ALLERGEN-SPECIFIC IMMUNOGLOBULINS

DURING PREGNANCY

by

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A thesis submitted in partial fulfilment of the requirements of Sheffield Hallam University for the degree of Doctor of Philosophy.

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February 1993

ACKNOWLEDGEMENT

I would like to express my sincere thanks to my supervisor, Professor A E Bolton for his guidance, advice and for providing the facilities for this study. Professor Bolton's infectious enthusiasm in the field of pregnancy is undoubtedly an important factor in pursuing such a research project.

I am indebted to many of my colleagues, namely Dr A Milford-Ward, who kindly agreed to act as my second supervisor, Dr C L Corbett at Victoria Hospital, Worksop, for his support, Dr J M Rice-Oxley for his advice, Dr Mazzadri, of the State University, New York, USA, for access to his patients' medical records and finally, to Professor E Middleton, Jnr, who provided clinical and laboratory facilities at The State University of New York, to advance my clinical experience.

I would also like to express my sincere thanks to practice patients at Langold Health Centre for agreeing to participate in the study. Except for the continuous support and efficiency of the practice staff, this project would not have been possible. Mrs Marguerite Lyons has typed and formatted this thesis and Mr Dennis Johnson provided technical assistance.

Lastly, my thanks are due to my wife, Bani and children, Gayatri and Neil, who provided the initial impetus for such a research project.

I would like to acknowledge the generosity of Pharmacia Diagnostics (Sweden) for providing test reagents at a reduced price.

This project was partially supported by a Travel Grant to the United States by the Wellcome Trust, London, in July 1990.

programme of research in partial fulfilment of the requirements for the Degree of Ph.D.

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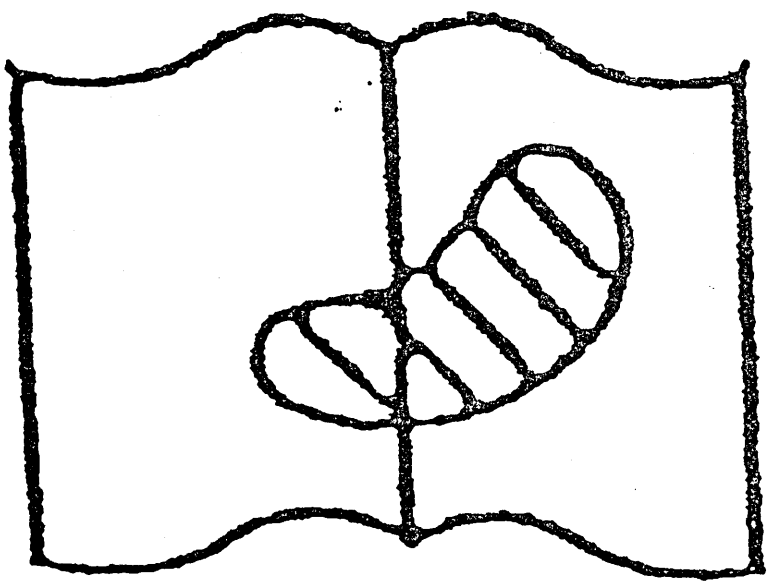
The Department of Health, UK, approved a period of nine months prolonged study leave to undertake this study project.

VARIABLE PRINT QUALITY



DEDICATION

This work is dedicated to my father

Sjt. Golokeswar Barua.

CONTENTS

Ab	Antibody
Ag	Antigen
A.S.	Ankylosing spondylosis
A-S	Allergen specific
A.N.A.	Anti-nuclear antibody
°C	Degrees Celsius
C_3C_4	Complement C_3 and C_4
CV	Coefficient variation
cAMP	Cyclic 3′,5′-adenosine monophosphate
d	Diameter
ECF-A	Eosinophilic chemotactic factor of anaphylaxis
Fc	Antibody receptor binding portion of antibody
g	gram
Hb	Haemoglobin
HCG	Human Chorionic Gonadotrophin
HETE	Lipid Chemotactic Factor
HLA	Human Leucocyte Antigen
HPETE	Hydroxyperoxy eicosatetraenoic acid
HPL	Human placental lactogen
Ig	Immunoglobulin
IgG	Immunoglobulin G
PAF	Platelet Activating Factor
PG	Prostaglandins
PGG_2	Prostaglandin G_2
PGH_2	Prostaglandin H_2
PP	Placental Protein

PNU	Protein Nitrogen Unit
PRU	Protein Reference Unit
RA	Rheumatoid Arthritis
RIA	Radioimmunoassay
SLE	Systemic Lupus erythrometosis
SRSA	Slow Reacting Substances of Anaphylaxis
TAME	Toxyl-N-arginine-methyl-ester
TXB	Thromboxenes
Vol	Volume
wt	weight
IgG4	Immunoglobulin G Sub-Class 4
IgE	Immunoglobulin E
IL-1	Interleukin 1
IL-2	Interleukin 2
IL-4	Interleukin 4
IL-5	Interleukin 5
IL-6	Interleukin 6
IVF	In vitro fertilization
kDa	Kilo Dalton
kU	Kilo Unit
L	litre
LR	Low Responder
LTA_4	Leukotriene A_4
mg	milligram (10^{-3} gram
ug	microgram (10^{-6} gram)
ul	millilitre (10^{-6} litre)

MR	Molecular Radius
NCF-A	Neutrophil Chemotactic Factor of Analphylaxis
nm	nanometric (10^{-9} metre)

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Historical Background

The first scientific approach to hay fever was documented and published in the "Medico-chirugical Transactions of London" in 1819 by John Bostock. It was entitled "Case of a Periodical Affection of the Eyes and Chest" and was an account of a single case, the patient himself, a London physician. In 1828 Bostock termed the illness "hay asthma" and heat was thought to be the causative factor. Gordon in 1829 attributed the cause to "the aroma emitted by the flowers of grass". However, his view was not widely accepted by the medical profession until 1859 when further research by Professor Von Philip of Geissen reinforced this concept. In 1873, Charles Blackley, a Manchester homeopathic physician, and a sufferer himself, published the classic book, "Experimental Researches on the Cause and Nature of Catarrus aestivus (Hay Fever or Hay Asthma)", and identified pollen as the causative factor.

The term allergy, which is derived from the Greek 'allos' (altered) and 'ergon' (reaction) was first used by the German pathologist Clemens Peter Von Pirquet in 1906. Since that time the immunological basis of allergies has been slowly unravelled. In 1913 Henry Dale made the fundamental discovery that the anaphylactic contraction of plain muscle was begun by antibodies fixed on cells, a concept that was totally foreign to the scientists of the time. Dale showed that the anaphylactic reaction released histamine, heparin and other substances. Frankland used controlled trial methods at St Mary's Hospital, London, to show the potential effectiveness of vaccines and also the role of released histamine and other inflammatory mediators.

Atopy or "out of place" was the term used by two Americans, Coca and Cooke, in 1923, referring to the differences between the allergic and non-allergic population.

Since Dale's day allergists have been aware of a possible "reaginic antibody" but it was not until 1966 that Ishizaka and colleagues confirmed Immunoglobulin E as the definitive reaginic antibody. Further work on the immunological aspects of allergy identified immunoglobulin G (IgG) as having a protective or "blocking" role in the allergic reaction. Sub-class IgG4 was further investigated as the possible specific antibody sub-class involved in blocking the allergic reaction.

In this study the serum concentration of IgE and IgG4 (total and allergen specific taking Timothy grass pollen as the model allergen) have been investigated prospectively during and after pregnancy in healthy women and women suffering from allergic rhinitis.

The results show that the total serum IgG concentration remained unchanged in both groups during pregnancy. There was no significant difference in the serum concentration of IgG 4 between pregnant allergic women and non-pregnant allergic women. Levels of IgG4 were approximately twice as high ($p< 0.01$) in non-allergic pregnant women compared to the non-allergic non-pregnant control group. Total IgG4 concentrations were similar in allergic and non-allergic women during pregnancy; however, in the non-pregnant state allergic women had significantly ($p = 0.017$) higher levels of IgG4 than non-allergic women.

The results show that both during pregnancy and in the non-pregnant state there was a highly significantly ($p<0.001$) greater serum concentration of total IgE in allergic than non-allergic subjects. Although the level of IgE was significantly ($p=0.004$) lower in pregnancy, the differences are relatively small and seem unlikely to be of great physiological significance. Allergic symptomatology did not correspond to IgE levels during pregnancy. In both the pregnant and non-pregnant women the serum concentration of antigen-specific IgE was highly significantly greater in the allergic than the non-allergic subjects. However, in allergic women, the concentration of antigen-specific IgE was very much lower during pregnancy, at about 6% of the non-pregnant level ($p<0.001$). The concentration of antigen-specific IgG4 was also reduced in pregnancy in allergy sufferers, being about half of the level found in the non-pregnant individuals ($p<0.001$).

There appeared to be an increase in spontaneous first trimester abortion in women who suffered symptoms of allergy. From the case histories of all 418 pregnancies at the Langold Health Centre ante-natal clinic attending between September 1976 and December 1990, 192 were to allergy sufferers and 226 were to normal women. The abortion rate was 16.7% in the allergic group and 5.3% in the normal pregnant women ($p < 0.001$).

CHAPTER ONE

THE IMMUNOBIOLOGY OF ALLERGIC RESPONSE

1.1 Atopy and the Allergic Reaction.

Atopic individuals mount an immune response to an environmental antigen with the production of IgE. When this specific IgE interacts with the eliciting allergen, cross-linking of the F^{ϵ} receptors on mast cells occurs, resulting in their degranulation and release of preformed mediators of the allergic reaction such as histamine and heparin. In addition, newly-formed mediators, such as certain prostaglandins and leukotrienes, are synthesised. Mast cell triggering also requires lymphokines, particularly IL4, produced by activated lymphocytes.

T-cell responses to mitogen stimulation are reduced in some atopics, and both histamine and some prostaglandins can suppress in vitro mitogen-stimulated lymphocyte transformation. Both defects of T-cell activity and abnormal responses to mediators of allergy have been implicated as the cause of allergic reactions. Chowdury and Chandra (1989) recently reviewed studies on immunoregulatory T-cell abnormalities in patients with atopic disease. Of 18 studies, 12 reported a reduction of total T-cell numbers and also showed that suppressor T-cells are reduced in atopic patients. In infants, the reduction of suppressor T-cell numbers precedes the development of allergies. Thus, a deficiency of suppressor T-cells would result in an increase in IgE production and thus enhance the risk of atopy. In addition to control at the cellular level, the function of IgG4 as a blocking antibody in the allergic reaction in general and among atopic individuals in particular is now well established.

1.2 Types of Hypersensitivity Reactions.

In 1963 Gell and Coombs classified hypersensitivity reactions into four types - Types I, II, III and IV. A fifth type, 'stimulatory', has since been added

in which non complement-fixing antibodies directed against certain cell surface components may actually stimulate rather than destroy the cell. Theoretically stimulation could also occur through the development of antibodies to naturally occurring mitotic inhibitors in the circulation (Roitt, 1984). Table 1 (page 4) shows the important features of reaction Types 1-1V.

Type I reactions are discussed in full detail in Section 1.3.

1.3 Development of Type 1 Hypersensitivity Reactions. (IgE Mediated Hypersensitivity).

Such allergic reactions proceed in three phases - the sensitization phase, the activation or challenge phase and the effecter phase.

Phase 1 - The Sensitization Phase.

The reaction begins when a genetically pre-disposed individual encounters a protein antigen of the appropriate molecular configuration to serve as an allergen. Such allergens can be airborne or ingested or arrive in the blood stream directly, e.g. by injection. At this point, interaction with cells of the immune system must occur for sensitization to be completed. IgE antibody synthesis is a T cell-dependent immune process. Antigen-presenting cells, often macrophages, present processed antigen in association with MHC class II molecules on their surface to helper T-cells (TH). IL-1, secreted by the macrophages at the time of antigen presentation, stimulates precursor helper TH cells to differentiate and mature. Mature TH cells secrete a second soluble factor, interleukin 2 (IL-2), which promotes clonal expansion of TH cells and stimulates the differentiation of B cells into antibody-secreting plasma cells. IgE antibody synthesis is a T cell-dependent immune process. Thus, cooperation

TABLE 1

Types of Hypersensitivity Reaction.

Type I	Type II	Type III	Type IV
Immediate anaphylactic	Cytototoxic	Antigen-antibody	Delayed hyper-sensitivity cell-mediated
Onset within 10 minutes	Onset after several hours	Onset in four to six hours	Onset after several hours or days
Resolves in three hours	Duration 24-48 hours	Maximal at six hours, fades over next few hours	Extended duration
Examples: Hay Fever/ Bee/Wasp stings	Examples: Hypersensitivity Haemolytic disease of the newborn Transfusion reactions	Examples: Serum sickness Allergic aspergillosis Industrial inhalants Farmers lung Asthma	Examples: Tuberculosis Asthma Contact eczema Asthma

From "Allergy Therapeutics". Eaton et al., (1982)

takes place between B and T lymphocytes and antigen presenting cells in the induction of an antibody response to most protein antigens.

Once this process is complete, each plasma cell elaborates antibody of a single antigen-binding specificity, although switching between immunoglobulin classes can occur. The IgE antibody molecules produced are specific and interact through their Fab region only with the antigen that stimulated their synthesis (Ishizaka et al., 1975).

Synthesised IgG antibodies either sensitise mast cells locally at the site of IgE production or are transported in the blood and sensitise mast cells and basophils distal to the site of production. Sensitisation occurs when IgE binds to high affinity Fc receptors previously on mast cells, but also on blood basophils. Blood-borne IgE antibodies are synthesized in respiratory or gastrointestinal lymphoid tissue and consequentially bind to mast cells in the skin and other organs. The binding of IgE with mast cells is reversible.

Phase 2 - The Activation or Challenge Phase

Following a second exposure to and interaction of sensitized host mast cells with an allergen, the antigen bridges two adjacent cell bound IgE molecules of identical specificity and brings the two receptor molecules into proximity (Fadal et al., 1981). This cross-linking of cell-bound IgE results in complex, enzyme-associated biochemical events which culminate in a decline of intracellular cAMP concentration and the facilitation of degranulation of mast cells with the release into the micro-environment of granules containing histamine and other preformed mediators into the micro-environment.

Phase 3 - The Effector Phase.

The principal preformed mediators released during the degranulation of mast cells are histamine and heparin. Acting via H1 receptors histamine causes constriction of smooth muscle resulting in, for example, bronchoconstriction and increased capillary permeability. Acting via H_2 receptors histamine stimulates mucus gland secretion. Heparin has a blood anti-coagulant effect. The eosinophil chemotactic factor ECF-A, also a preformed mediator, results in eosinophil accumulation. The histamine enzyme of eosinophils may act in a regulating capacity. Other observed phenomena, such as platelet aggregation, neutrophil accumulation and other post-inflammatory activities are mediated by newly synthesised products of mast cells.

1.4 Immunoglobulins and Allergens

Of the five classes of immunoglobulins, two, IgE and IgG, are of proven importance in allergic reactions.

1.4.1 Immunoglobulin E - The Human Reaginic Antibody.

The presence of reaginic antibodies in the sera of atopic patients was first demonstrated by Prausnitz on his patient Kustner. In 1966, Ishizaka et al. discovered the presence of IgE and established its structure, properties and defined its role in allergic reactions. These immunoglobulins are present in small amounts in the peripheral circulation compared to IgG and IgM. Allergen-specific IgE occurs at even lower concentration and is present at picogram/ml levels. For comparison, IgE normally occurs at 1/10,000th - 1/500,000th of the concentration of IgG. 99.99 per cent of IgE in blood remains free and is not bound to blood basophils (Fadal 1985).

IgE is responsible for Type I immediate hypersensitivity reactions and is implicated in anaphylaxis, allergic rhinitis, allergic asthma, immediate food allergy, atopic dermatitis, hypmenoptera sensitivities and certain drug and chemical reactions. In previously sensitised patients, a small allergic stimulus may evoke immediate hypersensitivity reactions following the interaction of antigen and specific IgE antibody on the surface of the mast cell. This results in anaphylactic degranulation with granule content extrusion from either the mast cell or the basophil. Thus IgE acts as an efficient liberator of histamine and other pharmacological mediators. This release is dependent on the activation of adenyl cyclase within the plasma membrane of mediator-containing cells and does not involve complement activation or cell damage.

1.4.2 The Structure and Physiological Activities of IgE

IgE protein has kappa and lambda light chains and possesses characteristic antigenic determinants not shared by other immunoglobulins of known classes and sub-classes. The antigenic structure of IgE as well as association of antibody activity with the protein indicate that IgE represents a distinct immunoglobulin class. Physicochemically, IgE is a glycoprotein with a sedimentation coefficient of 8S and molecular weight of around 200,000. The protein is composed of two heavy and two light polypeptide chains. The specific antigenic determinants are present in the Fc portion of the heavy chains. The carboxyl terminal of the Fc portion of IgE is essential for sensitization, and IgE molecules combine with receptors on target cells through the Fc portion of the immunoglobulin molecules.

Some important biological activities of IgE are shown in Table 2 (page 9).

