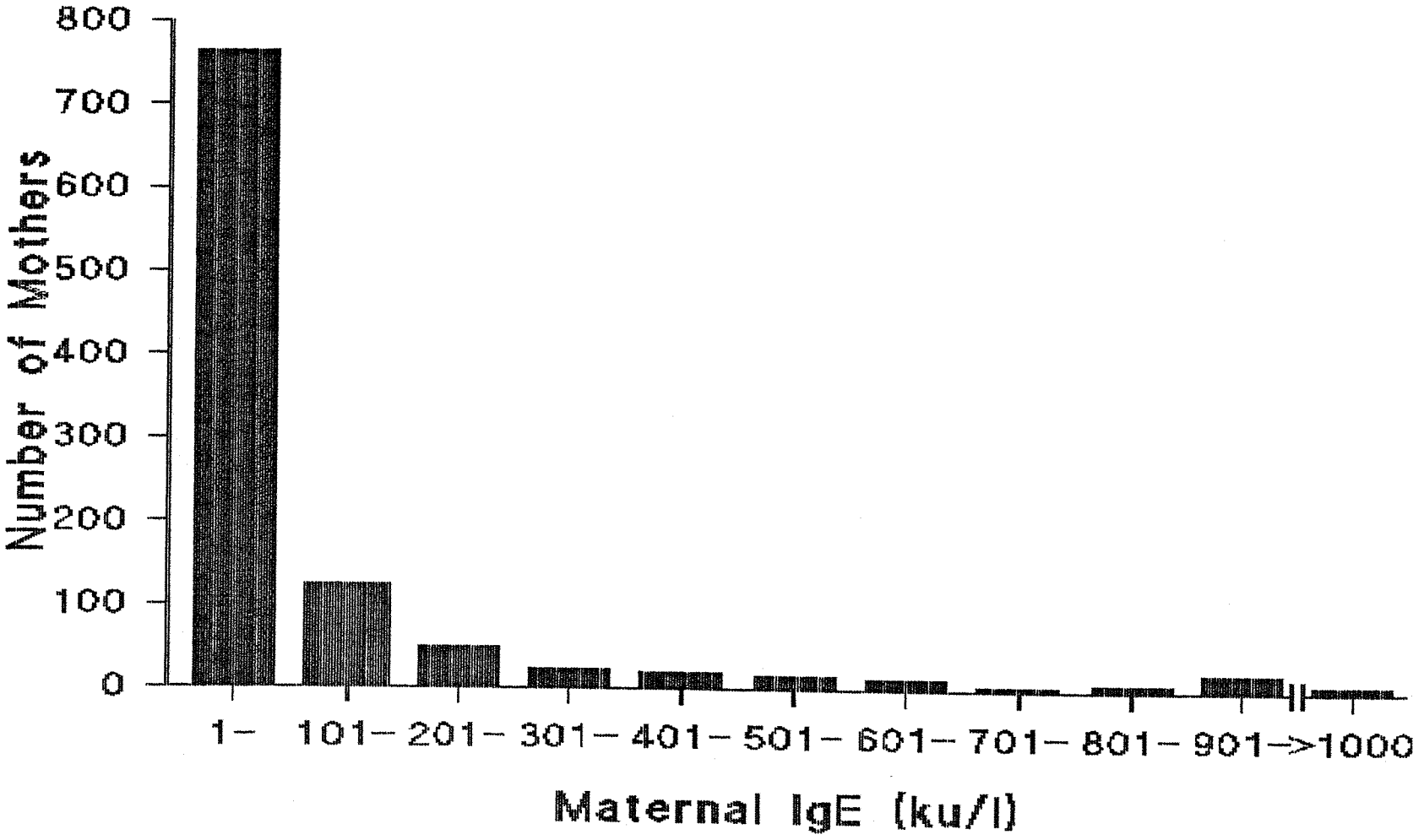

Fig. 6.3. Frequency distribution of Maternal IgE values (n=1056).

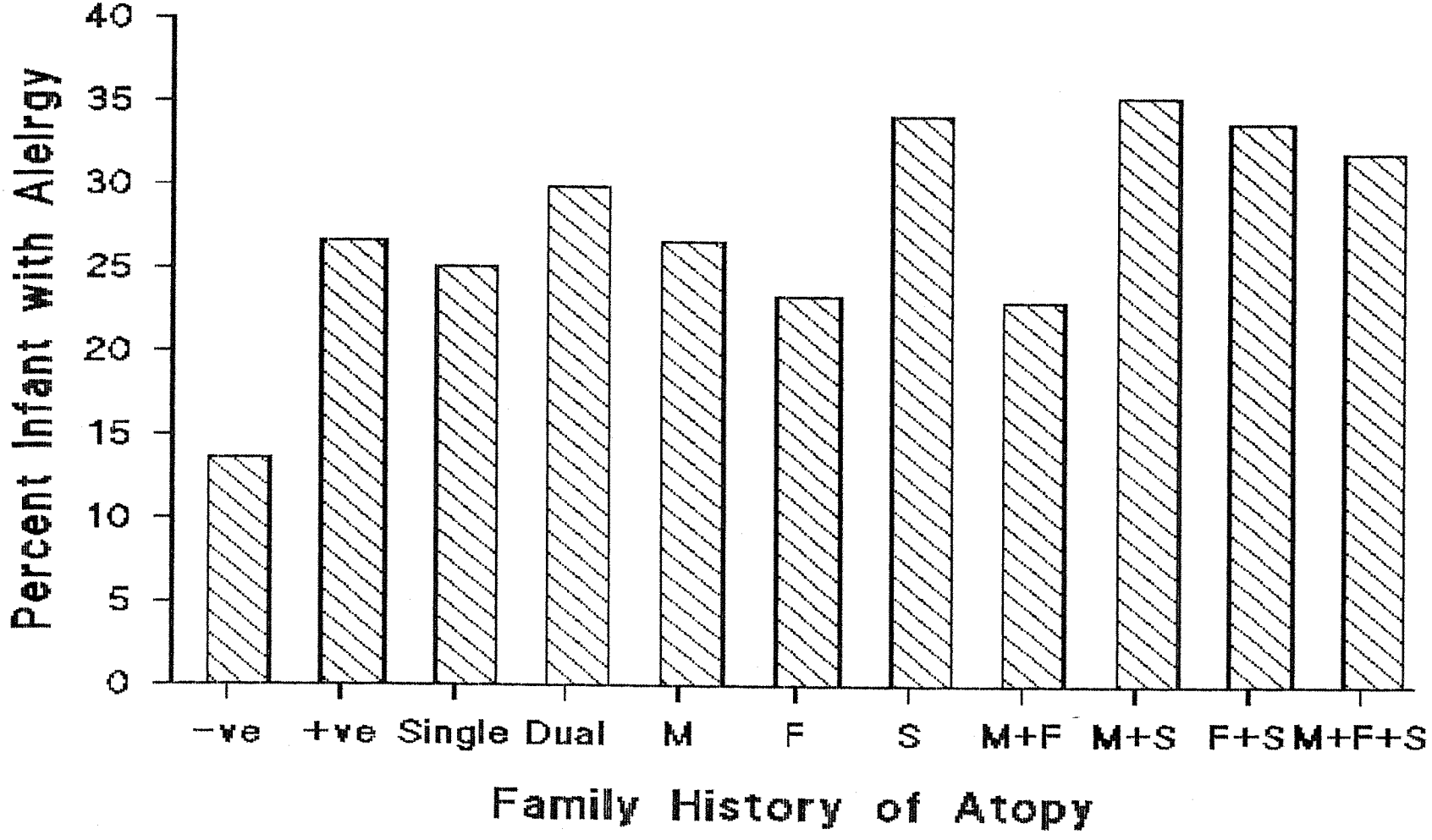

Fig. 7.1. History of atopy in the immediate family and allergy in infancy. (M=Mother, F=Father, S=Sibling)