1.4.3 Regulation of IgE Production and Synthesis

The regulation of IgE production is not clear. B cells bearing IgE are detectable at 11 weeks in the fetus, but production of IgE in utero is negligible and IgE is undetectable in umbilical cord blood in most infants. Maternal IgE antibody does not efficiently cross the placenta. Observations confirm that children with increased IgE levels in the first year of life have a significantly greater chance of developing allergy later in childhood and also there is a familial tendency in allergic disease.

IgE is produced by T cell-regulated B cells, which transform into antibody secreting plasma cells. These are primarily in lymphoid tissue of the respiratory and gastrointestinal tract. Tonsils and adenoid tissue contain the highest concentrations of such cells. After local production, IgE is transported in the blood or diffuses into tissues and mucosal secretions. Once in the blood, only 0.01% IgE fixes to basophils and the rest circulates to other tissues for mast cell binding. The most important biological property of IgE antibody is the ability to sensitise homologous tissue for allergic reactions by reversibly binding with high affinity to specific membrane receptors on basophils and mast cells. Mast cells are of primary importance in mediator release and the cells thus precipitate allergic inflammation. Mast cells and basophils contain upwards of 80,000 IgE receptor sites per cell.

In normal human subjects, peripheral blood B-lymphocytes do not synthesise IgE. However, in atopic individuals peripheral blood lymphocytes

TABLE 2

IMPORTANT BIOLOGICAL PROPERTIES OF IgE

Tissue half-life	21 days
Serum half-life	2.5 days
Serum levels	< 0.5 mg/L
Distribution in secretions	+
Placental transfer	-
PK reactivity	+ (10 pg of antibody required)
Complement fixation	+ (alternate pathway only)
Receptors for Fc on	
Basophils	+
Mast cells	+
Molecular weight of heavy chains	72×10^3 Da
Carbohydrate	12 per cent
IgE molecular weight (Daltons)	19×10^4

(Fadal, 1985)

spontaneously synthesize IgE in vitro and in such individuals the concentration of IgE increases following exposure to allergen during the pollen season.

The IgE-specific regulatory T cells with helper or suppressor activity have been demonstrated to bear Fc receptors for IgE. These receptors are shed into the micro-environment and can be detected as IgE binding factors because of their ability to bind IgE. These IgE binding factors stimulate IgE-producing memory B cells and regulate their differentiation into plasma cells. Plasma cells secrete more IgE antibody or provide suppressor signals to shut off IgE antibody production. The balance between these two signals will determine the magnitude of the IgE antibody response. Helper T-cells (CD_4+ cells) and suppressor T cells (CD_8+ cells) are responsible for modulating such activities (Geha et al., 1984).

1.5.1 Immunoglobulin G (IgG) and its Subclasses

IgG is the major circulating immunoglobulin. There are four IgG subclasses, differing both immunochemically and functionally (Heiner, 1984). Different subclasses appear to relate to different disease stages (Oxelius, 1984). IgG subclass 4 has a role in allergic disease.

1.5.2 IgG Subclass 4 - its Distribution and Significance in Human Diseases

In a community survey of IgG4 antibody levels in the U.K. (Merrett et al., 1983) precise and specific radioimmunoassays were developed to quantify total IgG4 and IgG4 antibodies. It was demonstrated that men have significantly higher total serum IgG4 levels than women (mean value in males 0.581 mg/ml; in females 0.302 mg/ml) comprising 0.7% of total serum IgG, the range 15 mg - 185 mg/ml, (Shakib, 1975)) and quantitatively makes it the least abundant of

the four IgG sub-classes. Van Der Giessen (1975) reported that serum IgG4 levels increase slowly from birth until reaching adult levels around 12 years of age. Circulating levels of IgG4 antibodies with specificities directed against food components, especially egg and milk, are normally higher than those against common inhalant allergens, such as grass pollen, house dust mite and cat epithelium.

The role of IgG4 as part of general common mucosal immunological reactions and as protective in allergy in particular is gaining increasing support from the work of many investigators (Heiner 1980, Perelmutter 1983, Aalberase 1983). It has been suggested that sub-types IgG4a and IgG4b are responsible for the anaphylactic and blocking activities of IgG4 subclass immunoglobulins respectively (Gwenn, 1978; Perelmutter, 1983). In recent reviews (Halpern, 1983; Perelmutter, 1983) it has been postulated that the possible mechanisms for these activities are that IgG4 sensitises target cells for mediator release on antigenic challenge and at the same time acts as a protective antibody in allergic disease.

The role of IgG4 in allergy is still controversial and conflicting reports of IgG4 involvement in allergy are common. In studies on histamine release from basophils by anti-IgG4, three positive and one negative report have appeared. Similarly in the case of membrane receptors for IgG4 on mast cells three positive and three negative reports have been published (Nakagawa, 1983). In addition, the sera from atopic patients have been shown to be capable of positively sensitising leucocytes from non-atopics for histamine release with anti-IgG4 (Vijoy, 1978).

In order to assess the passive protection by IgG antibodies, Lessof et al., (1978) isolated the gammaglobulin fraction of plasma from bee keepers and injected it into five subjects allergic to bee venom. The bee keepers globulin conferred passive protection. However, this protection was transient. Nevertheless a rapid IgG antibody response was noticed and confirmed that IgG antibody may indeed have a 'blocking' role. Kemney (1983) studied the role of different sub-classes of IgG in an attempt to categorise the role of the antibody response. His study showed, as Aalberse (1983) had initially demonstrated, that an initial IgG1 response to bee venom switches to a more sustained IgG4 response.

Heiner (1980) was the first investigator who produced evidence that IgG4 might be a protective or blocking antibody as well as a skin sensitising antibody when he examined the IgG4 serum levels in novice and experienced bee keepers. Since then a number of papers have been published on IgG4 and bee keepers. With every bee sting roughly 50 ug of venom is deposited under the skin. In a sensitised subject the reaction to a bee sting may range from a mild or a severe local reaction, to a generalised response entailing urticaria, angiodema, asthma or life threatening anaphylaxis. In a recent study published in Sweden, Lojmnitzer et al., (1988) suggested that there may be two immunological types of bee allergic patients. The first type behave as expected by producing high levels of IgE, IgG and lymphocyte proliferation in response to the bee venom. The second type, however, show very low levels of these immunological responses. There was no clinical differentiation in both groups as they had equally severe allergic reactions and positive skin tests to bee venom.

Jarisch (1986) has shown that non-allergic volunteers produce either no response to bee venom injections or a transient IgE antibody response, which disappears within six months. In contrast, after being stung by live bees, atopic people with a tendency to allergy produce a much more vigorous and prolonged sensitising response, which can also be seen in some non-allergic subjects. Inevitably this may be seen as supporting evidence for the view that allergy is a failure of the mechanism that normally suppresses IgE after the first sensitisation has passed but might still be goaded into action by repeated injections of antigen (Lessof, 1986).

1.5.3 Structure, Antigenic Characteristics and Distribution of IgG Sub-Class 4

The four human IgG sub-classes differ in the number and/or distribution of interchain disulphide bonds, which undoubtedly influence their varying susceptibility to proteolysis. Soloman and co-workers (1978) found that IgG2 and IgG4 were more resistant to digestion by neutrophil elastase than were IgG1 and IgG3 and suggested that this stability contributed to effective phagocytosis and bacterial killing in tissues in which there is active inflammation.

It has been suggested that the carbohydrate prosthetic group of the four human IgG sub-classes may have inherent, distinctive structural differences which could be of biological significance (Stanworth, 1983). For example, in addition to the differences in amino acid sequence within the heavy chains of the four human IgG sub-classes, other genetic differences are seen. These allotypic differences in IgG4 are divided into two types, IgG4a and IgG4b. These antigenic determinants, located in the heavy chain, are related to

determinants shared with other IgG sub-classes. It has been shown that the distinctive antigen of the common 4a type is shared with all IgG1 and IgG2 sub-classes (Kunkel, 1970). In addition, IgG4 is distinguished from the other three IgG sub-classes at the physicochemical level by its faster electrophoretic mobility.

Biologically, IgG4 cannot activate the classical complement pathway, differentiating it from the other IgG sub-classes. Studies have shown that the Clq binding site is present on IgG4 protein but this is shielded by the molecules Fab region (Stanworth, 1983). It has also been reported that IgG4 is functionally univalent, this being discovered when testing IgG4 antibody in a system with antigen coated polystyrene tubes and radio-labelled antigen (Aalberse, 1983). This univalent characteristic is shared by IgE molecules. On the other hand IgG sub-classes 1, 2 and 3 are bivalent and can readily form insoluble immune complexes.

1.6.1 The Primary Effectors of Immediate Hypersensitivity: The Mast Cell

The mast cell was first described by Ehrlich in 1877 and is involved in a wide variety of inflammatory processes. Mast cell degranulation was observed in the 1930s following intraperitoneal injection of various irritants in rats which induced generalized urticarial reactions. The reaction appeared to be anaphylactic, a phenomenon first described by Portier and Richet in 1902. In man, mast cells are thought to be derived either from lymphoid precursors, thymic epithelial cells, histiocytes, undifferentiated mesenchymal cells or reticular cells (Mitchell, 1983). In the mouse, mast cells appear to be of bone marrow origin. Their precursor cells leave the marrow and enter the tissues where they slowly differentiate into mast cells. However, as

lymphocyte-derived factors can promote proliferation in tissue culture, at least some mast cells appear to be thymic dependent (Ginsburgh et al., 1978). Mast cells are ovoid-shaped, 10-30 um in length and contain many dense cytoplasmic granules. These granules stains metachromatically using a variety of dyes and average 0.2 to 0.4 um in diameter. In human mast cells, the granules can appear to have a scroll-like structure.

The suggested biological functions of mast cells include parasite rejection, repair of connective tissue, regulation of microvasculature, regulation of gastric acid secretion and detoxification of surrounding tissues. These cells are the primary effectors of immediate hypersensitivity reactions. Among all the populations of immunologically active cells, they are unique in that they possess a recognition system already in situ. Sensitized IgE-bearing mast cells can recognise foreign configurations (non-self) without recruitment of other cells from the immune system. These cells are widely distributed but are noticeably present in connective tissue, particularly surrounding blood vessels, nerves and glandular ducts. The cells are prominent in the skin, lymphoid tissue, lung, bladder, uterus, synovium, mesentery and submucosal and subserosal layers of the digestive tract.

1.6.2 Mast Cell Heterogeneity in the Human

Two types of human mast cells have been predicted, based upon the characterization in rodents of mucosal and connective tissue types of mast cells. Each type of rodent mast cell contains histamine and undergoes IgE-mediated activation and IgE mediated secretion, but they differ in their staining and fixation characteristics, growth conditions, susceptibility to non IgE-dependent secretagogues and in the specific proteases, and proteoglycans present in their

secretory granules. Their designation as mucosal and connective tissue mast cells has been based on the predominance of the former type in the small bowel mucosa and the later type in skin. Both sub-sets, however, are often present together in the same tissue. In humans, heterogeneity based on fixation or staining characteristics has not been as clear as in rodents. Nevertheless, two mast cell types are now recognized by their different protease compositions. Those containing tryptase alone have been termed T mast cells and those containing tryptase together with chymase have been termed TC mast cells.

Further functional and compositional properties of each subset need to be determined. T mast cells are present at about 21,000/mm^3 in small bowel sub-mucosa; TC mast cells at 8,500/mm^2 in small bowel sub-mucosa and 3,000/mm^3 in skin.

The distribution of mast cell types in human lung is as follows:
the percentage of T mast cells in alveolar wall and in bronchial/bronchiolar subepithelium is greater than 90%, in bronchial/bronchiolar subepithelium is 70% and in nasal mucosa is about 50%. In human lung, concentrations of mast cells are highest in alveolar tissue (26,500/mm^3) and in bronchial/bronchiolar subepithelium (18,000/mm^3).

1.7.1 Generation and Release of Mediators of Allergy in Man.

Allergic reactions are expressions of amplification mechanisms that are selectively triggered by recognition of an allergen. These intrinsic reactions are pre-determined and have characteristics which are determined by the dose of the allergen. The clinical manifestations of allergic reactions are mostly physiological responses to the release or generation of predominantly low molecular weight mediators with considerable biological potency. When

allergens interact with circulating immunoglobulin, with cell bound immunoglobulin or with antigen recognition sites on mast cells, this results in the liberation of both preformed and newly synthesized mediators, which together orchestrate the development of the inflammatory response. Newly generated mediators are synthesized de-novo and secreted after IgE dependent activation by mechanisms independent of secretory granule exocytosis. They are not stored in appreciable quantities in the mast cell. They include metabolites of arachidonic acid Leukotriene C_4 [LTC_4] and Prostaglandin D_2 [PGD_2] the phospholipid derivative PAF and the nucleoside adenosine.

1.7.2 Preformed Mediators

Preformed mediators of mast cells include histamine, heparin and chemotactic factors.

Histamine is the only biogenic amine detected in human mast cells. Histamine is synthesized in mast cells by the action of histidine decarboxylase on histidine and is stored in cytoplasmic secretory granules. These granules are acidic and histamine is probably bound to negatively charged carboxyl or sulphate groups in the proteoglycan-protein (heparin) matrix of the granule. After release into the neutral pH extra-cellular environment, histamine becomes less positively charged, dissociates from the complex and is freely soluble. Histamine expresses its biological activity by binding to H_1 and H_2 receptors on the membranes of target cells.

In skin, histamine elicits a response consisting of an initial local erythema due to arteriolar vasodilation mediated via an axon reflex, and later a central edematous wheal due to enhanced capillary permeability.

Enhancement of local vasopermeability by released histamine facilitates the tissue deposition of plasma components, and permits the interaction in tissues of plasma components with released mast cell neutral proteases. Contraction of bronchial and gastrointestinal smooth muscle occurs via H_1 receptors, whereas gastric acid secretion by parietal cells is mediated via H_2 receptors. Histamine is an airway constrictor. It also increases vascular permeability to plasma components at the level of the postcapillary venule by increasing the pore size between adjacent endothelial cells. Intravenous infusion of histamine causes increased heart rate and pulse pressure along with diastolic hypotension, flushing, and headache.

In vitro histamine expresses other activities, which are not yet fully evaluated in vivo. Thus, the activation of histamine H_2 receptors (Melmon & Rocklin, 1981) results in the down-regulation of T-lymphocyte-mediated cytotoxicity, the release of lymphokines and the proliferation of lymphocytes. Stimulation and the secretion of granule mediators by neutrophils, basophils and mast cells along with augmentation of T lymphocyte suppression activity also occurs.

Histamine is also chemokinetic for eosinophils and neutrophils (Lewis, 1984). Although histamine is not chemotactic for the migration of these cells, it does increase their random non-directional movement. Chemokinetic influences on cells makes them more able to respond to chemotactic factors. Thus, histamine is an effector of neutrophil and eosinophil migration by rendering the migratory cells more active, while not directing their movement. Histamine thereby increases the potency of eosinophil chemotactic factor of anaphylaxis (ECF-A).

The biological half-life of released histamine is short, greater than 90% being metabolized after one pass through the circulation. The main catabolic pathway involves the action of histamine methyl transferase and diamine oxidase.

In clinical situations, such as in patients suffering from anaphylaxis provoked by exercise or antigen, mastocytosis, angiodema and cold urticaria, serum histamine levels are found to be elevated (Heavey et al., 1986). After challenge by specific antigens in patients with atopy, histamine has been demonstrated in nasal lavage fluid and skin-blister fluid.

1.7.3 Chemotactic Factors

Mast cell degranulation results in the influx of secondary cells, particularly eosinophils, neutrophils, and lymphocytes. Mast cells are thought to release factors capable of recruiting these secondary cells to the sites of immediate hypersensitivity reactions. Mediators chemotactic for eosinophils are collectively called "eosinophil chemotactic factors of anaphylaxis." These include two acidic tetrapeptides, Val-Gly-Ser-Glu and Ala-Gly-Ser-Glu, which account for part of the low molecular-weight chemotactic activity and are active at concentrations as low as 10^{-8}M. In addition to causing chemotaxis, exposure to these tetrapeptides desensitises eosinophils to any further chemotactic effects and increases the expression of receptors for complement components C4b and C3b on these cells. Another protein presumably of mast cell origin, of molecular weight 750,000 and which attracts neutrophils (Neutrophil Chemotactic Factor, NCF), has been found to be released into the venous effluent during experimentally-induced attacks of physical allergy in patients with cold or

cholinergic urticaria. This protein has been implicated in bronchospasm induced by specific allergens. The release of these chemotactic factors into the circulation is antigen and dose dependent. This release is inhibited by pre-treatment with sodium chromoglycate.

1.7.4 Proteases

Mast cells are rich in intragranular proteases which are released during immunological activation. These are strongly positively charged at physiological pH (isoelectric point pH 9) and are active at neutral pH. In some species, such proteases constitute up to 50 per cent of the total mast cell protein. The functional relevance of these enzymes in the mast cell response is indicated by the diminution of skin lesions in experimental hypersensitivity after protease inhibitors are administered. The two major neutral proteases of human mast cells are tryptase and chymase. Tryptase is the principal protease of the secretory granules of human mast cells and can also be detected, by immunoassay, in experimental skin chamber fluid after cutaneous allergen challenge. No other cell type - including those found in normal lung, small intestine and skin, or eosinophils, basophils, neutrophils, monocytes or lymphocytes has been found to contain appreciable amounts of tryptase. Tryptase release is therefore a useful marker of mast cell activation (Schwartz et al., 1981).