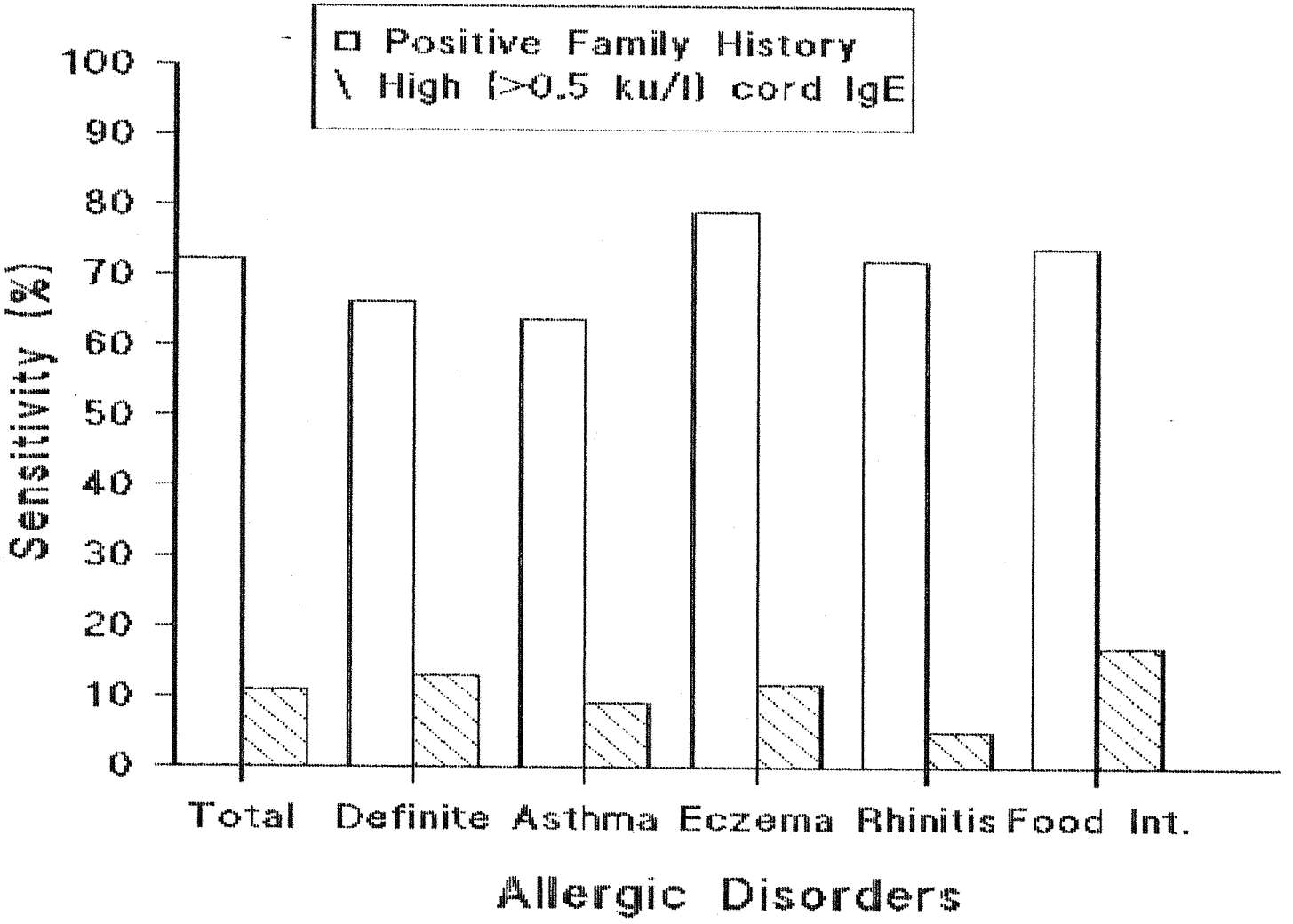

Fig. 7.2. The sensitivity of family history of atopy and high cord IgE for the development of allergic disorders in infancy. (Food Int.=Food Intolerance)

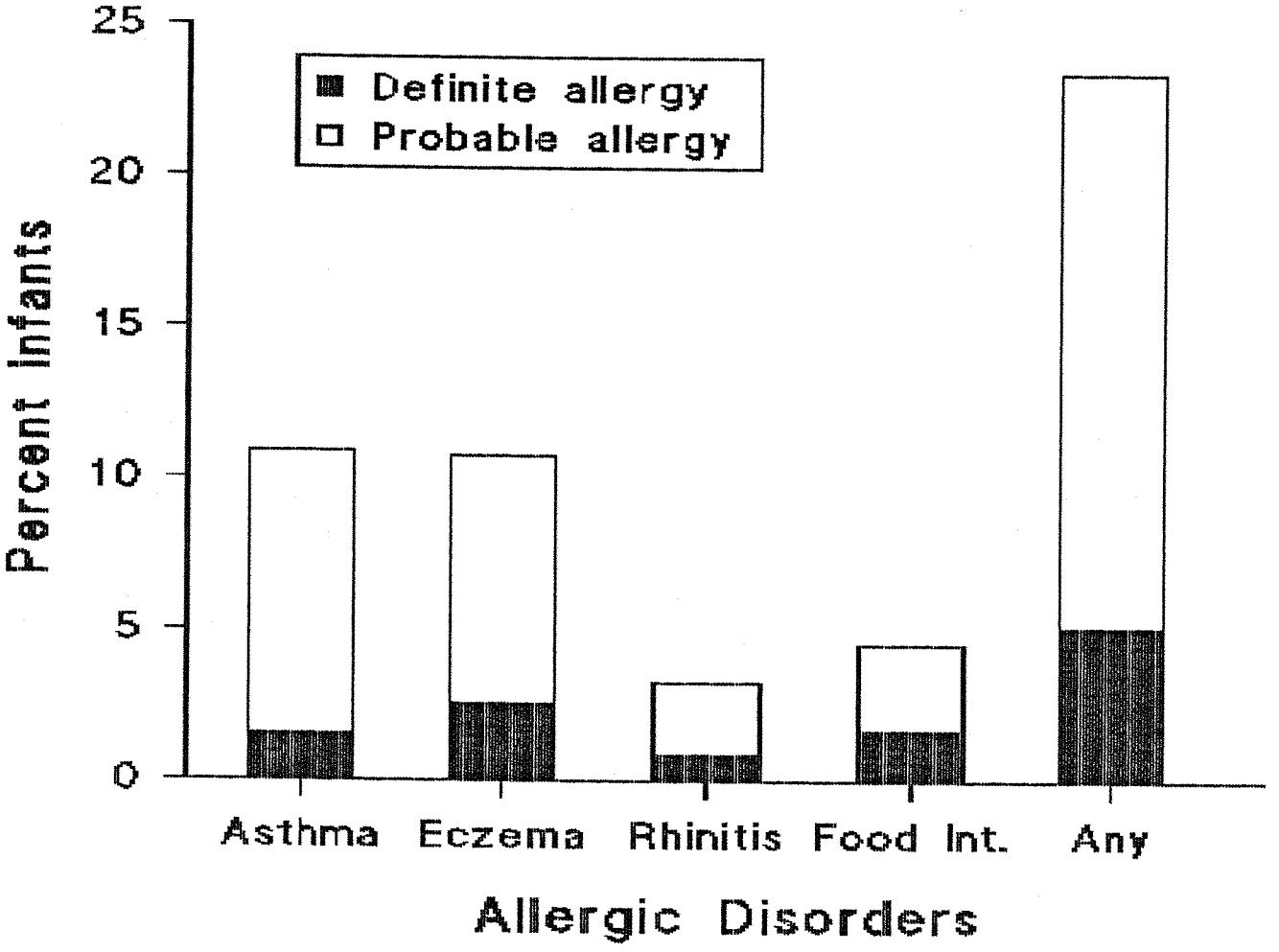

Fig. 9.1. Prevalence of allergic disorders during the second year of life. (n=1174)

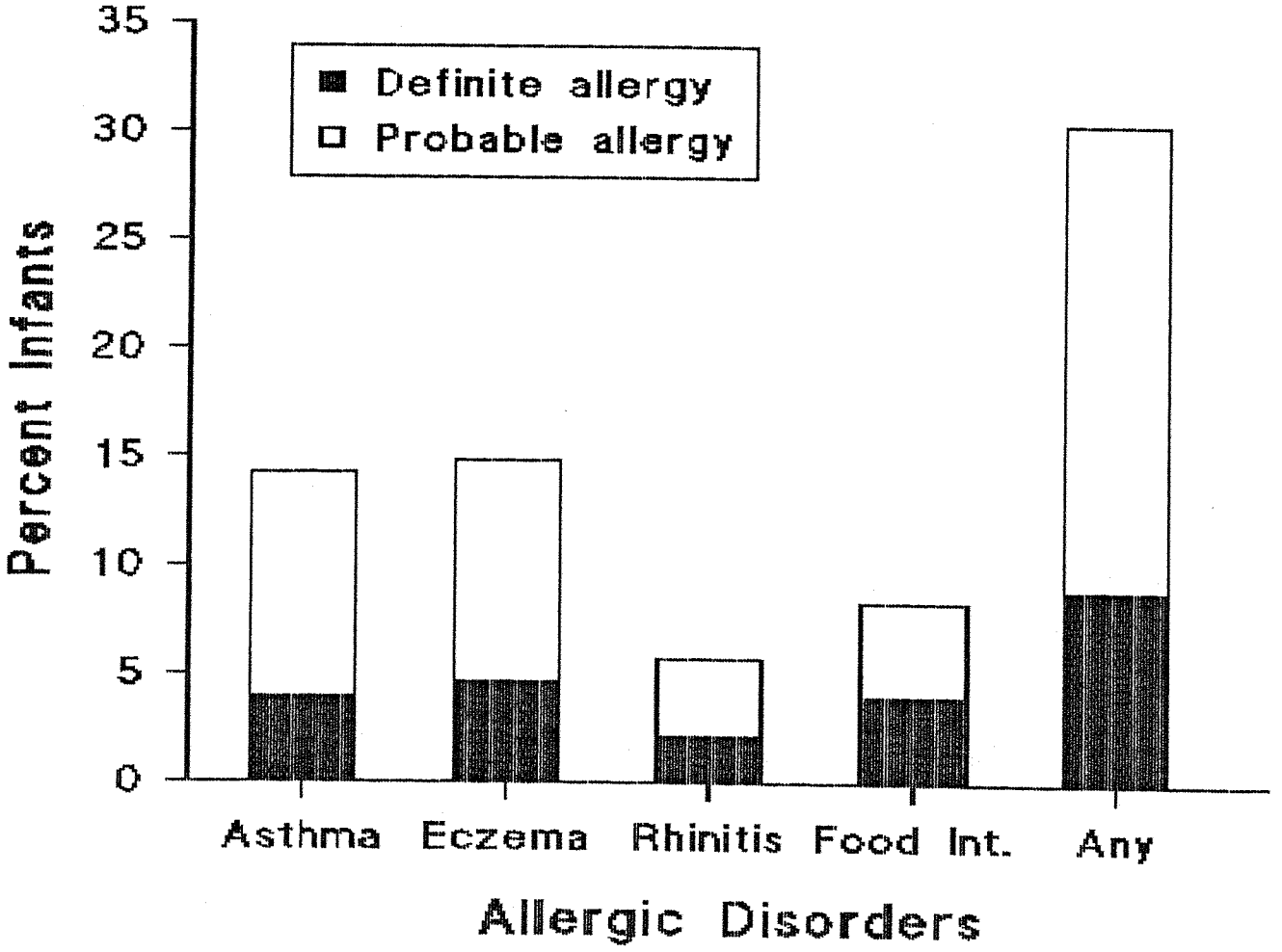

Fig. 10.1. Cumulative prevalence of allergic disorders during the first tw years of life (n=1174).