Tryptase is stored and secreted as a tetramer composed of two 35 kilodalton KDa and two 37 KDa sub-units (Schwartz, et al., 1981). In recent work by Schwartz and Bradford (1986) it was found to be a tetrameric serine endoprotease of 134,000 Mr (monomer) with sub-units of 33,000 Mr and 34,000 Mr each with active sites. The sub-units also share antigenic sites. It is not

known whether the Mr difference between the two sub-units reflect distinct primary amino acid sequence differences or differences in post translational processing of identical gene products. Like pancreatic trypsin, tryptase is an endopeptidase. Tryptase stored in secretory granules is fully active and is not inhibited by any protease inhibitors found in plasma. The enzyme is stabilized in its active tetrameric form by association with the proteoglycan heparin. When free in solution, tryptase sub-units rapidly dissociate into inactive monomers.

The possible biological functions of tryptase and chymase include digestion of blood-vessel basement membrane with resultant increased vascular permeability, degradation of connective-tissue components such as the peptide core of proteoglycans allowing the influx of secondary inflammatory cells and the degradation of debris and activation of growth factors to promote wound healing. Plasma proteins, including angiotensin 1 and complement C3, have been shown to be substrates for mast-cell proteases in vitro. Tryptase however has no effect on complement component C5. Tryptase rapidly inactivates fibrinogen as a coagulable substrate for thrombin.

Chymase is the second protease found in secretory granules of human mast cells, particularly those from the skin and intestinal submucosa. It exhibits a cleavage pattern similar to that of pancreatic chymotrypsin. Unlike tryptase, chymase is not present in all mast cells. Chymase is a serine endoprotease of 30,000 Mr (monomer). Like tryptase, chymase is stored in mast cell secretory granules as a fully active enzyme and is presumably bound to the negatively charged granular proteoglycan. Unlike tryptase, chymase is inhibited by plasma proteinase inhibitors.

Additional proteases associated with human mast cells include those with Hageman factor-deriving, Hageman factor-activating, Kalikrein, pre Kalikrein activating, elastase, and cathepsin G1 activities.

1.7.5 Proteoglycans

Proteoglycans consists of glycosaminoglycan side chains (repeating disaccharide units of uronic acid and hexosamine that are variably sulphated) covalently linked to a protein core via a trisaccharide-protein linkage sequence of galactose-xylose. These are present in the secretory granules of mast cells. Two classes of proteoglycan, heparin and chondroitin sulphate have been localized in distinct rodent mast cell subsets (Katz et al.. 1985). A similar distribution has been suggested in humans where heparin has been found associated with lung and skin mast cells and chondroitin sulphate has been associated with intestinal mast cells.

The molecular weight of the heparin proteoglycan in human mast cells is 60 to 150 KDa. A high degree of sulphation - 300 to 1000 sulphate residues per molecule - makes heparin the most negatively charged molecule in the body and probably accounts for the characteristic metachromatic staining of mast cells by certain basic aniline dyes, such as toluidine blue. Heparin has a structure with an average of 2.5 sulphate groups per disaccharide The protein core in rat heparin consists predominantly of the amino acids glycine and serine (43%).

Chondroitin monosulphates are sulphated either in position 4 (chondroitin 4-sulphate, chondroitin sulphate-A) or position 6 (chondroitin 6-sulphate, chondroitin sulphate-C) of galactosamine residues. Disulphated disaccharides have been found with sulphate groups in positions 4 and 6 of galactosamine

(chondroitin 4,6-disulphate, chondroitin sulphate E) and in positions 4 of galactosamine and 2 of iduronic acid (disulphated chondroitin sulphate B, chondroitin sulphate di-B). The core protein structure of chondroitin sulphate E proteoglycan (averaging 1.5 sulphate groups per disaccharide) from mouse bone marrow derived mast cells consists predominantly of glycine (43%) serine (17%) and glutamic acid (10%). The chondroitin sulphate di-B (1-2 sulphate groups per disaccharide) proteoglycan from rat bone marrow derived mast cells is predominantly glycine (35%) serine (23%) and alanine (10%). All these protein cores are markedly resistant to digestion by a variety of proteases.

The biological function of proteoglycans is to facilitate the packaging of proteases within the granules. They remain attached to the protease after the granule contents are released. The presence of large protease-proteoglycan complexes in the extracellular environment may serve to prevent extensive diffusion of the enzymes and enhance their activity by impairing their inactivation by inhibitors. The stabilization effect of heparin on human tryptase activity may be of crucial importance in mast cell mediated events. Heparin also acts as an anticoagulant by enhancing the inhibitory action of antithrombin-3, has anti-complement activity and facilitates Hageman factor autoactivation.

1.7.6 Newly Generated Mediators

The mast cell produces several prostaglandins via the cyclo-oxygenase pathway of arachidonic acid metabolism. Oxidation of arachidonic acid (5,8,11, 14-eicosatetraenoic acid) by cyclo-oxygenase gives rise to compounds which are collectively called the eicosanoids and includes prostaglandins, prostacyclin and the thromboxanes. Another group of eicosanoids, the leukotrienes, are synthesized from arachidonic acid by a second enzyme system - the

lipoxygenase pathway (Samuelson, et al., 1980). Leukotrienes also appear to have a role in inflammation.

1.7.6.1 Eicosanoids; The Biology and Pathophysiology of Eicosanoids in Inflammation

Eicosanoids are derived from naturally occurring eicosapolyenoic acids. Arachidonic acid, which is either derived from the diet or synthesized from linoleic acid, is widely distributed in the body and is usually stored covalently bound in its esterified form in the phospholipid fraction of the cell membrane of most body cells. Following an inflammatory stimulus, an acylhydrolase, phospholipase A_2, acts to release the arachidonic acid from its phospholipid pool. This free arachidonate is either immediately re-esterified or metabolized by one of the two possible enzyme pathways. The cyclooxygenase enzyme system will convert arachidonate to cyclic endoperoxide - PGG_2. This is acted upon by another enzyme to yield PGH_2, a cyclic hydroxy endoperoxide which subsequently gives rise to the primary prostaglandins, PGD_2, PGE_2, and PGF_2 as well as prostacyclin (PGI_2) and non-prostanoate thromboxanes A_2 and B_2 (TXA_2 and TXB_2). Members of this group of eicosanoids have been implicated in every phase of inflammation.

1.7.6.2 Cyclo-oxygenase Products

Prostaglandin D_2 is the most important cyclo-oxygenase product of mast cells (Lewis et al., 1982). Human basophils do not produce PGD_2. Prostaglandin D_2 is also generated by human platelets (Oelz et al., 1977). Prostaglandin D_2 in humans is produced by dispersed human lung mast cells where there is an approximate 10:1 ratio of T to TC human mast cells (Lewis et al., 1982). Prostaglandin D_2 is also found in nasal lavage fluid after allergen

challenge (Creticos et al., 1984). When injected into human skin in nanomolar amounts, prostaglandin D_2 causes increased vasodilation and vasopermeability, resulting in an erythematous wheal-and-flare reaction similar to that seen with histamine, except that it is nonpuritic. The reaction persists for as long as two hours and is accompanied by an influx of polymorphonuclear leukocytes. When inhaled, prostaglandin D_2 produces bronchoconstriction, with a potency about 10 times greater than that of histamine (Holgate et al., 1984). That prostaglandin D_2 may be an important mediator of anaphylaxis is suggested by the finding of markedly increased production of prostaglandin D_2 metabolites in the urine of patients with systemic masocytosis who have attacks of flushing and severe hypotension (Roberts et al., 1979). Prostaglandin D_2 is a potent inhibitor of platelet aggregation (Mills et al., 1974).

The other two biologically active cyclo-oxygenase products are prostacyclin (a biocyclic prostaglandin) and the thromboxanes, in which a six member oxane ring replaces the cyclopentane ring of the prostaglandins. These products, the prostanoids, are not stored in situ and several are very unstable (Piper et al., 1971). In aqueous solution at pH 7.5 and 37°C, the half life of cyclic endoperoxides PGG_2 and PGH_2 is 4 and 3.4 minutes respectively before they are converted to primary prostaglandins. The half life of PGI_2 is 4 minutes and TXA_2 is 32 seconds (Salmon et al, 1979). $PG1_2$ and TXA_2 are rapidly hydrolyzed to the inactive but stable products 6-keto-PGF and TXB_2. Only about 3% of PGE_2 remains unchanged in the plasma 90 seconds after intravenous administration. Release of prostanoids, of which PGE_2 is usually predominant, is generally accepted to be an integral part of the inflammatory process.

Prostanoids are also implicated in symptoms involving reactions against endotoxins. Prostaglandins are also found in the synovial fluid of human patients with arthritic conditions, particularly rheumatoid arthritis. Haemodynamic changes attributed to PGI_2-induced vasodilation lead to systemic hypotension. Low concentrations of PGE_1 have been shown to cause hyperalgesia or enhanced susceptibility to pain, and PGI_2 has also been shown to be a potentiator of pain responses. Raised concentrations of PGE_2 have been recovered from cerebrospinal fluids of human patients with high temperatures suffering from typhoid/viral encephalitis. The febrile effect is due to an action on the anterior hypothallamus and can be blocked by cyclo-oxygenase inhibitors (Schwartz, 1987).

1.7.6.3 Lipoxygenase Products

Lipoxygenase enzyme activity in mammalian tissues was first reported from platelets by Hamberg and Samuelsson (1974) who showed that arachidonic acid could be converted to 12-hydroxyperoxy-5,8,10,14- eicosatetraenoic acid (12-HPETE). Turner et al., (1975) demonstrated that 12-hydroxy-5,8,10,14-eicosatetraenoic acid (12 HETE), derived from 12-HPETE, was a potent mediator for the chemotaxis of polymorphonuclear leukocytes. Walter (1976) showed that the accumulation of leukocytes in inflamed tissues was independent of cyclo-oxygenase activity. In 1976, Borgeat and co-workers had shown that polymorphonuclear leukocytes themselves formed 5-HETE, an isomer of 12 - HETE. In 1979 the unstable 5,6-epoxide of arachidonic acid was reported to be formed in polymorphs from an open chain hydroxy acid 5-HPETE and named leukotriene. The unstable epoxide was given the name leukotriene A_4 (LTA_4). Leukotriene A_4 is the most important lipoxygenase product, the first in a series

of chemical slow reacting substances of anaphylaxis. Leukotriene C_4 (LTC_4) is synthesized inside cells by addition of the tripeptide glutathione (Glu-cys-gly) to biologically inactive LTA_4. Once secreted, LTC_4 can be metabolized sequentially to the two other major slow reacting substances of anaphylaxis by extracellular enzymes, LTD_4 by removal of glutamine and LTE_4 by removal of glycine. Inactivation occurs by further oxidation and/or removal of cysteine. Leukotriene B_4 is another important arachidonic acid derivative, a leukotriene produced from LTA_4 by the enzyme epoxide hydrolase.

In humans, LTC_4 can be generated by the dispersed human lung mast cells (T:TC mast cells in lung = 10:1) and after cutaneous allergen challenge. Human basophils also generate LTC_4 (MacGlashan, 1986) as do eosinophils and certain macrophages. Leukotriene C_4, like LTD_4, acts through cell receptors and is a potent bronchoconstrictor, enhancing mucus production, increasing vascular permeability and acting on vascular smooth muscle. Leukotriene B_4 is a potent chemotaxin for human neutrophils and is the predominant lipoxygenase product of human neutrophils, monocytes and alveolar macrophages.

CHAPTER TWO

PREGNANCY, IMMUNOLOGY AND ALLERGY

2.1 An Overview of Pregnancy

In humans, fertilization of the ovum by the sperm and the initiation of cell division usually starts in the mid-portion of the fallopian tube. A successful pregnancy requires highly synchronised responses including social interactions, sexual behaviour and immunomodulatory mechanisms at the various stages of pregnancy, orchestrated by the effects of hormones. These processes can be broadly divided into three stages:

Stage 1 - the initiation of pregnancy

Stage 2 - the maintenance of pregnancy

Stage 3 - parturation, lactation and maternal behaviour.

2.1.1 The Establishment of Pregnancy

Following fertilization, the embryo remains in the oviductal site for a period of three to four days. During this period in the oviduct, the embryo cells continue to divide from the two cell to the eight cell stage when the cleaving embryo is termed the morula. The morula arrives at the uterus through the tubul isthmus. At the 32-64 cell stage, further morphological changes occur with the transition of the morula to a blastocyst. Secretions of the uterus provide the blastocyst with dissolved oxygen, nutrients and a vehicle for waste metabolite removal, permitting its survival. By this time the zona pellucida is shed due to activity of the trophoblast. This enables the trophoblast to come into direct contact with the endometrium allowing attachment and subsequent implantation. Trophoblastic invasion leads to destruction of maternal epithelium, connective tissue stroma and also the endothelium of the maternal blood vessels, leaving the maternal blood bathing the fetal chorion -

haemochorial placentation. Thus, fetal tissue is directly exposed to cells of the maternal immune system in the maternal blood circulation.

The presence of a fertilised ovum and its subsequent implantation provides the mother with a series of signals prominent among which is human chorionic gonadotrophin (hCG). This has a luteotrophic function preventing luteolysis and maintaining a functional corpus luteum. Progesterone from the corpus luteum enables the continuing development of the feto-placental unit. Luteal function is maintained until there is sufficient progestagen production by the placenta. In addition to an endocrine function, as the placenta develops it takes over as the main organ of nutrition for the fetus.

Thus, the initiation and maintenance of pregnancy is a complex physiological event, requiring nutritional, endocrine and immunological modulation.

2.1.2 The Mechanisms of Acceptance of the Fetal Allograft - Theories and Current Views.

The fetoplacental unit contains a complement of paternally derived antigens. Why then does not the immunocompetent mother reject the fetal semi-allograft? A number of mechanisms have been proposed - see paragraphs 2.1.2.1. - 2.1.2.7. below.

A summary of some of the reported changes in the maternal immune system during pregnancy are shown in Table 3. (page 31)

2.1.2.1 Lack of expression of paternal MHC and other paternal antigens

Although the fertilised embryo is potentially exposed to maternal immune system cells while present in the lumen of the oviduct and uterus, for much of this time it is surrounded by the zona pellucida. The initial intimate

TABLE 3

SUMMARY OF MATERNAL IMMUNOLOGIC CHANGES DURING PREGNANCY

PARAMETER	CHANGE DURING PREGNANCY
Cell counts	
Total white count	Increased
Neutrophils	Increased
Lymphocytes	Decreased
Monocytes	Increased
T/B cell ratio	No change
Neutrophil function	Decreased chemotaxis/superoxide anion generation
Immunoglobulins	
IgG	Decreased
IgA	No change or slight decrease
IgM	No change
IgE	No consistent change
Antigen-specific antibody response	No change
Autoantibodies	
De novo production	Rare
Preformed	Decreased (anti-insulin, antithyroid antigens) or unchanged (rheumatoid factor)
Immune complexes	Absent or rare
Delayed skin tests	No change or slight decrease
T4 positive cells	Variable, probably not of biologic significance
In vitro lymphocyte responsiveness	
PHA	Unchanged in majority of studies
Antigen	Conflicting data
Mixed lymphocyte	No change
Pregnancy serum	
Effect of lymphocyte responsiveness to PHA	Decreased
Effect on mixed lymphocyte reactivity	Decreased
Mediators of immediate hypersensitivity	
Cyclic AMP (urinary)	Increased
Cyclic GMP (urinary)	Increased
Histamine	Decreased
Histaminase	Increased
Prostaglandin	Increased
Prostaglandin A	Increased
Prostaglandin E	Increased (third trimester)
Prostacyclin (I_2)	Increased
Thromboxane A_2	Increased

From, "Principle and Practice of Allergy", Ed. E. Middleton, Jnr, 1985.

physical contact between mother and fetus occurs at implantation - approximately seven days following ovulation, at a time when the trophoblast is first forming. At ten or eleven days, the trophoblast differentiates into the inner cytotrophoblast and the outer syncytiotrophoblast, a covering layer where the cell membranes fuse to produce a multinucleated cell mass. The mature placental villus possesses the following layers - at the maternal interface an outer layer of degenerated cellular debris, "Roht's" layer; the syncytiotrophoblast; the cytotrophoblast; and an inner mesenchymal stroma separated from the cytotrophoblast by a basement membrane. Transplacental exchange of immunogenic cells and macromolecules can occur in both directions, although maternal immunocompetant lymphocytes are usually effectively barred from the fetus by the syncytiotrophoblast layer.

The main area of contact between mother and fetus is the trophoblast, which expresses paternal antigens. These do not appear to be products of the major histocompatibility loci (MHC) or ABO or RH blood group antigens, although MHC antigens are present on fetal embryonic cells from around the time of implantation and are detectable throughout pregnancy. For this reason, the presence of maternal anti-HLA antibodies, which have been demonstrated in up to 50% of multigravidous women, do not compromise the success of pregnancy. The presence of such antibodies indicate that the normal pregnant woman may show immunological reactions against the conceptus. The trophoblastic barrier is supplemented by a variety of modifications in immunologic responses in both mother and foetus, which allow the pregnancy to continue. Factors are present in retroplacental sera which inhibit the expression of MHC class 2 antigens on the human monocytic cell line U937 by

recombinant gamma interferon (Nicholas, et al., 1986). Such factors may at least in part be responsible for the lack of MHC class 2 expression by the trophoblast.

2.1.2.2 Antigen masking

The masking of cell surface alloantigens on the trophoblast has been proposed as one immunomodulatory factor operating during pregnancy, in addtion to the lack of MHC antigen expression on this organ. In the rabbit, uteroglobulin, a 15.8 KDa protein synthesised in the uterus in early pregnancy and cross-linked with beta2-microglobulin, results in the masking of MHC antigens on the cell surface (Mukherjee et al, 1982). Transferrin bound to specific receptors on the trophoblast and IgG bound to trophoblastic Fc receptors may have similar masking effect in the human (Johnson et al, 1981).

2.1.2.3 The uterus as an immunologically priveleged site.

It has been proposed that the uterus acts as a highly selective site, an environment in which the cell mediated immune system is ineffective. Head & Gaede (1986) demonstrated Ia antigen-expressing cells in the uterus of virgin rats. Ia antigens are MHC class 2 antigens, expressed presenting cells, and this finding suggests that the uterus is capable of local immune responses can be decidual and myometrial lymphatic drainage and infiltration can be observed adjacent to the trophoblast.

2.1.2.4 The placenta as an Immunological Filter

The fetus and its circulating blood are separated from the maternal circulation by the placenta. It has been proposed that the placenta prevents immunogenic fetal cell from reaching the maternal circulation thus

circumventing immunological attack by maternal lymphocytes. However, fetal erythrocytes readily cross the placenta.

2.1.2.5 Immunological Tolerance and functional immune deficiency During Pregnancy

General or specific maternal immune reponsiveness is maintained with only minor alterations during pregnancy. Up to the 20th week of gestation there is a fall in the total number of circulating T cells. However, this reduction appears to be due to a redistribution of T-cells into different lymphoid compartments. An increase in B-cells numbers has been noted, perhaps as a compensatory mechanism for the reduction in T-cells (Strelkaukas et al., 1975). The presence of sperm antigens expressed on the early fetus suggests that a certain degree of immunological tolerance to paternal antigens may develop in pregnant women.

Observations in vitro that cytotxic responses to trophoblast antigens are impaired during pregnancy suggest that maternal cell-mediated immune responses are impaired. The presence of suppressor cells associated with the decidua (Clark et at., 1986b) and also the presence of immunoregulatory molecules such as pregnancy-specific and pregnancy-associated proteins (Sargent & Redman, 1985) are of interest in this context.

A number of observations on cell-mediated immune responses during human pregnancy have been published. Hawes et al (1981) studied cell-mediated immune responses and skin tests reactions to house dust, house dust mite, Candida albicans extracts and tetanus toxoid. The skin test reactions were found to be unchanged during pregnancy. However, in vitro lymphocyte transformations induced by these antigens were enhanced during pregnancy.

There is evidence of a pregnancy-associated immune deficiency syndrome and the susceptibility to infection by poliomyelitis virus and Plasmodium is two to seven times greater in pregnant than in non-pregnant women (Weber & Nelson, 1986). The incidence of the following infective diseases is also reported to increase in women during pregnancy: hepatitis A, influenza A, variola, Entamoeba histolytica infections (Weber & Nelson, 1986).

The diagnosis of carcinoma of the breast is 1.8 times and cervical carcinoma 13.5 times more common during pregnancy, although in part this is likely to result from the closer clinical supervision of individuals at time. Tumours first diagnosed during pregnancy, especially during the third trimester, have a poorer prognosis than those diagnosed in the non-pregnant state. The five year survival rate of patients with malignant melanoma is reduced with each successive pregnancy (Weber & Nelson, 1986). Although these observations may indicate some compromise of other factors including the secretion of increased amounts of steroids and other cell growth factors are also likely to be an important contributory factor.

Thus, there is some evidence that cell-mediated immunity is depressed during pregnancy, but not to a profound degree. Comparative studies on pregnant and non-pregnant women related to delayed hypersensitivity, skin test responses, skin graft rejection and in vitro stimulation of lymphocytes with plant mitogens, recall antigens or allogeneic lymphocytes appear to vary.

The effect of pregnancy on humoral immunity is controversial. Ostensen et al (1983) studied humoral immunity in a series of 31 randomly selected healthy pregnant women aged 18-36 years, 13 women with definitive or probable AS aged 22-34 years and 10 patients with RA aged 18-34 years. Levels

of IgG and IgA were decreased in all pregnant women while IgM antibodies remained normal compared to the non-pregnant state. Multiparous women had higher levels of IgG during pregnancy than primigravidae. No differences in the frequency of either rheumatoid factor (RF) or antinuclear antibodies (ANF) were found when healthy pregnant women were compared to healthy non-pregnant controls. In another study (Pasca, 1977) serum samples taken at 14 weeks gestation, at delivery and at 30-45 days post partum revealed that, after delivery, humoral immunity to rubella virus returned to the levels of early gestation.

Autoimmune diseases show a variable response to pregnancy, although the incidence of such diseases in pregnancy is low (Donat, 1986). While rheumatoid arthritis (RA) appears to improve in the majority of pregnant patients, ankylosing spondylosis (AS) patients generally have unchanged disease activity during gestation (CeCere et al, 1981, Bulmarsh, 1979). A postpartum disease flare-up occurs commonly in both RA and AS. A decrease in circulating immune complexes has been found in RA patients remitting during pregnancy. No single serum factor or combination of factors responsible for such gestational remission has yet been identified. Generally, however, blood parameters in autoimmune patients generally reflect the biochemical changes of a normal pregnancy.

2.1.2.6 The Blocking Antibodies of Pregnancy

The significance of the presence of antibodies which may block cell-mediated immune responses during pregnancy is a controversial area. In 1964, Kaliss and Dagg proposed that successful allogenic pregnancies might be protected by such antibodies. Hellstrom et al (1969) suggested that IgG

antibodies may be the putative blocking factor as these were present in sera from primiparous and multiparous mice after allogeneic but not syngeneic matings. Blocking antibodies are essential for a normal gestation in the human and are absent in the sera of spontaneous aborters - no such antibodies were detected in any of a series of eight habitual aborters, whereas all eight normal control multiparous women had such antibodies in their sera (Rocklin et al, 1976). These blocking antibody activities could not be removed by absorption to platelets, indicating that the target antigens for these antibodies were not HLA-A, -B or -C antigens.

It has been shown that maternal serum can block the cell-mediated immune reactivity of maternal cells against paternal antigens in vitro and also maternal versus either fetal or paternal mixed lymphocyte reactions (Rocklin et al, 1979).

Macrophage migration inhibitory factor (MIF) is a lymphokine produced by activated lymphocytes. MIF is produced by maternal lymphocytes in response to genetically foreign cells (Rocklin et al, 1973), a response inhibited by autologous maternal serum. However, it appears that the blocking factor which inhibits MIF release is absent from the serum of chronic habitual aborters (Rocklin et al, 1976).

Bell & Billington (1980) reported that female mice produce predominantly non-complement fixing IgG antibodies against the fetus during pregnancy. Specific antibody production, including IgG antibodies against a number of paternal class 1 and 2 HLA antigens, were noted during pregnancy and these were shown to be capable of blocking specific maternal cytotoxic lymphocytes (Morin-Papunen et al, 1984). Also found are antibody specificities directed

towards non-HLA antigens produced by the syncytiotrophoblastic layer of the placenta - these may combine with the coresponding antigens to form immune complexes in the maternal circulation.

Faulk and McIntyre (1983) reported the involvement of non-cytotoxic antibodies to the TLX antigens as being of importance in the survival of a pregnancy. The TLX antigens are a group of trophpoblast-lymphocyte cross-reacting molecules closely associated with the HLA antigens. Thus, partners sharing common HLA types are likely to have similar TLX antigens and consequently are less likely to generate anti-TLX antibodies. They could, therefore, be a greater risk of pregnancy loss resulting from such matings. In practice it has been found that immunization with paternal cells may reduce the rate of pregnancy loss in women suffering recurrent habitual abortion (Mowbray et al, 1984). Blocking antibodies to TLX or similar antigens may play a role here.

2.1.2.7 Pregnancy Proteins and Immunosupression During Pregnancy

A number of proteins have been identified in human pregnancy. These can be classified broadly into two groups: those which are specifically secreted during pregnancy (pregnancy-specific proteins); and proteins which are present in the non-pregnant state, but found in increased amounts during pregnancy (pregnancy-associated proteins). A number of functions have been proposed for these pregnancy proteins including suppression of the maternal immune system thereby facilitating fetal allograft survival.

The pattern of secretion of pregnancy proteins can be two general types:-

Pattern A: those present at maximum level during the first few weeks of gestation, e.g. human chorionic gonadotrophin, hCG (Braunstein, 1970).

Pattern B: those the secretion of which appears to relate to the overall size of the placenta - levels of these proteins increase throughout gestation, often reaching a plateau a few weeks before term, e.g. human placental lactogen, hPL (Chard, 1982).

Human chorionic gonadotrophin was the first placental protein identified (Ascheim and Zandek, 1927). Human Placental Lactogen (hPL) was isolated from pregnancy serum and placenta in 1962 by Josimovich and McLaren. Several new placental proteins were described during the early seventies, but only a few of these fit the definition of pregnancy proteins in that they are synthesised uniquely by the placenta. Chard et al. (1985) reviewed pregnancy proteins, suggesting redefinition as "reproductive proteins", dividing them into three functional groupings thus:

Group 1	**Group 2**	**Group 3**
Enzymes	PP5	Binding proteins
hCG, hPL	PAPP-A	PZP
SP1	PP12	

Group 1 products are of trophoblast origin and control mechanisms are dependent upon trophoblast mass and uteroplacental blood flow. The postulated functions are as hormones and enzymes. Group 2 products are also released by the trophoblast and possibly other as yet unidentified sources. Postulated functions are activities related to local immunological responses and effects on blood coagulation. Group 3 products are of maternal hepatic/endometrial/decidual origin. These are controlled by oestrogen/progesterone and bind to small molecules, e.g. thyroxine and steroids, as a maternal response to pregnancy.

There are around forty proteins which have been extracted from placental tissues to date. Of those which have been implicated in modulating maternal immune responses and which, therefore, could influence allergic reactions, two are reviewed below. Table 4 (page 41) shows some soluble placental tissue proteins and their characteristics.

2.2 Reproductive proteins potentially implicated in modulating allergic reactions during pregnancy

Although many of the identified reproductive or pregnancy proteins have been ascribed immunomodulatory (usually immunosuppressive) functions, reliable experimental evidence has been published for only a few of these. Of these, two, pregnancy-associated plasma protein A (PAPP-A) and placental protein 14 (PP14), which may potentially influence allergic reactions are reviewed here.

2.2.1 Placental Protein 14(PP14)

Placental protein 14 was originally isolated from term placentae (Bohn et al., 1982). It is a dimeric 56 KDa glycoprotein with homologous 28 KDa subunits (Westwood et al, 1988). It is highly glycosylated (30%) and highly hydophobic. The sequence of the first 24 N-terminal amino acids is as follows: Met Asp Ile Pro Gln Thr Lys Gln Asp Leu Glu Leu Pro Lys Leu Ala Gly Thr Glu His Glu Met Ala Met

P14 is a pregnancy-associated protein which is also present in the endometrium and peripheral blood of non-pregnant women. Levels increase rapidly in early pregnancy - a rise can be detected in the peripheral circulation as soon as 2-12 days after embryo replacement in in vitro fertilization (Julkunen et al, 1985). Maximal concentrations of the protein appear in the maternal

TABLE 4

PLACENTAL PROTEIN	NAME, FUNCTION OR SYNONYM	MOLECULAR WEIGHT	CARBOHYDRATE CONTENT (%)
PP2	Ferritin	500 KDa	
PP5	Protease Inhibitor	36.6 KDa	19.8
PP7	Glutathione-transferase	37.1 KDa	5.4
PP12	Chorionic alpha$_2$-microglobulin (CAG-1)	25.2 KDa	4.3
PP14	Chorionic alpha$_2$-microglobulin (CAG-2)	43.0 KDa	17.5
PP15	Immunosuppressive	30.7 KDa	3.3

(Bohn 1985)

circulation between 6 and 12 weeks of gestation and decline thereafter. Highest levels are found in decidual tissue in which PP14 comprises some 10% of the total soluble protein by the 7th-8th week of gestation. High levels of PP14 are also present in seminal plasma (Bolton et al., 1986a).

PP14 is active in inhibiting T-lymphocyte proliferation in vitro and is thought to be an immune regulatory molecule (Bolton et al, 1986b, 1987). Indeed, Bolton et al (1987) showed that the immunosuppressive activity of decidual tissue can largely be explained in terms of its PP14 content. The secretion of PP14 is progesterone dependent (Julkunen et al., 1986b, Li et al, 1991). Progesterone itself has been proposed as an important immunosuppressive agent in pregnancy as it directly interferes with cell-cell contact (Clemens et al., 1979) and alters the local exchange of immunological information (Van Vlasselaar et al., 1986). However, PP14 does not effect cell-cell contact, indicating that the immunosuppressive action of PP14 is independent of any shown by progesterone (Pockley & Bolton, 1988).

The mechanism by which PP14 appears to act has been investigated in some detail. It exhibits an inhibitory effect on Interleukin-2 (IL2) production by T-cells (Pockley & Bolton, 1989), probably operating via an inhibitory effect on IL-1 production or release (Pockley and Bolton, 1990). The resulting inhibition of T-lymphocyte activation and proliferation could potentially have widespread effects on the immune system, including modulation of allergic reactions - activated T-lymphocytes (CD4+ cells) produce IL4, the lymphokine responsible for B-lympocyte proliferation (Stein et al, 1986), differentiation (De France et al, 1987) and IgE isotype selection (Snapper et al, 1988).

2.2.2 Pregnancy-associated plasma protein-A(PPAP-P)

Pregnancy-associated plasma protein-A is a high Mr glycoprotein (750 KDa)(Lin et al, 1974). An immunosuppressive role for PAPP-A has been proposed as it inhibits lectin-induced lymphocyte stimulation (Bischof et al, 1982). However, such activity has been questioned by Sinsich et al (1984); furthrmore PAPP-A shows no suppressive activity in allogeneic mixed lymphocyte cultures (McIntyre et al, 1981).

PAPP-A binds to heparin (Sinosich et al, 1981) and inhibits the activity of certain proteolytic lysosmal enzymes such as granulocyte elastase (Sinosich et al, 1982). Thus, PAPP-A could potentially interfere with the putative role of heparin, released from mast cells during allergic reactions, in enhancing local protease activity around stimulated mast cells (see page 23).

2.3 Pregnancy and Allergy

The role of allergy in the pregnant state is not well-defined. There is a general but apparently unsubstantiated feeling among practising obstertricians that allergic conditions improve during pregnancy. Holbreich (1982) in a review on asthma and other allergic disorders in pregnancy, described asthma as a complicating factor in approximately 1% of all pregnancies. Other allergic diseases, such as seasonal rhinitis, vasomotor rhinitis, and urticaria, may occur in up to 15% of all pregnancies. It was estimated that asthma will improve in one third, remain the same in one third and become worse in one third. In general, women with severe asthma are more likely to have exacerbations in the condition during pregnancy than those with milder disease.

2.3.1 Asthma in Pregnancy

Gluck and Gluck (1976) reviewed retrospective studies encompassing 1,087 pregnancies and noted a considerable conflict among these reports regarding the influence of pregnancy on the course of asthma. These authors also reported that a serum IgE level that increased or remained the same during pregnancy tended to be associated with worsening symptoms asthma, although this observation was based on data from only 28 patients. In a study from Helsinki (Stemius-Aarnala et al., 1988), 198 pregnancies in asthmatic women were monitored and followed for up to six months postpartum. The patients were divided into four groups on the basis of severity as defined by the required treatment, and classified as atopic or non-atopic by skin or RAST testing. A control group was matched for age, parity and time of delivery. In line with previous studies it was found that approximatley 40% required the same treatment, 40% required an increase and 20% required a reduction in treatment compared to their pre-pregnant state. The most severe groups requiring a high dose of medication contained significantly more non-atopic cases (those with negetive skin test or RAST) and the majority of these (56%) improved after delivery; only 22% improved in the atopic group after delivery.

2.3.2 Allergic rhinitis and pregnancy

Very little has been reported on the effect of pregnancy on allergic rhinitis. Nelson (1980) stated that women who have allergic rhinitis may suffer worsening of their symptoms during pregnancy, although some women may have a Paradoxical improvement of their nasal symptoms. In any case, nasal congestion is a common complaint during pregnancy. In one study of 327 women, 309 complained of frequent or constant nasal symptoms (Mabry, 1980).

Eighteen patients had had similar symptoms before pregnancy, but most experienced worsening during pregnancy. Congestion of the nasal mucosa develops by the end of the first trimester and progresses thereafter. Mucosal edema is prominent at the last two months of gestation. Even pregnant women without overt nasal symptoms show glandular hyperactivity, increased phagocytic activity and increased mucopolysaccharides in ground substance (Toppozada, 1982). These changes parallel increases in levels of oestrogen and progesterone, which are the probable cause. Paradoxically some women may show improvement of chronic rhinitis in pregnancy.