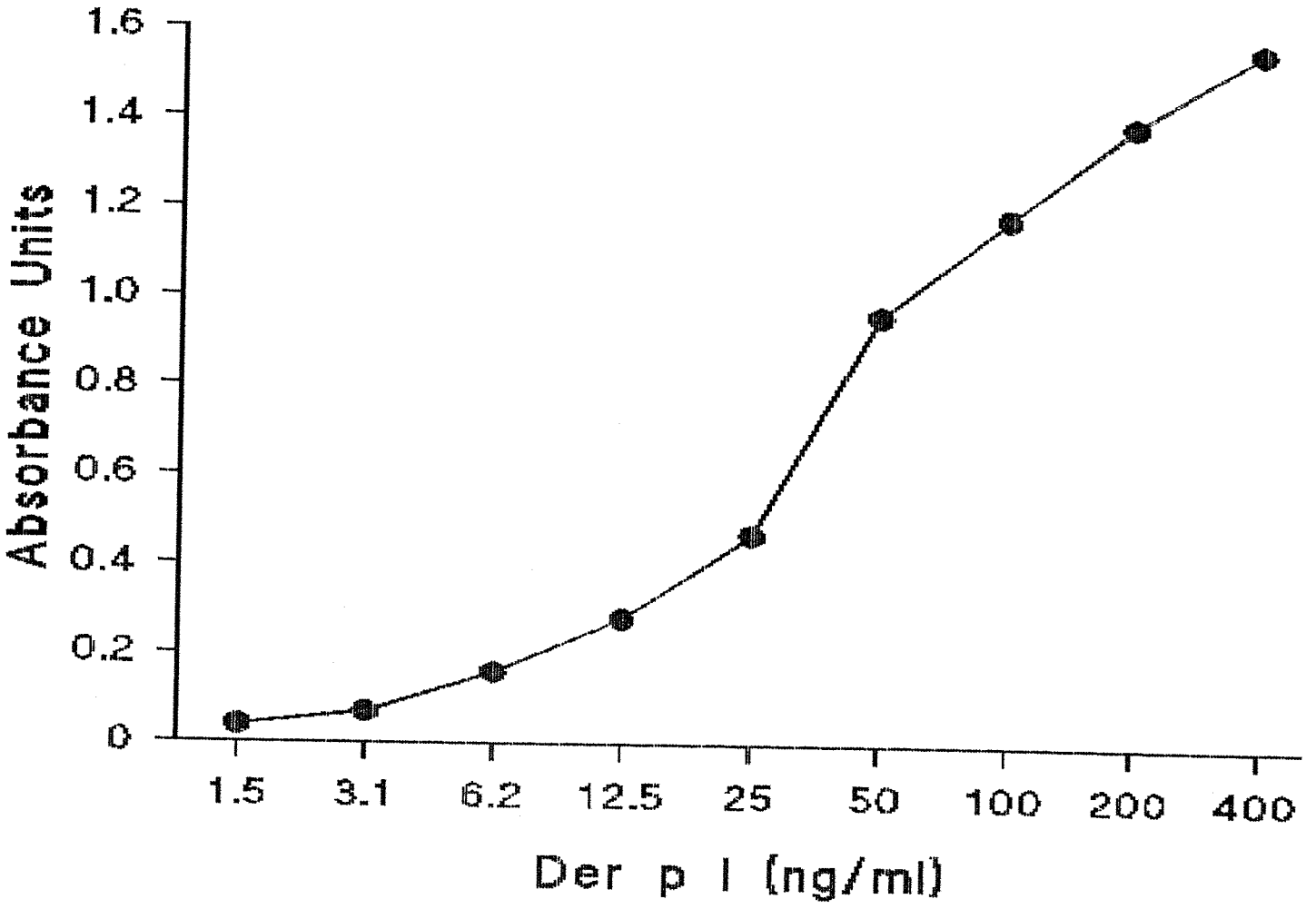

Fig. 11.1. Standard curve for ELISA Der p I test.