2.3.3 Dermatitis in pregnancy

The effect of pregnancy on atopic dermatitis is unpredictable. Atopic dermatitis appears to be largely unchanged during pregnancy. In one large series of patients, 3% showed an improvement and 1% a worsening of symptoms, while 96% remained unchanged (Nelson, 1980). Urticaria may be limited to or associated with pregnancy and recurrences may occur in subsequent pregnancies (Chapman, 1969). Progesterone has been implicated in pre-menstrul urticaria, bullous, eczematoid, maculopapular or urticarial lesions and immediate or delayed hypersensetivity to progesterone has been demostrated (Hart, 1977).

2.3.4 Womb sensitisation and Allergy

Recently, Jacobson(*1990) postulated the womb sensitisation theory in allergies. Jacobson reported that some babies who are allergic to cow's milk had been exposed to high levels of beta-lactoglobulin in utero. Antibodies from

* Personal Communication

high level of milk protein sensitise the fetus which itself is immuno-competent and able to produce IgE anitbodies.

2.3.5 Atopy and Prematurity

Premature babies may be at greater risk of developing asthma and eczema than full-term infants. Lucas et al., (1990) investigated 777 infants in a multicentre study and noted wheezing or asthma was common in 23 per cent and eczema in 19 per cent of pre-term babies. Such findings indicated that pre-term infants are vunerable to an increased incidence of later allergic reactions. Lung damage due to mechanical ventilation in this group is unlikely to be the sole reason. This is because wheezing and asthma was noted to be very common even in those infants who received no mechanical ventilation at all and it was also strongly associated with family history of atopy. Incidence of wheezing or asthma was correlated with that of eczema.

CHAPTER THREE

THE IMMUNOLOGICAL PROCESSES OF ALLERGIC RHINITIS

3.1 Allergic Rhinitis - Clinical Manifestation

Allergic rhinitis or hay fever is defined as a seasonal affliction of eyes, throat and nose. Classical symptoms are due to grass pollen and appear in the grass pollen season, usually in June and early July in the U.K. In atopic individuals exposure to such allergen manifests, at the initial stage, as itchy red eyes, sneezing and dry non-productive cough. In the second stage secretion follows. The eyes water and nose runs. In the lung the cough becomes productive and narrowing of bronchi causes diminished airflow resulting in bronchospasm. This bronchospasm is clinically manifested as wheezing or seasonal asthma. In the third or final stage, oedema causes marked swelling of the sclera which, due to secondary infection, often become blood red and exude yellowish gelatinous materials. As the cornea is clear, no visual impairment occurs. Peripheral involvement of the eyelids result in swelling or blepharitis and the conjunctiva takes on a "cobblestone" appearance in severe cases. In such cases, if treatment is not available, ulceration may occur resulting in irreversible visual impairment. A blocked nose precipitates constant difficulty in breathing and chest symptoms may vary from mild discomfort to serious asthma.

3.1.1 Patho-physiology of Hayfever and Asthma

Experimental animals have been used to study the immunological mechanisms of asthma, which is the single most important clinical entity of the trilogy of atopic diseases. The animals are naturally or artificially sensitised to specific antigens to which they are subsequently exposed by inhalation. Antigen(allergen)-antibody interaction on the surface of mast cells stimulates the

Triggers for "Allergic" Reactions

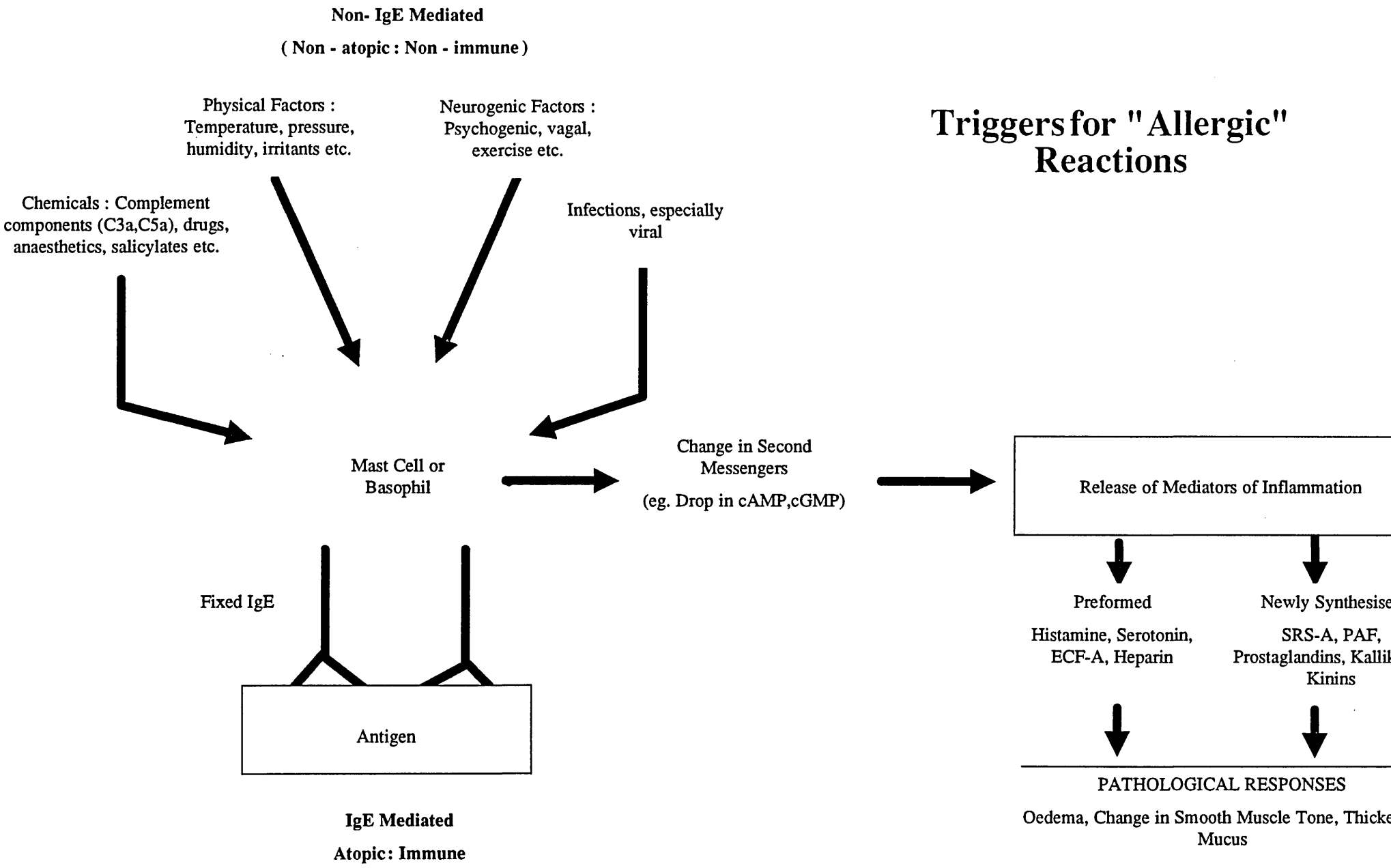


Fig 1

release of mediators of asthma attacks. Triggers of allergic reactions are shown in Fig. 1, page 49.

Most of the bronchial mast cells are, however, not on the mucosal surface, but in the sub-mucosa adjacent to blood vessels and neural ganglia. It has been suggested that the antigen binds to the small proportion of sensitised mast cells in the bronchial lumen or on the mucosal surface. Access to antigen to submucosal mast cells is then enhanced by increased permeability of the epithelium, with loosening of the normal "tight junctions" between cells, resulting from mast cell mediator release. The reaction is thus amplified (Hogg, 1978). While this pattern of response is more likely in hayfever asthma among atopic individuals, it does not give a clear picture in clinical asthma.

3.1.2 Adrenergic Hypothesis of Reversible Airways Obstruction

The vagus nerve carries both efferent stimuli to the muscle of the bronchial tree and afferent information from sensory irritant receptors in the airways. Vagal stimulation provokes broncho-constriction via a reflex arc. Adrenoreceptors (alpha-receptors) on the bronchial muscles, promote broncho-constriction and are constrictors, are few and of doubtful significance; beta-receptors, which promote broncho-dilation are much more important. These are stimulated naturally by circulating catecholamines. Though the presence of adrenoreceptors is now accepted, there is no evidence for a direct adrenergic nerve supply to human bronchial smooth muscles. The sympathetic nerves in the airways are distributed mainly to small vessels and also to autonomic ganglia, where their activity may modulate the degree of parasympathetic tone. Szentivamyi (1968) suggested that a state of partial beta-blockade may underlie the asthmatic state.

The demonstration that a pharmacological blockade of beta receptors may enhance bronchial reactivity to inhaled allergens or metacholine in hayfever patients without a previous history of bronchial asthma is compatible with the beta-blockade theory.

3.1.3 Cholinergic Hypothesis

Atropine or ipratorium, which is an anti-cholinergic drug, abolishes bronchial hyperactivity induced by exposure to various noxious gases, such as ozone, nitrogen dioxide or sulphur dioxide, and to bronchial virus infection. There may be a similar bronchospasm in hay fever, but here the bronchial hyper-activity is less intense, more transient and not associated with hypertrophy of the smooth muscle. This gives rise to the suspicion that the cholinergic system may be overactive in reversible airways obstruction.

The effects of cholinergic blockade in asthma are variable. In acute challenge with antigen, both in sensitised dogs and in allergic asthmatic patients, some studies have shown a complete inhibition of symptoms, while others have shown either no protection or only an effect compatible with the degree of broncho-dilation produced. The effect of histamine is as a direct constrictor of bronchial smooth muscle but is also known to stimulate irritant receptors and may have an indirect effect via a cholinergic reflex. The role of the parasympathetic nervous system is not yet fully understood. However, it seems likely to be relevant to the transient acquired hyper-reactivity of normal subjects.

3.1.4 Purinergic Hypothesis

Richardson and Beland (1976) postulate a non-adrenergic inhibitory "purinergic" nerve supply to the airways. Abnormality of nerves in the embryologically-related gastro-intestinal tract have been associated with defective smooth muscle function in Hirschsprung's Disease. Whether similar nerves supplying the bronchi may be relevant to asthma is not established. Another possible contributor to asthma is the reported abnormal permeability of the bronchial epithelium, allowing greater access of noxious agents to irritant receptors or to the smooth muscle itself.

3.1.5 The Concept of a "Shock Organ"

The idea of a "shock organ" appears to be determined by the site of antigen-antibody interactions, for example the skin in local anaphylaxis. However, such an idea does not satisfactorily explain why, for instance, one person reacts with hayfever and another with asthma to the same inhaled allergen. Nor does it explain why the pharmacological hypersensitivity is limited to the target cells of one or more tissues instead of affecting all the target cells uniformly. In asthmatic patients, for example, the injection of an otherwise non-toxic amount of histamine induces wheezing but not hives. In urticaria it induces hives, but not wheezing. The ultimate clinical manifestation of atopic abnormality, i.e. the organ or tissue localization of the disease, is determined by which type is primarily harbouring the postulated abnormality.

3.2.1 Eosinophilia in Allergic Reactions

A conspicuous association of eosinophils with allergic reactions has been well-documented but the nature of this association is not clear. Of the

catecholamines, both epinephrine and isoproternon produce eosinophilia, thus eosinophilia may be defined as a beta adrenergic action of catecholamines. Consequently, an impairment of beta-adrenergic mechanisms would be expected to eliminate one of the important suppressing influences in the homeostatic control of eosinophils and possibly result in eosinophilia. In a study using biopsies of nasal mucus membrane, Lozewicz et al. (1989) reported that eosinophil infiltration of mucus membrane occurs at the same time as mast cell degranulation.

3.2.2 Newer concepts of the pathogenesis of Allergic Rhinitis

In Chapter 1, some aspects of inflammatory processes have been discussed. Much of this knowledge has been gained using experimental situations and animal models. However, in recent times, with the advent of fibre optic bronchoscopy and technical advance in tissue fixation procedures, it is now possible to study the active physiological and pathological changes occurring in human volunteers. The biopsy evidence suggests that inflammation plays a fundamental role in the pathology of asthma. Town et al. (1989), Laitiner et al. (1985), Djukanvic et al. (1989) have all reported that such inflammation can be associated with profound epithelial damage and collagen deposition in the submucosa. Roche et al. (1989) reported that the collagen deposited in mild asthmatics is of Type III and Type V. Roche et al. also reported the presence of fibronectin in the lamina reticularis below the basal lamina.

Among recently identified pro-inflammatory mediators are platelet-activating factor acether (PAF-acether), free radicals, chemotactic factors, eosinophil-derived products, interleukins and a series of neuropeptides. Barnes

(1989) suggested that bronchial epithelium itself releases a relaxing factor and bronchial epithelial cell damage may decrease its release.

3.2.3 Platelet-activating factor acether (PAF-acether)

This is identified as an important mediator of inflammation, which exerts a significant effect inducing airway hyper-responsiveness in animals. Recent experiments have also shown that PAF can increase non-specific reactivity and broncho-constriction in normal human subjects.

PAF is an ether-linked phospholipid released from membrane phospholipid by the action of phospholipase A_2. It is generated by eosinophils (Lee et al., 1984) and alveolar macrophages (Arnoux et al., 1983) but not by mast cells. PAF acether was first discovered after the observation that lymphocytes sensitised with specific IgE antibody and challenged in vitro release a potent substance which causes platelet activation (Benveniste et al., 1972). O'Neill et al., (1984) reported alterations to platelet physiology immediately following conception in the human caused by a factor which had similar biochemical and physiological properties to the potent platelet activating factor 1-0-alkyl-2-acetyl-yn-glycerol-3 phosphocholine (PAF acether). Embryo-derived PAF may be the embryonic factor which signals the presence of a fertilized embryo in the uterus during the pre-implantation stages of pregnancy (Orozco et al. 1986). PAF is also a potent chemotactic factor for eosinophils and preactivates them for mediator release (Wardlow and Kay, 1986).

3.2.4 Oxygen Radicals

Oxygen is used in human metabolism to transform nutrients to energy, to oxidise endogenous compounds and to detoxify xenobiotics. These processes

generally transform stable molecule oxygen (O_2) to stable reduced states, such as $C0_2$ and H_2O. In the process four electrons are added to each oxygen molecule. Under certain circumstances, however, the reduction of oxygen may be incomplete, yielding a series of unstable oxygen species, including oxygen-derived free radicals (ODFR) 0_2^- (Superoxide) and 0H (Hydroxyl). During inflammation, polymorphonuclear leucocytes and cells of the monocyte/ macrophage lineage elaborate superoxide when activated (Babior 1978). This normally serves to lyse invading cells, to alter permeability of endothelium and to generate a chemotactic factor from a plasma-derived substrate, thereby defending the host against infection (Joyce, 1987).

Defects exist in superoxide production by leucocytes. Hereditary defects also exist in the cellular mechanisms which protect against ODFR, for example glucose-6-phosphate dehydrogenase deficiency. The resulting increase in concentration of ODFR leads to accelerated haemolysis and ODFR have also been implicated in altered vascular permeability and disturbing smooth muscle calcium homeostasis.

3.2.5 Lymphokines

Lymphokines, particularly IL4, are of particular interest in patients suffering from hyper IgE syndrome and also in some atopic individuals (Callard, 1987). IgE responses in the human can be obtained by B cell stimulation with IL4 secreting T-cell clones (Maggi et al., 1988). Soluble CD23 (sCD23) has been found to enhance ongoing IgE secretion by B-cells from atopic patients. IL4 is a potent stimulant of IgG1 as well as IgE production (Paul, 1987), and also stimulates Ig Class switching. Heiner (1984) reported that properties of IgG1 and IgG4 are similar in certain respects and specific

combined deficiencies tend to be associated with certain diseases. It is possible that IL4 exerts significant effects as a growth and differentiation factor in atopic individuals.

3.3.1 Hayfever Distribution and the Dimension of the Problem

Hayfever is the commonest allergic disease in the U.K. and affects approximately ten percent of the population - one in four of the participants surveyed by "Which?" Magazine in 1985 reported a history of hayfever symptoms in the household. It has been reported that among the general public nearly one in five professional people suffer from hayfever compared with only one in ten of the general population. There are regional variations of incidence among the UK population. The condition usually occurs in childhood or early adult life, but it can arise for the first time in middle age. In a survey among the general public it was found that symptoms generally manifested before the age of 15 and were suffered for 17 years on average. Both sexes are equally affected. Some people grow out of their hayfever symptoms in their late twenties, thirties or forties, although it may return later in life.

It is estimated that about 30 to 40 per cent of hayfever sufferers get summer asthma and each year a quarter of a million people are obliged to be absent from work from hayfever. Among known asthmatics, 30 per cent are incapacitated occasionally and 15 per cent frequently during the hayfever season (Lane & Starr, 1979).

The weather has a particular influence upon the incidence of hayfever by affecting the release of pollen into the atmosphere and the subsequent increase in pollen count. For example, due to the sudden burst of hot weather in late June 1983, there occurred a rise above the expected rates of non-infective

wheeze and the dry, sunny April in 1984 produced a similar rise ("Doctor", May 1984).

3.3.2 Allergens

All allergens were thought to be functionally identical, acting non-specifically on the same sensitive target common to all atopic people. However, isolation of house dust allergen did not confirm chemically that there is a unique allergen molecule and many distant allergens have now been identified.

Allergens are mostly brown in aqueous solution, thermostable, resistant to proteolytic digestion and weakly immunogenic in laboratory animals. They migrate fast anodically in an electric field at slightly alkaline pH, and are relatively deficient in free amino groups. In the semi-solid state, allergens are highly hygroscopic.

3.3.3 Pollen Allergens

Pollen extracts (March et al., 1970) contain a number of distinct groups of antigenically different allergenic glycoproteins displaying immunochemical and allergological cross-reactivity only within each group. Pollen grains are generally between 12 and 25 microns in size and the molecular weight of pollen allergens is usually in the order of 20 KDa. Clinical and immunological evidence confirms that, apart from a general response to allergenic determinants, the body reacts to these particular allergens by the production of specific antibody directed against the antigenic determinants.

TABLE 5. Biological Properties of Timothy Grass Pollen

Family - Graminae.

Sub family - Festucoideae.

Tribe - Agrostideae.

Allergen number - 28.

Allergen group - B (mol. wt. 19,000 and under).

Antigen content - Group I antigen.

Antigen B only found in Timothy.

Allergen 19 and 25 with molecular weight 15,000 account for 85% of total allergenicity.

Electrophoretic migration velocity: positive in relation to human albumin.

Analysis of 11 of the 28 antigens were shown to be allergic to human.

Protein determination shows antigen No. 3,19,24 as pure amino acids and others to be glycoproteins.

3.3.4 Timothy Grass (Phleum pratense) Pollen

Timothy grass is common throughout the British Isles, occurring naturally in meadows. Pollination occurs in July. Timothy grass allergen cross-reacts with many other grass pollens, making this a particularly useful species for study. Table 5 shows the biological properties of Timothy grass pollens.

AIMS OF THE STUDY

(a) To investigate and determine the levels of total and allergen-specific IgG4 and IgE in non-atopic and atopic patients during pregnancy and to compare these levels with those in the same patients after pregnancy.

(b) To correlate the laboratory findings with that of clinical manifestations of allergic symptoms.

CHAPTER FOUR

PATIENTS AND CLINICAL METHODS

4.1 Study Location

This project was undertaken at the Langold Health Centre, Worksop in Nottinghamshire. The Health Centre cares for a population of 7000 patients. Almost the entire population is of European stock. Most of the patients are in the mining industry and some are involved in agriculture and professions, e.g. teachers, office workers, etc. The practice area is surrounded by agricultural lands and woodlands.

4.2 Preliminary Environmental Survey

It is well established that clinical manifestations of hayfever relate to environmental factors, particularly the pollen count. A preliminary survey was carried out to investigate the environmental factors pollen count, rainfall and temperature and also to identify the principal plant species known to generate allergenic pollen found in the geographical location of the clinical study area.

The local pollen counts, obtained from the National Pollen Count Centre at Rotherham - 10 miles north of Langold, were analyzed for three consecutive years during the pollen seasons in 1984, 1985 and 1986 (Appendix I). Fig. 2, Appendix I, shows monthly pollen count, rainfall and temperature from May to August 1985.

The principal allergic pollen-generating species in the area are shown in Appendix I. It can be seen that the parishes from which the practice population are drawn contain over 1000 ha of grassland and it was thus likely that grass pollen would be a common allergen in this population.

4.3 Preliminary Patient Selection

A preliminary survey was undertaken to determine the most common allergen affecting the population so that this could be examined in the

subsequent study of pregnancy. This preliminary study also enabled a clear understanding of the clinical manifestations of hayfever and of any significant local variations to be obtained. It also provided a basis for a comparison of the clinical symptoms in the pregnant and non-pregnant population. Practice hayfever sufferers were invited to join the preliminary study. In response to a notification that allergy screening facilities were available in the practice, a total of 75 known hayfever patients attended for allergy screening.

4.4 Diagnostic Procedures

Clinical History

In allergy, as in any other branch of medicine, history taking is all important. In order to achieve an accurate history comparable between individual cases and to arrive at a clear presumptive diagnosis, an allergy case history from (Form 1, Appendix II) was designed.

Clinical Examination

This included a general examination with special attention to chest symptoms such as asthma, nasal polyp, deviated nasal septum and eczema lesions.

Skin Testing

For the purpose of identification of local allergen distribution among the practice population and also for the identification of a model allergen for further laboratory investigations, the modified skin prick test was used.

A single drop of allergen extract was placed on the skin, on the inside of the forearm. Allergen testing solutions were prepared from aqueous allergen extracts containing 50 per cent glycerin and 0.4 per cent phenol in 3 ml dropper bottles. These solutions are standardised by the manufacturers by iso-electric

focusing to ensure constant potency and reproducible results. Allergy concentrations are quoted as protein nitrogen units (PNU) per unit volume. The allergen was dissolved in normal saline and a stilette introduced through the drop of allergen into the skin at an acute angle and followed by a single vertical lift. Test solutions were separated from each other and results were compared with negative and positive controls. The responses, which appeared as wheals of different sizes, were evaluated after 10 to 20 minutes. The results recorded on form III (Appendix II). Skin test kits were produced and provided by E. Merck Pharmaceuticals, Alton, U.K.

Interpretation of Results of Skin Test

The wheals were graded as either +, ++, +++, ++++, or +++++, according to diameter (Table 6). In order to avoid confusion and operator error, these tests were carried out by a single operator.

4.5 Summary of Results of Preliminary Screening

The study was conducted in a rural setting in England covering a population of approximately 7000 people. Pollen counts in the summer months of three consecutive years showed that June 1984 and June 1986 had similar daily average pollen count of 103 where as June 1985 had an average pollen count of 62 for the same period.

A total of seventy five patients with allergic symptoms attended for preliminary screening (Table 7). Forty two of these were male and thirty three were female. Among the male patients, seven suffered from perennial symptoms, twenty eight with seasonal symptoms and seven with perennial symptoms with seasonal exacerbations. Among the female patients, ten had perennial symptoms, twenty one seasonal and two reported seasonal exacerbation of symptoms.

Extracts of thirty two different allergens were tested, depending on the history of individual allergic symptoms. The top three positive reactions to plant pollens were against grass (54) barley (35) and wheat (19). Appendix III Table 8 shows the distribution of skin test reactions.

The age of onset of symptoms in the attendant group was reported to be from the very young (under 2 years of age) up to the age of 48 years (Appendix III Tables 9 and 10).

Appendix III gives full details of the patients screened and the results of the skin tests.

Skin testing confirmed grass pollen to be the most common allergen among the test population. Phadebas IgE PRIST (Pharmacia) and IgE RAST (Pharmacia) tests were employed for quantitative determination of immunoglobulins against Timothy grass allergen which was confirmed to be present in all twelve test samples randomly taken from the selected patient group with allergic symptoms.

4.6 The Selection of Pregnant Patients

Pregnant women were initially interviewed while attending the antenatal clinic, collecting the data using Form II, Appendix II. Appropriate individuals were entered into the study. No skin tests were carried out on these women to avoid potential detrimental effects of such procedures during pregnancy. Also, the interpretation of skin test results are likely to be anomalous in pregnancy. Patients participating in the study were requested to record their own allergic symptoms daily using Form IV (Appendix II).

TABLE 6. Assessment of skin test reactions by the prick test.

+	A raised weal of diameter up to 3 mm
++	A raised weal of diameter from 3 to 8 mm
+++	A raised weal of diameter from 8 mm to 16 mm
++++	A raised weal of diameter from 16 mm to 20 mm
+++++	A raised weal of diameter of greater than 20 mm

TABLE 7. Number and sex of allergy patients attending for skin test.

SEX	PERENNIAL	SEASONAL	SEASONAL AND PERENNIAL	TOTAL
Male	7	28	7	42
Female	10	21	2	33
Total	17	49	9	75

The groups selected for study were:

Group 1	Pregnant	Allergic	N = 18
Group 2	Pregnant	Non-allergic	N = 16
Group 3	Non-pregnant	Allergic	N = 15
Group 4	Non-pregnant	Non-allergic	N = 18

Participating patients joined the study voluntarily. Blood samples were collected at about 10 weeks and at 32-36 weeks of pregnancy. Follow-up specimens were collected at 6 weeks and about 6 months following delivery.

CHAPTER FIVE

LABORATORY MATERIALS AND METHODS

5.1.1 Specimen Collection and Storage

Blood samples (10 ml) were collected in plain containers by venipuncture from each of the four groups of individuals as defined below:-

Group 1 - pregnant allergic women;

Group 2 - pregnant non-allergic women;

Group 3 - age matched non-pregnant allergic women;

Group 4 - non-pregnant, non-allergic women.

The specimens were allowed to clot for 1 hour at room temperature, followed by centrifugation at 700 x g for 10 min at room temperature. The serum was stored at -20°C until tested.

5.1.2 Assay Method. Enzyme-linked Immunosorbent Assay

Commercial assay kits were used to measure the levels of allergen-specific circulating IgE (Phadezym PRIST™, Pharmacia Biotecnology) and IgG4 (RAST™, Pharmacia Biotechnology) antibodies. These immunoassay systems are based on the principle of the allergosorbent test. Details of the reagents provided are given in Appendix IV.

5.1.3 Principle of the Procedure for the Determination of Circulating Allergen-Specific IgE

The allergen of interest (Timothy grass pollen), covalently coupled to a paper disc, reacts with specific IgE in the patient serum sample. After washing, a disc-allergen-IgE complex remains. Anti-IgE, conjugated with the enzyme B-galactosidase via a thiol sensitive disulphide link, reacts with any IgE bound to allergen on the disc. After washing away surplus loosely adsorbed reagent, a disc-allergen-IgE-anti-IgE enzyme complex remains. The enzyme is released from the complex by the addition of glutathione. The enzyme then reacts with

the chromogenic substrate 0-nitro-phenyl-B-galactoside to form a yellow-coloured product, 0-nitrophenol, and colourless galactose. The enzymatic hydrolysis is stopped after a fixed reaction time by addition of sodium carbonate. The absorbance measured at 420 nm is directly proportional to the concentration of enzyme bound to the allergen disc and hence to the level of allergen-specific IgE.

5.1.4 Test Procedure

Details of the reagents supplied for this assay are given in Appendix IV. The paper discs with bound specific allergen were incubated with the test sera at room temperature for three hours. The discs were then washed three times with the washing solution. A solution of anti human-IgE enzyme complex was added and the incubation continued overnight at room temperature. Discs were then washed three more times after which the development solution, containing enzyme substrate, was added to all tubes. Tubes were covered with plastic film and incubated at 37°C for 2 hours exactly. The reaction was then terminated by adding stop solution and the absorbance of the coloured product was measured at 420 nm using an LKB spectrophotometer.

5.1.5 Calculation of Results

The absorbance value of each reference serum and unknown sample was measured. Results were expressed as a percentage of the reference serum response (units - PRU/ml) by calculating:-

$$\frac{\text{X absorbance of unknown}}{\text{X absorbance of reference}} \times 100$$

5.1.6 Principle and Procedure for the Determination of Total Serum IgE

The IgE PRIST™ (Paper Radioimmunosorbent Test) is based on the standard sandwich immunoassay technique using paper discs as a solid phase. Anti-IgE, covalently coupled to the paper disc, is allowed to react during the first incubation with the IgE in the sample. After washing, a fixed amount of enzyme-labelled anti-antibody is added, which forms a complex with the IgE molecules bound to the paper disc during the first incubation. Bound and free anti-human IgE-enzyme complex are separated by washing the disc. After incubation with the developing agent containing the enzyme substrate and stopping the reaction, the absorbance of the yellow coloured product is measured at 420 nm. The colour is directly proportional to the concentration of IgE antibodies in the sample.

Each determination was performed in duplicate.

5.1.7 Test Procedure

Patient samples were diluted 10 fold with sample diluent (horse serum). A standard curve was prepared for each individual assay.

Details of the reagents supplied for this assay are given in Appendix IV. Paper discs containing sheep-anti-IgE were incubated with the test sera at room temperature for 3 hours. The discs were then washed three times with washing solution. Enzyme-labelled rabbit anti-human IgE solution was then added and incubated overnight at room temperature. Discs were washed three more times after which the development solution containing the chromogenic substrate 0-nitro-phenyl-B-galactoside was added. The reaction was allowed to continue for exactly 60 minutes at 37°C and then terminated by stop solution (sodium

carbonate). Measurement of the absorbance of the coloured product of the enzyme hydrolysis (0-nitrophenol) was carried out using an LKB spectrophotometer.

The results were calculated as described in paragraph 5.1.5 above.

5.2.1 Principle and Procedure for the Determination of Circulating Specific IgG4.

In the IgG RAST™ assay, the specific allergen (Timothy grass pollen) covalently coupled to the walls of plastic tubes reacts with the corresponding specific IgG in the patient serum sample. After washing away loosely adsorbed non-specific IgG, enzyme-labelled mouse monoclonal antibodies against human IgG4 are added. After washing, the enzyme is released from the complex and is quantitated as indicated above (paragraph 5.1.3). To evaluate the test results, absorbance values of patients' serum are compared directly with the absorbance value of a reference serum run in parallel.

5.2.2 Test Procedures

Patient serum samples were diluted 1:50 with sample diluent (containing chicken serum in buffer solution) supplied by the manufacturers of the test kits (Pharmacia Biotechnology).

After washing the allergen coated tubes, 100 ul of reference sera or diluted patient serum samples were added to appropriate tubes and allowed to incubate for 3 hours at room temperature. Tubes were washed 3 times with 10 ml of wash buffer and 100 ul of enzyme-labelled mouse anti-human IgG4 was then added to all tubes except the blanks. The reaction tubes were allowed to incubate overnight at room temperature after which they were washed a further

3 times as above. 200 ul aliquots of development solution (0-nitrophenyl-B-galactoside and glutathione) were then added to all tubes including the blanks. Following a further incubation of exactly 30 minutes at 37°C, 1 ml stop solution (sodium carbonate) was added to all tubes.

The absorbance of the coloured product was measured at 420 nm using an LKB spectrophotometer.

Results were expressed as a percentage of the reference response by calculating:

$$\frac{\text{Mean absorbance unknown}}{\text{Mean absorbance reference}} \times 100$$

5.3.1 Principle and Procedure for the Determination of Total IgG and Total Sub-class IgG4

The Radial Immunodiffusion (RID) Assay

The determination of a soluble antigen by radial immunodiffusion involves antigen diffusing from a cylindrical well through the liquid phase of an agarose gel. When the corresponding antibody is uniformly incorporated in the gel, immunoprecipitation will occur when the antigen diffusing outward reaches the concentration of equivalence with the antibody. The end point is reached when all of the antigen has reacted with the antibody forming a precipitation ring. The diameter of the precipitation ring is proportional to the antigen concentration, thus a standard curve may be generated by plotting the ring diameters of a number of standard solutions versus their concentrations. The concentration of the antigen in an unknown sample may then be determined by measuring the diameter of the ring produced by that sample and interpolating this value into the standard curve. The measurement may be

made after a fixed diffusion time or after equilibrium has been reached. In the present study, measurements were taken after equilibrium.

5.3.2 Specimen Preparation

Serum samples were collected as described in paragraph 5.1.1 above. The total IgG concentration of the samples was determined using undiluted patient serum according to the manufacturers instructions (The Binding Site Ltd., Birmingham, UK). For the measurement of total IgG4, serum samples were diluted 1:2 with the sheep serum included in the kit.

High, medium and low concentration calibrators or standards are supplied with each kit. These were reconstituted to the stated volume with distilled water. Each calibrator contains 0.2% sodium azide as a preservative and is physically and chemically similar to normal serum.

5.3.3 Procedure

Following removal of the lids, the RID plates were stood at room temperature for 10 mins to allow evaporation of any condensation.

The amount of antibody in each batch of plates is identical so that a calibration curve does not need to be made for each plate. 5 ul of calibrator in sample was added to each well in the plate using a Hamilton microsyringe. The plate lid was tightly closed and the assay was allowed to run to completion (48 hours for IgG and 96 hours for IgG4). The diameter of the rings measured. For maximal accuracy the rings must be allowed to diffuse to full size, a final ring size for the high calibrator of between 8.7 -9.3 mm being acceptable. Any reading outside this size was ignored and the tests were repeated. The

concentrations of protein were read from the RID reference table supplied with the assay, taking into account any dilution factor.

5.3.4 Calculation of Results

After completion of the assay ring diameters were measured using the calliper provided with the RID Reference Table. The total IgG and IgG4 subclass concentrations were then read directly from the appropriate column.

Using the same batch of plates, three sets of calibrators were run. A typical standard calibration curve for total IgG is shown in Fig. 3. All tests were completed under similar conditions as recommended by the manufacturers.

The between assay precision for the RID assays, using a pool of blood from non-pregnant females in 18 assays was:

IgG (total) - Mean = 9.06 g/l
- SD = 1.57 g/l
- CV = 17.3%

IgG4 - Mean = 223 mg/l
- SD = 43 mg/l
- CV = 19.3%

These values compare with the manufacturers figures of coefficients of variation of 17% for IgG and 28.5% for IgG4.