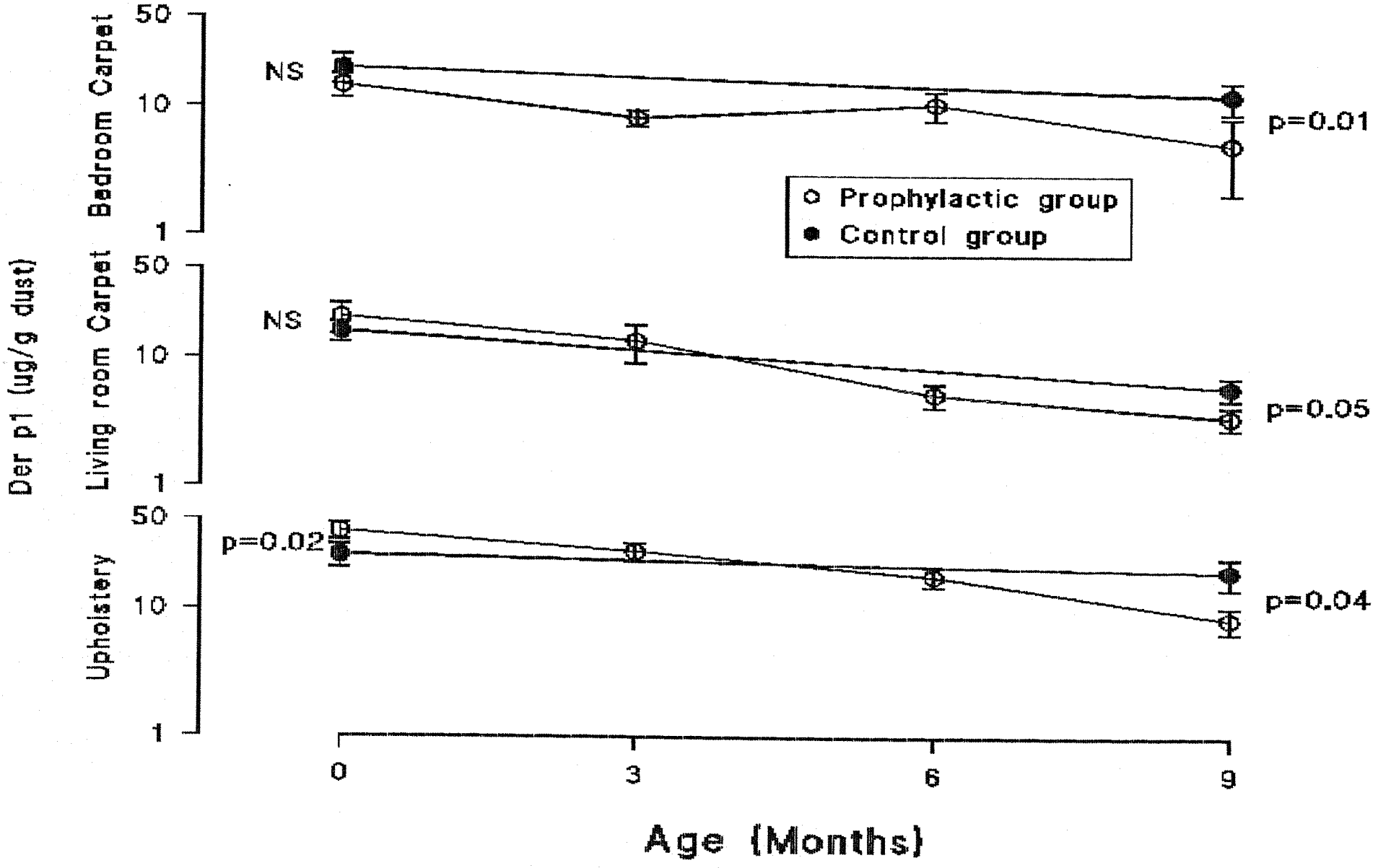

Fig. 11.2. Mean (±SEM) Der p I values in two groups from birth to 9 months.