Mean, Standard Deviation and Coefficient Variation are shown in Table 11A.

ıese results are discussed in full detail in Chapter Six.

able 11A

IgG TOTAL

	MEAN	SD	CV%	
1. Pregnant-Allergic Women	9046	2550	28%	n=48
2. Pregnant Non-Allergic Women	9408	2199	23%	n=34
3. Non-pregnant Allergic Women	9364	1797	19%	n=15
4. Non-pregnant Non-allergic Women	9063	1566	17%	n=18
Female Single Donors	10402	1768	17%	n=60

IgG4 (TOTAL)

	MEAN	SD	CV%	
1. Pregnant-Allergic Women	320	224	70%	n=46
2. Pregnant Non-Allergic Women	486	203	42%	n=32
3. Non-pregnant Allergic Women	351	181	52%	n=15
4. Non-pregnant Non-allergic Women	223	43	19%	n=18
Female Single Donors	321	192	60%	n=53

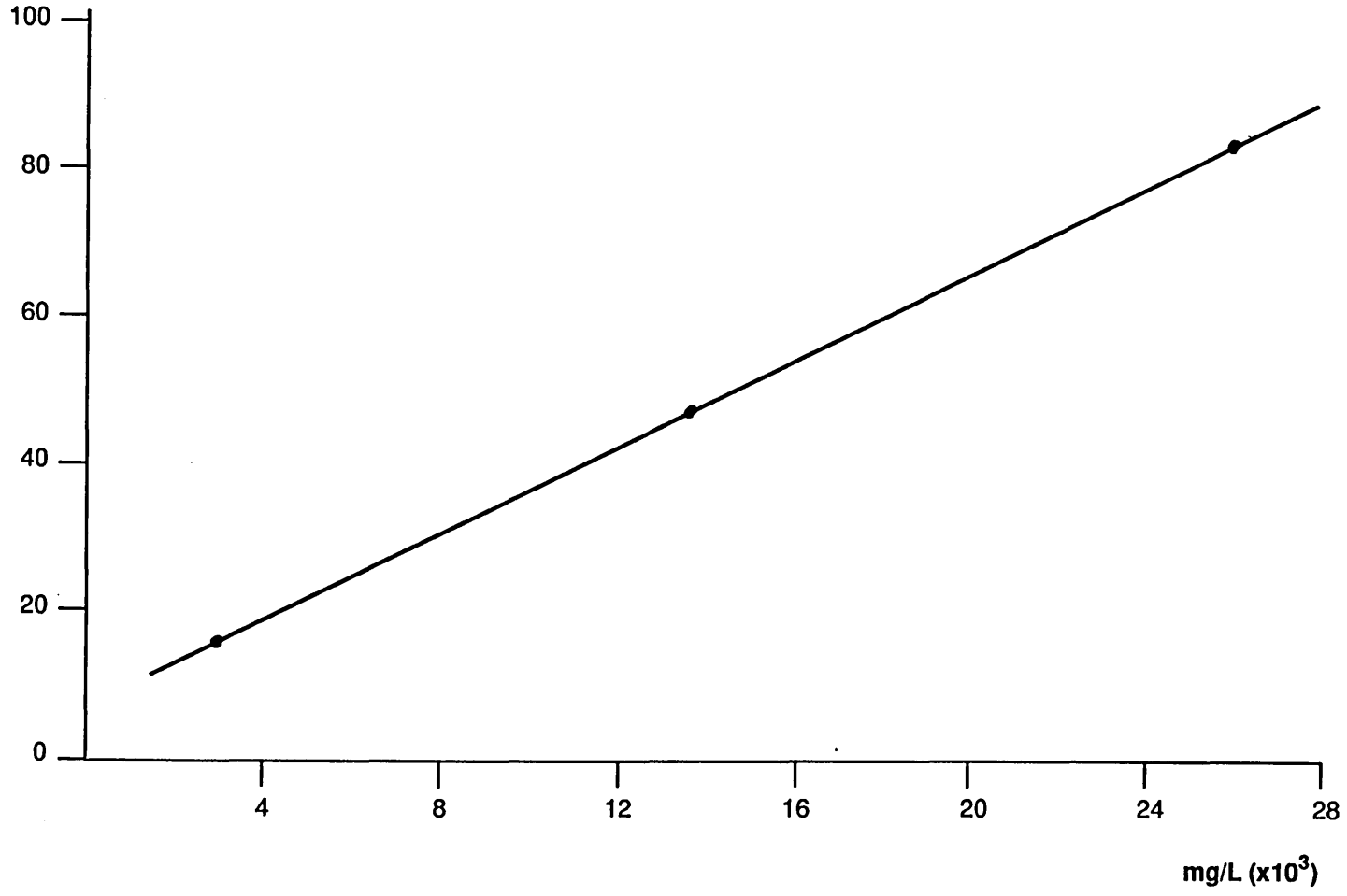


Fig 3 STANDARD CURVE FOR IgG ASSAY

Key :- d^2 Diameter in square (mm)

6. INTRODUCTION

Sufficient immunoglobulin data for subsequent analysis were obtained from a total of 67 individuals. Of these 18 were pregnant women who suffered allergic symptoms (pregnant allergic), 16 were pregnant women who suffered no such symptoms (pregnant non-allergic), 15 were non-pregnant women who suffered allergic symptoms (non-pregnant allergic) and 18 women were not pregnant and suffered no allergic symptoms (non-pregnant non-allergic).

6.1 Changes in serum concentrations of immunoglobulins during pregnancy in allergic and non-allergic women

The data on the 67 individuals were analyzed using the SPSS/PC + V 3.1 statistical package, which was also used to generate graphical representations of the data. Advice on statistical methods and their interpretation was provided by the Trent Regional Health Authority Statistical Unit, Sheffield (S. Saljano).

6.1.1 Description of the data used in the analysis

(a) Nature of the data

Data were obtained from 4 experimental groups thus:

Group 1 - pregnant allergic (n=18)

Group 2 - pregnant non-allergic (n=16)

Group 3 - non-pregnant allergic (n=15)

Group 4 - non-pregnant non-allergic (n=18)

Five serum immunoglobulin measurements were recorded for each woman:

Total immunoglobulin G [IgG (total)]

Total immunoglobulin G subclass 4 [IgG4 (total)]

Timothy grass antigen-specific IgG4 [IgG4 (a-s)]

Total immunoglobulin E [IgE (total)]

Timothy grass antigen-specific IgE [IgE (a-s)]

A single blood sample was taken from the women in Groups 3 & 4 for immunoglobulin measurements. These samples were collected in April 1986. Four blood samples were taken for immunoglobulin measurements from the women in Groups 1 & 2. These were taken at times after the beginning of the pregnancy and, therefore, they also exhibited seasonal differences. These can be summarized thus:

Stage A - early pregnancy (7 weeks +/- 3), season 1 (February - April).

Stage B - late pregnancy (33 weeks +/- 3), season 2 (May - July).

Stage C - first post-natal (six weeks after delivery) - season 3 (August - October).

Stage D - second post-natal (36 weeks +/- 3 after delivery) - season 4 (November - January).

An additional data item recorded was the parity of each of the pregnant women. These data are summarised in Table 12, a-d.

(b) Completeness of the data

The immunoglobulin data for Groups 3 & 4 are complete. For the pregnant women (Groups 1 & 2) some measurements are missing, particularly for the second post-natal measurement (Stage D).

(c) Undetectable levels

Undetectable levels are shown as less than the defined minimum detectable levels of each assay. These were:

for IgE (a-s) - <0.35 PRU/ml

for IgE (total) - <5 kU/l

for IgG4 (a-s) - <5 per cent

Table 12. The concentration of various immunoglobulins in the peripheral blood taken from pregnant allergic, pregnant non-allergic, non-pregnant allergic and non-pregnant non-allergic women.

Units

IgE(a-s) - PRU/ml

IgE (total) - kU/l

IgG (total) - g/l

IgG4 (total) - mg/l

IgG4 (a-s) - % of response of reference serum

(d) Derivation of a single immunoglobulin measurement for the pregnant women (Groups 1 & 2)

In order to compare the pregnant women in Groups 1 & 2 with the non-pregnant women in Groups 3 & 4 a composite single set of immunoglobulin measurements is required for each such woman. The average concentration of each immunoglobulin in samples taken at Stages A to C of pregnancy (ie from 10 weeks gestation - 6 weeks post partum) was used to derive a single measurement for each immunoglobulin for each pregnant woman. The second post natal sample (Stage D) was not used to derive this composite value as few measurements were available and also these samples were taken 6 months after the end of the pregnancy. This composite average value used as much data as was available for each individual. Thus, if only a single measurement was available in an individual pregnancy, this value was taken. If two or three values were available, these were meaned.

6.1.2 Statistical analysis of the immunoglobulin measurement data

The statistical analyses used were two-way analysis of variance (ANOVA), two sample T-tests, matched pairs T-test and repeated measures ANOVA. These techniques require the data to conform to the following assumptions:

1. Data for each immunoglobulin measurement within each of the four groups of women should be approximately normally distributed.
2. The variance of the data for each immunoglobulin measurement within each of the four groups of women should be approximately equal.

If it appears that these assumptions are violated then the data should be transformed.

Table 12a. Levels of Immunoglobulins in pregnant allergic women.

NO.	IgE (a-s)				IgE (Total)				IgG (Total)				IgG4 (Total)				IgG4 (a-s)				PREGNANCY
	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	
1	1.41	0.61			62.75	48.4			7920	7520			6.46	6.46			9.8	9.0			First Pregnancy
2	27.3				90.20				10000								27.3				First Pregnancy
3	1.135		1.57		249.7		122.6		10900				6.46		6.4		18.7		7.8		First Pregnancy
4	1.160				180.7				7130								10.8				Fifth Pregnancy
5	<0.35	0.98	0.88	<0.35	132.0	107.5	72.6	87.4	10500	8320	9150	11800	306	240	251	366	9.0	5.7	14.7		Third Pregnancy
6	<0.35	<0.35	<0.35		298.0	299.0	290.8		11400	6370	9580		646	135	295		17.2	9.0	<5		First Pregnancy
7	4.40	2.22	1.20	1.39	700.0	651.0	482.5	803.2	11400	10900	12800	13200	600	470	615	484	6.8	5.6	21.9	13.2	Second Pregnancy
8	<0.35	<0.35	<0.35		427.3	372.0	206.5		11800	7130	14200		443	273	555		7.0	<5	7.2		First Pregnancy
9	<0.35	<0.35	<0.35	0.66	117.9	116.9	82.2	72.2	8320	6370	9150	8740	391	330	366		<5	5.6	19.7		Second Pregnancy
10	0.85	0.60	0.66		138.6	99.9	90.6		6750	6370	7130		646	631	638		9.27	17.8	20.4		First Pregnancy
11	0.41	0.52			280.5	225.9			12800	9150			646	585			12.6	6.8			First Pregnancy
12	<0.35		<0.35		363.9		98.9		12300		11800		646		646		15.1		6.3		First Pregnancy
13	0.57	<0.35	<0.35		25.3	21.3	17.6		10900	10500	15200		64	57	57		<5	9.6	17.0	25.7	Fourth Pregnancy
14	0.55	<0.35	<0.35		36.7	11.9	11.9		8740	8740	8320		484	443	615		9.3	7.69	15.9		Second Pregnancy
15	1.78	0.60	<0.35		23.9	40.7	25.4		6750	5280	7920		262	110	171		8.1	9.72	12.8		Third Pregnancy
16	1.4	0.5	0.46		70.9	49.4	58.3		5640	3930	6750		171	127	220		8.0	9.9	14.9		Third Pregnancy
17	1.79	1.09	1.05		14.5	16.6	11.0		6370	6370	5640		57	50	135		6.5	10.8	16.0		First Pregnancy
18	1.31	1.24	1.03		23.2	28.9	16.7		9150	7520	9580		181	102	200		17.8	16.0	19.5		First Pregnancy

Table 12b. Levels of Immunoglobulins in Pregnant Non-Allergic Women

NO.	IgE (a-s)				IgE (Total)				IgG (Total)				IgG4 (Total)				IgG4 (a-s)				PREGNANCY
	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	
1	<0.35	<0.35	<0.35		6.10	6.32	<5		8740	8740	11400		57	57	79		16	20.81	20.36		Third Pregnancy
2	<0.35	<0.35	<0.35		7.10	7.32	<5		12800	12100	11800		646	646	646		14.43	16.21	15.8		Second Pregnancy
3	<0.35				8.30				6750								15.7				Fourth Pregnancy
4	<0.35	<0.35	<0.35		27.50	26.20	28.40		5640	9580	8740		630	600	615	630	32.1	17.19	21.71	19.68	Second Pregnancy
5	<0.35				6.70				13700								5.3				First Pregnancy
6	<0.35				20.50				7130								7.8				First Pregnancy
7	<0.35				<5				9150								22.3				Second Pregnancy
8	<0.35	<0.35	<0.35		<5	5.64	<5		10000	9150	9150		585	417	484		<5	24.88	28.73		Second Pregnancy
9	<0.35	<0.35	<0.35		<5	5.9	6.1		9580	6000	8740		600	443	600	555	<5	5.2	18.1	17.4	Third Pregnancy
10	<0.35	<0.35	<0.35		63.1	18.8	20.4		6370	5280	6450		585	484	450		8.0	7.2	7.4		Third Pregnancy
11	<0.35	<0.35	<0.35		55.9	37.3	39.6		9580	9580	9640		646	646	646		11.7	10.39	10.6		First Pregnancy
12	<0.35				8.69				10000				570				15.3				Third Pregnancy
13	<0.35				15.7				9150				512				16.5				Third Pregnancy
14	<0.35				18.5				9580				240				20.0				Second Pregnancy
15	<0.35	<0.35	0.57		18.9	17.2	14.0		11400	8320	13700		526	600	646		10.6	11.3	12.6		First Pregnancy
16	<0.35	<0.35	<0.35		10.6	9.8	10.8		12300	11300	8320		64	584	71		8.6	9.8	17.0		Third Pregnancy
17																					
18																					

Table 12c. Levels of Immunoglobulins in Non-Pregnant Allergic Women

No.	IgE (a-s)	IgE (Total)	IgG (Total)	IgG4 (Total	IgG4 (a-s)
1	1.70	213.30	9 150	470	16.0
2	5.35	89.43	10 000	110	12.7
3	12.60	109.70	9 150	404	43.7
4	19.70	96.90	12 300	202	16.3
5	28.80	309.1	8 320	110	7.8
6	0.65	63.0	11 400	284	16.0
7	21.85	612.7	5 280	330	21.3
8	15.80	315.7	7 130	118	20.7
9	19.30	152.1	9 150	181	7.2
10	21.90	638.2	11 400	600	20.7
11	29.70	259.7	9 150	646	23.0
12	10.60	117.4	8 740	512	18.4
13	6.50	117.1	9 150	512	16.6
14	98.60	833.6	8 740	470	19.6
15	214.3	151.5	11 400	318	29.8

Table 12d. Levels of Immunoglobulins in Non-Pregnant Non-Allergic Women

No.	IgE (a-s)	IgE (Total)	IgG (Total)	IgG4 (Total	IgG4 (a-s)
1	<0.35	25.74	7 130	171	16.4
2	<0.35	20.20	7 920	190	20.26
3	<0.35	51.00	9 150	220	26.26
4	<0.35	46.20	10 500	144	20.9
5	<0.35	13.60	6 000	262	12.8
6	<0.35	14.85	10 900	262	24.17
7	<0.35	21.30	10 900	262	32.53
8	<0.35	50.90	10 500	251	17.90
9	<0.35	24.80	9 150	306	60.9
10	0.54	21.30	11 800	220	17.1
11	<0.35	63.00	10 000	284	19.7
12	<0.35	<5.00	7 920	240	10.3
13	<0.35	25.00	9 150	190	10.3
14	<0.35	11.70	9 150	220	13.7
15	<0.35	<5.00	6 750	220	13.5
16	20.35	22.32	9 150	162	28.8
17	<0.35	21.40	7 920	220	11.0
18	<0.35	7.69	9 150	200	15.48

Table 13a. Summary of Means, Standard Deviation and 95% Confidence Intervals.