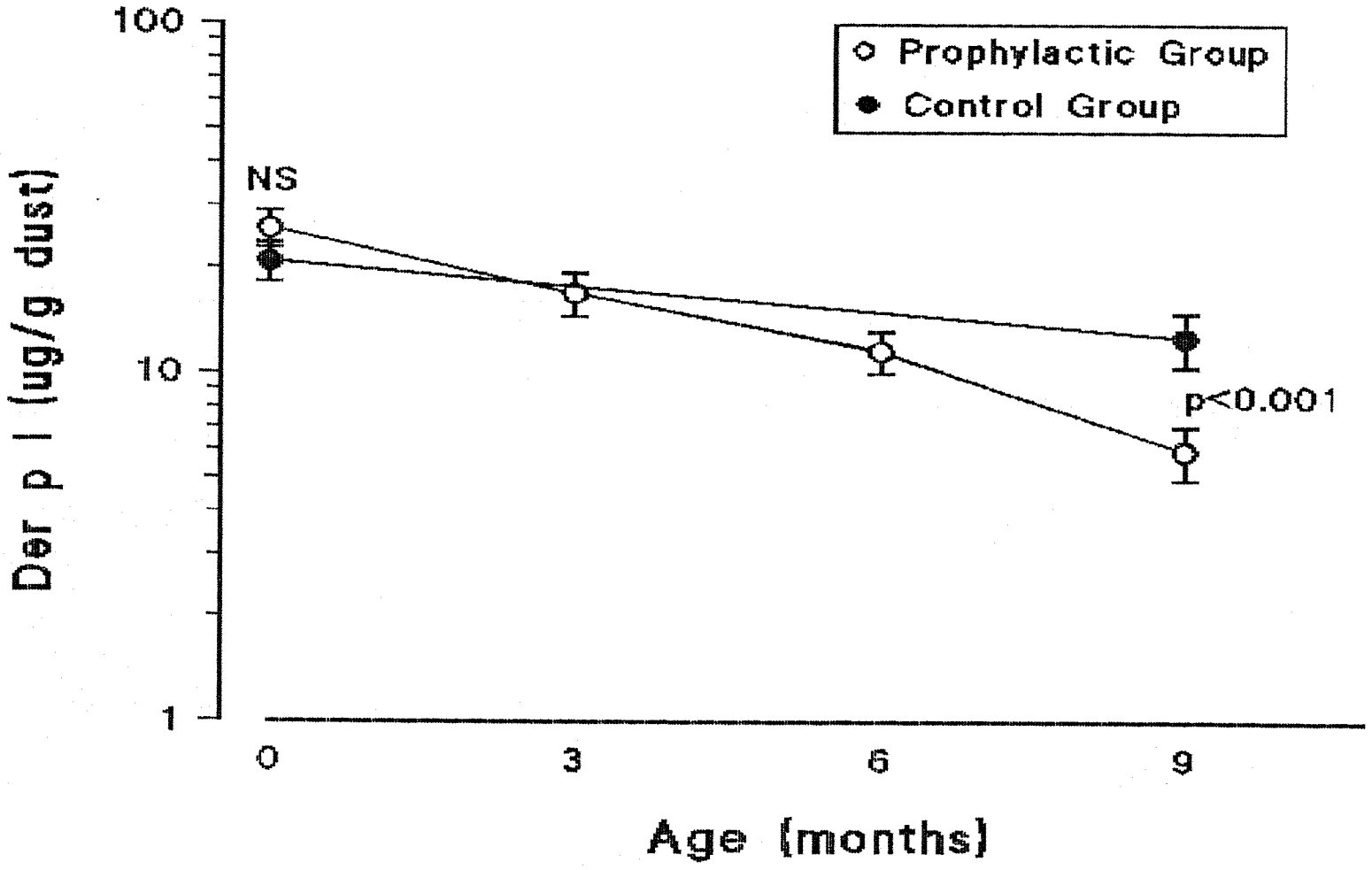

Fig. 11.3. Mean (± SEM) Der p I values of all areas at 0, 3, 6 and 9 months.

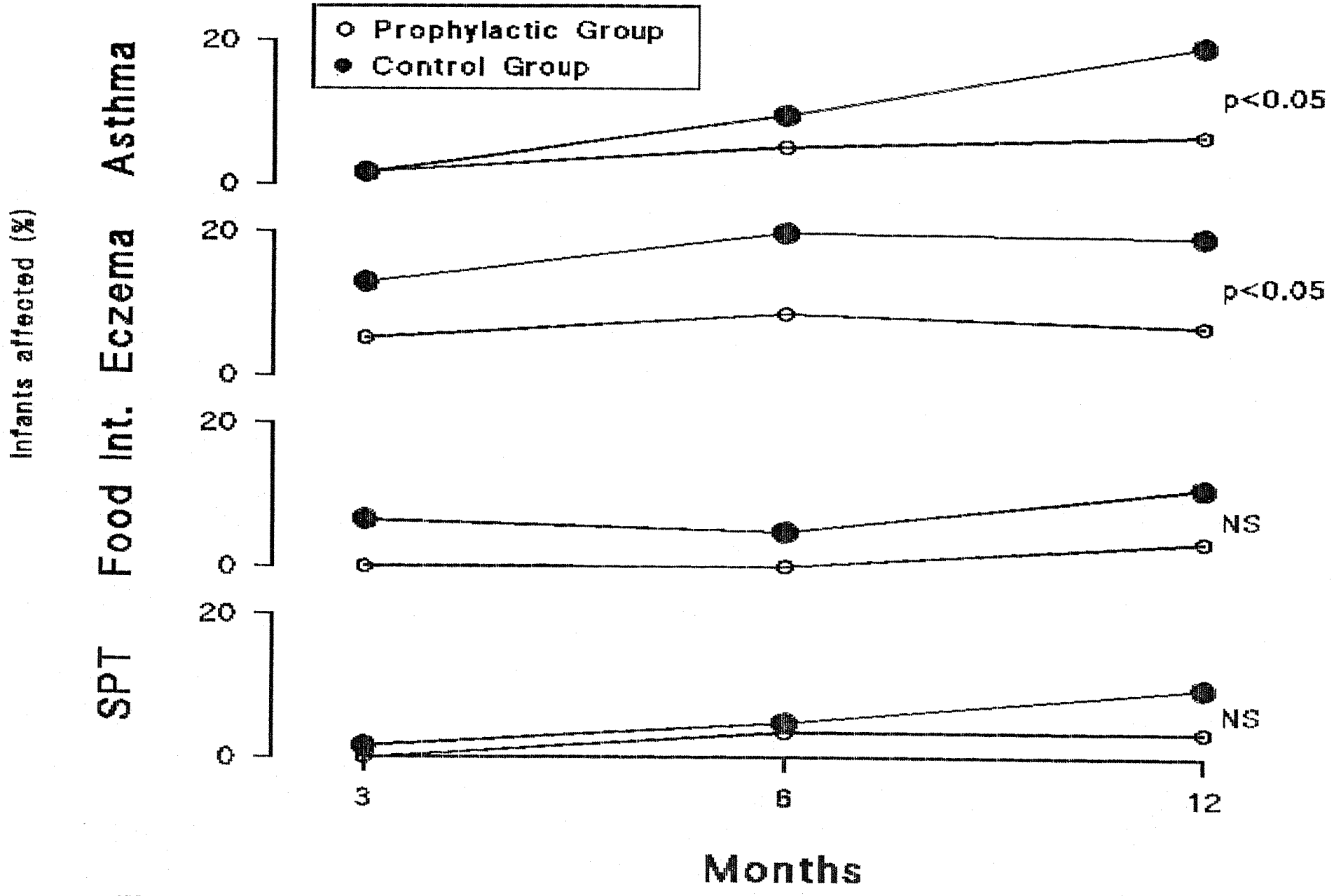

Fig. 11.4. Period prevalence of allergic disorders at 3, 6 and 12 months.

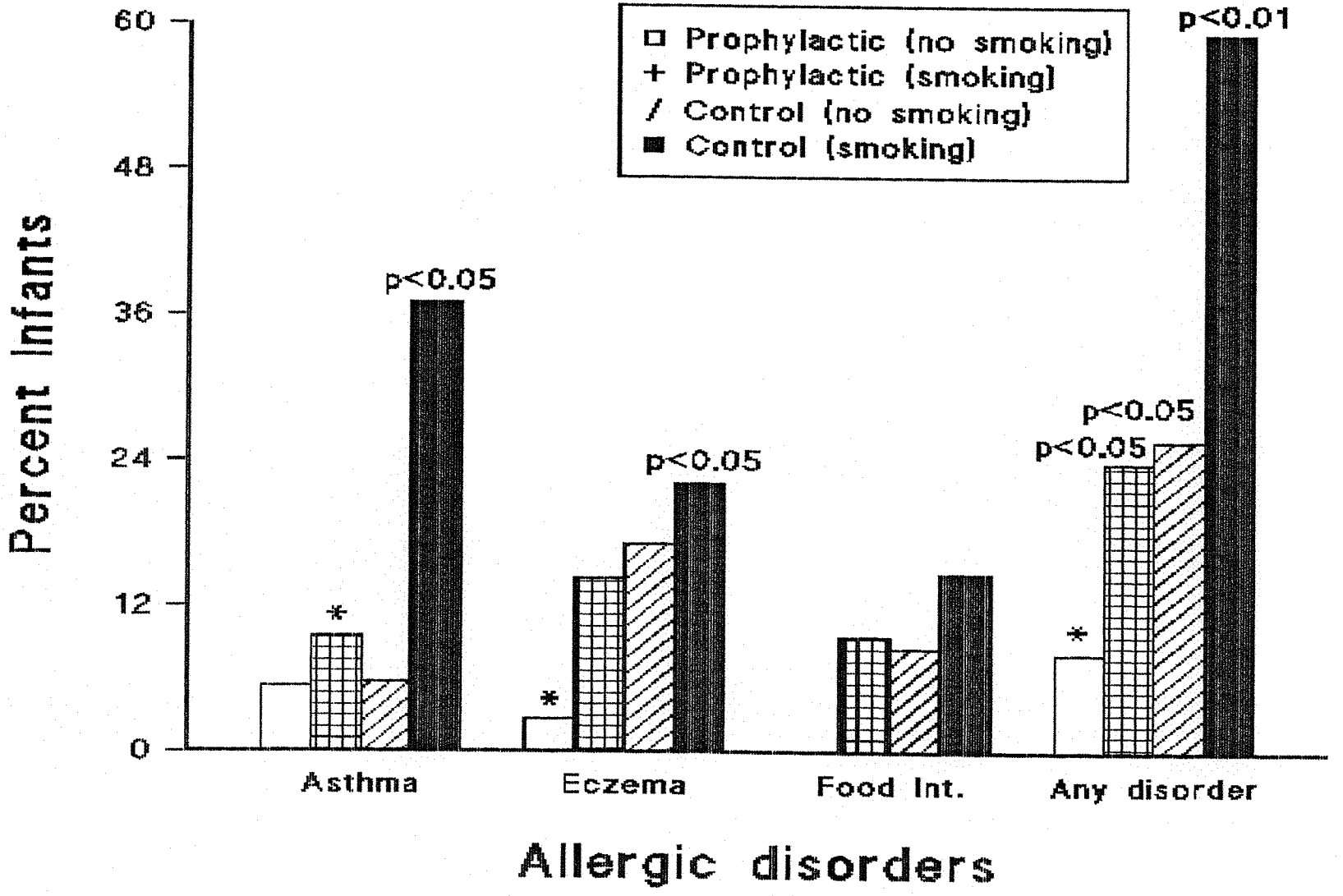

Fig. 11.5. Prevalence of allergic disorders in the prophylactic and control and control groups according to parental smoking.

(* = significant comparison made from this subgroup)