A IgE (A-S)
Means and Standard Deviations

	Mean	Std. Dev.	N	95 % Conf	Interval
PREGNANT ALLERGIC	1.22	2.72	14	0.68	2.17
PREGNANT NON-ALLERGIC	--	--	1	--	--
NON-PREGNANT ALLERGIC	17.48	4.45	15	7.64	40.00
NON-PREGNANT NON-ALLERGIC	--	--	1	--	--
For entire sample	4.20	6.46	31	2.12	8.33

B IgE (Total)
Means and Standard Deviations

	Mean	Std. Dev.	N	95 % Conf	Interval
PREGNANT ALLERGIC	103.2	3.3	18	57.3	186.0
PREGNANT NON-ALLERGIC	12.6	2.0	15	8.6	18.3
NON-PREGNANT ALLERGIC	200.3	2.2	15	129.4	309.8
NON-PREGNANT NON-ALLERGIC	23.5	1.8	15	17.0	32.7
For entire sample	51.5	4.0	63	36.3	73.0

C IgG (Total)
Means and Standard Deviations

	Mean	Std. Dev.	N	95 % Conf	Interval
PREGNANT ALLERGIC	9083	2185	18	7996	10169
PREGNANT NON-ALLERGIC	9392	1980	16	8337	10447
NON-PREGNANT ALLERGIC	9364	1797	15	8369	10359
NON-PREGNANT NON-ALLERGIC	9063	1566	18	8285	9842
For entire sample	9214	1860	67	8761	9668

D IgG4 (Total)
Means and Standard Deviations

	Mean	Std. Dev.	N	95 % Conf	Interval
PREGNANT ALLERGIC	396	219	16	279	513
PREGNANT NON-ALLERGIC	473	188	12	353	592
NON-PREGNANT ALLERGIC	351	181	15	251	451
NON-PREGNANT NON-ALLERGIC	224	43	18	202	245
For entire sample	349	187	61	301	397

E IgG4 (A-S)
Means and Standard Deviations

	Mean	Std. Dev.	N	95 % Conf	Interval
PREGNANT ALLERGIC	11.73	657.46	18	10.34	13.52
PREGNANT NON-ALLERGIC	13.13	229.57	16	10.34	17.22
NON-PREGNANT ALLERGIC	16.66	297.27	15	13.03	22.04
NON-PREGNANT NON-ALLERGIC	17.65	416.49	18	14.57	22.04
For entire sample	14.46	307.79	67	13.03	16.13

6.1.2.1 Evaluation of the assumption of a normal distribution of the untransformed immunoglobulin data

For each immunoglobulin, and for each of the four groups of women, normal probability plots were visually inspected to check normality. In a normal probability plot each observed value is plotted against its expected value from a normal distribution. If the sample is from a normal distribution the points should fall, more or less, on a straight line.

Initially, such a visual evaluation was carried out and a total of 32 plots reviewed. The visual inspection revealed that the observed and expected points for the groups of women did not cluster around a straight line in all cases.

This subjective, visual assessment of the data was further investigated using the Lilliefors test which provides a test of the null hypothesis that the data are from a normal distribution. This test confirmed that the untransformed data for IgG (total) and IgG4 (total) showed a normal distribution.

6.1.2.2 Evaluation of the assumption of equality of variance of the untransformed immunoglobulin data.

The Levene test for the homogeneity of variance is particularly useful in evaluating whether data are suitable for use with ANOVA procedures. The results of this analysis showed that the untransformed data for IgE (a-s), IgE (total) and IgG4 (total) did not conform to the assumption of homogeneity of variance.

In the case of IgE (a-s), IgE (total) and IgG4 (a-s), where the assumptions of either normality of distribution or equality of variance were not valid, the data were transformed.

6.1.2.3 Determination of the required transformation

A power transformation is frequently used to stabilise variances and also quite often overcomes departures from normality. Of the data from the five immunoglobulin measurements in Sections 6.1.2.1 and 6.1.2.2 above, only the values for IgG (total) conformed to both assumptions of normality and equality of variance.

Various power transformations for the data of the other four immunoglobulins were investigated. The significance levels of the Levene and Lilliefors statistic for the transformed variable were compared with their untransformed equivalents. All transformations for IgG4 (total) were rejected as the inequality of variance was not corrected and non-normal data were generated.

Following this analysis, subsequent statistical studies were based on the following:

For IgE (a-s) - natural logarithm

For IgE (total) - natural logarithm

For IgG4 (a-s) - reciprocal of the square root.

The data for IgG (total) and IgG4 (total) were analyzed untransformed. A summary of the means, standard deviations and 95% confidence intervals for these data are given in Table 13, A-E. It should be noted that, in the case of the transformed data, the values of the means and confidence intervals given in Table 13 a-e were obtained by applying the inverse transformation to convert them back to the original units of measurement. Thus, the standard deviations quoted in this Table have no meaning in the untransformed state when transformed data have been analyzed.

6.1.3 Comparisons of immunoglobulin concentrations

A two sample T-test was carried out for paired group comparisons within each set of immunoglobulin data. A p value of less than 0.05 indicates that the mean immunoglobulin concentrations of the two groups are significantly different at the 5% level.

Significances of differences were confirmed using analysis of variance (ANOVA).

6.1.4 Concentration of IgG (total) in serum during pregnancy in allergic and non-allergic women

There were no significant differences in the levels of IgG (total) between pregnant allergic (9.083 +/- 2.185 g/L) and pregnant non-allergic women (9.392 +/- 1.980 g/L) and both were similar to the concentrations found in the non-pregnant women (9.364 +/- 1.797, allergic women; 9.063 +/- 1.566, non-allergic women) (Table 13C; Fig. 4). The concentration of IgG (total) remained unchanged in both groups during pregnancy, remaining unaffected by the period of gestation.

6.1.5 Concentration of total and antigen-specific IgG4 in serum during pregnancy in allergic and non-allergic women.

The serum concentration of IgG4 (total) was 224 +/- 43 mg/L in the group of normal non-pregnant women. Significantly higher levels (351 +/- 181, p=0.017) were found in non-pregnant allergic women, a finding reported previously and thought to be related to the production of "blocking antibodies". In pregnant non-allergic women, serum levels of IgG4 (total) were significantly higher (473 +/- 188, p=0.001) than in the non-pregnant group with no allergic symptoms, again, presumably, representing some blocking antibody response,

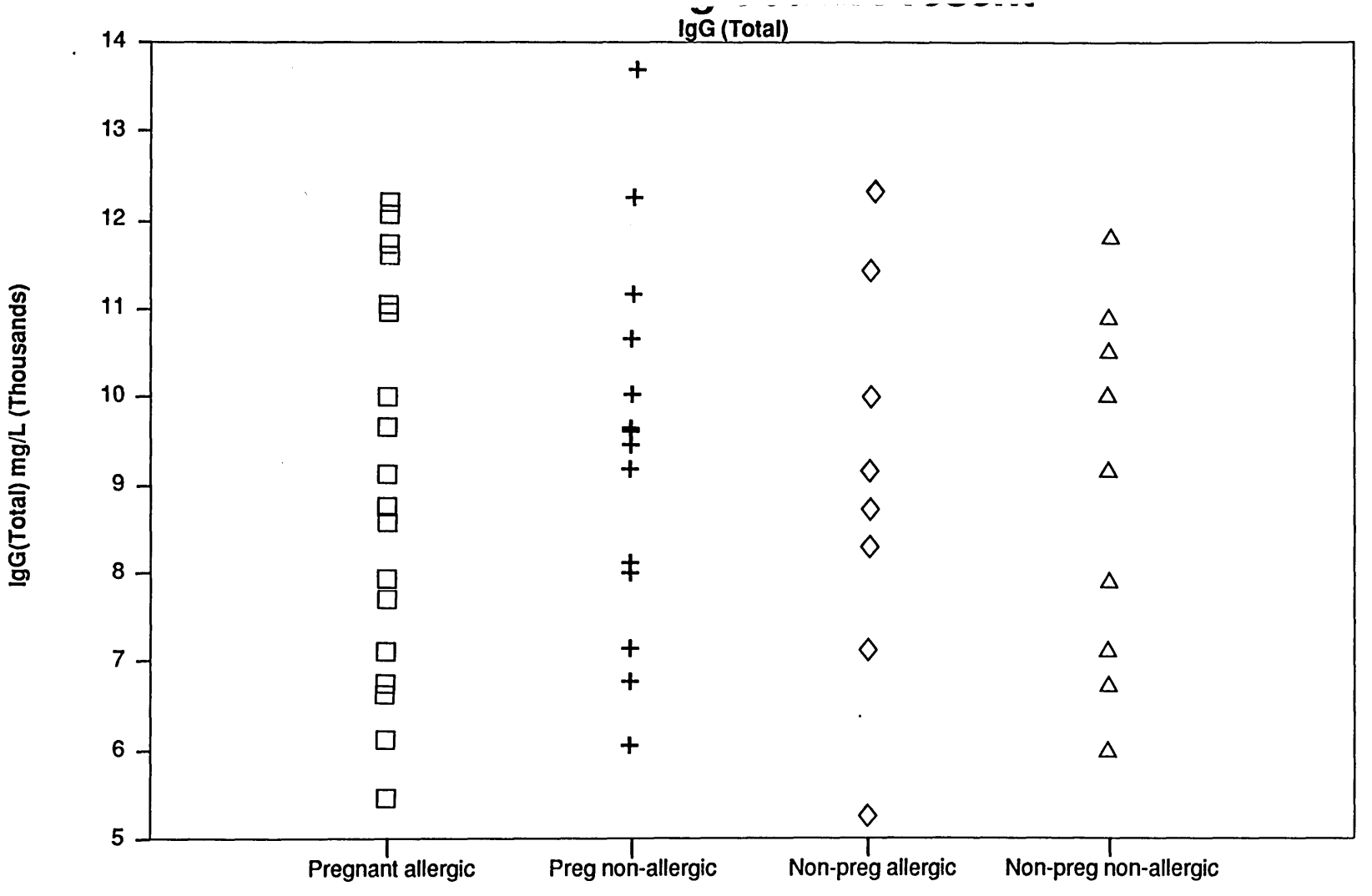

IgG (Total)
IgG(Total) mg/L (Thousands)
14
13
12
11
10
9
8
7
6
5
Pregnant allergic
Preg non-allergic
Non-preg allergic
Non-preg non-allergic

Fig 4

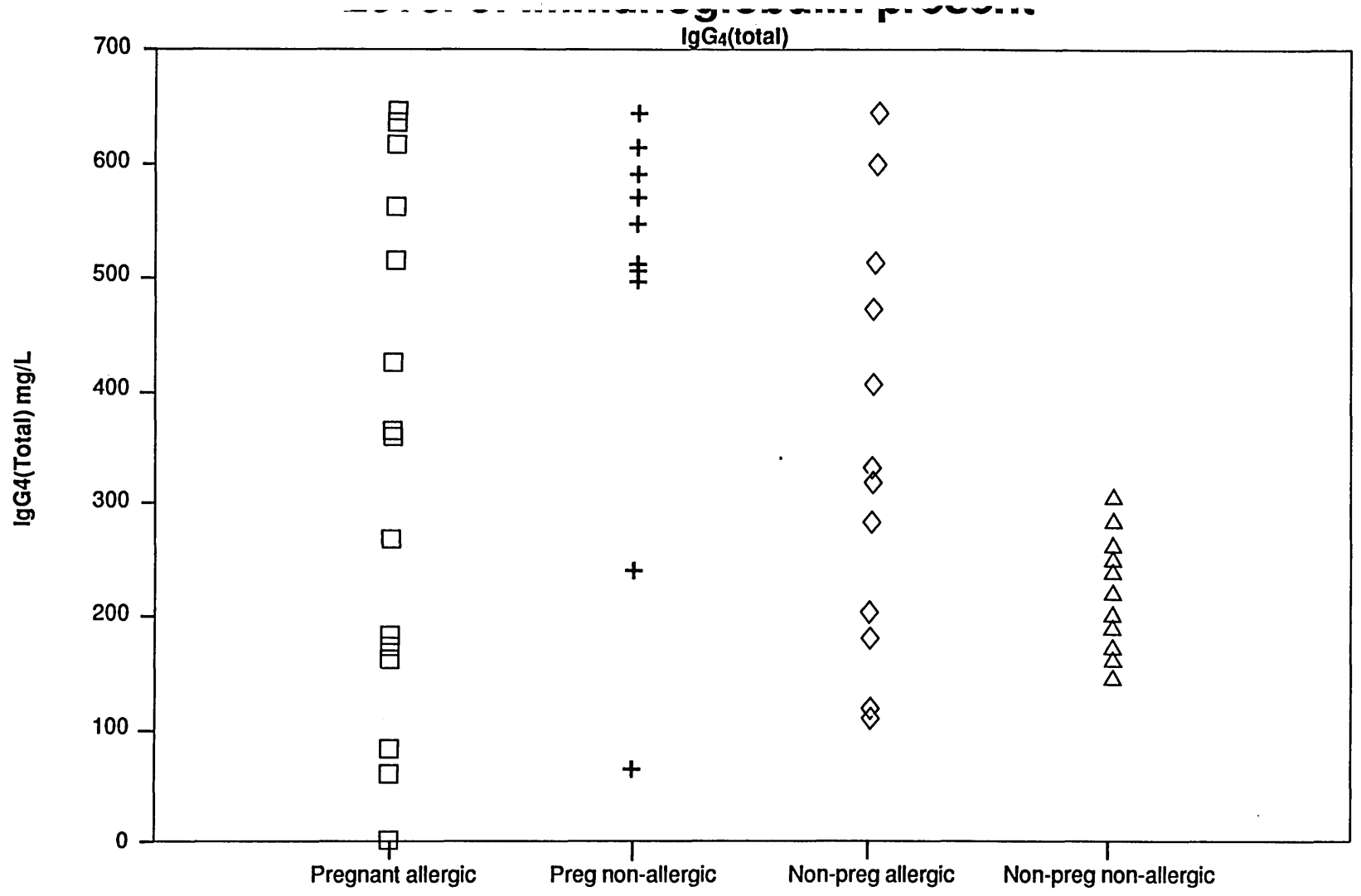

IgG4(total)
IgG4(Total) mg/L
700
600
500
400
300
200
100
0
Pregnant allergic
Preg non-allergic
Non-preg allergic
Non-preg non-allergic

Fig 5

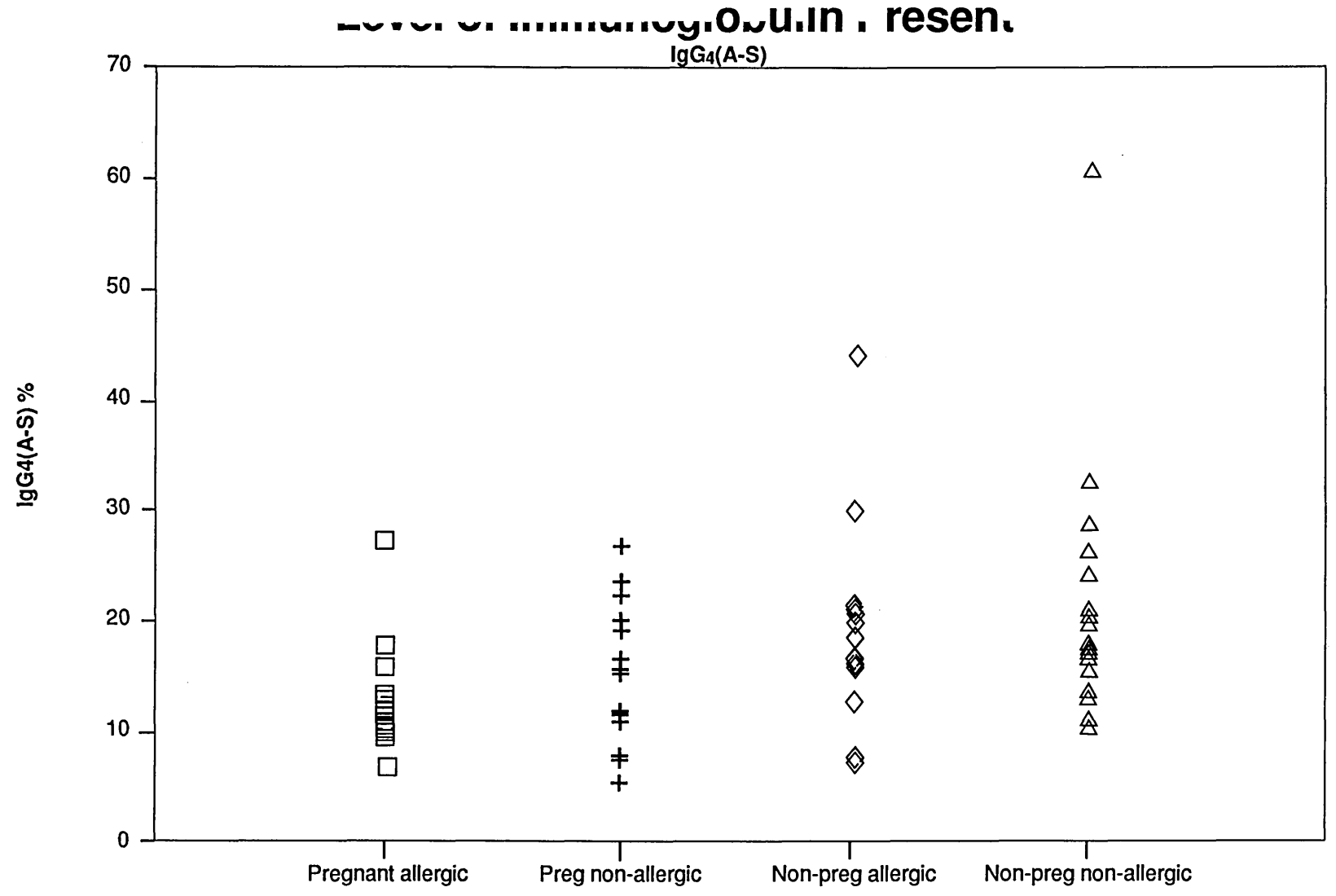

IgG4(A-S)
IgG4(A-S) %
70
60
50
40
30
20
10
0
Pregnant allergic
Preg non-allergic
Non-preg allergic
Non-preg non-allergic

Fig 6

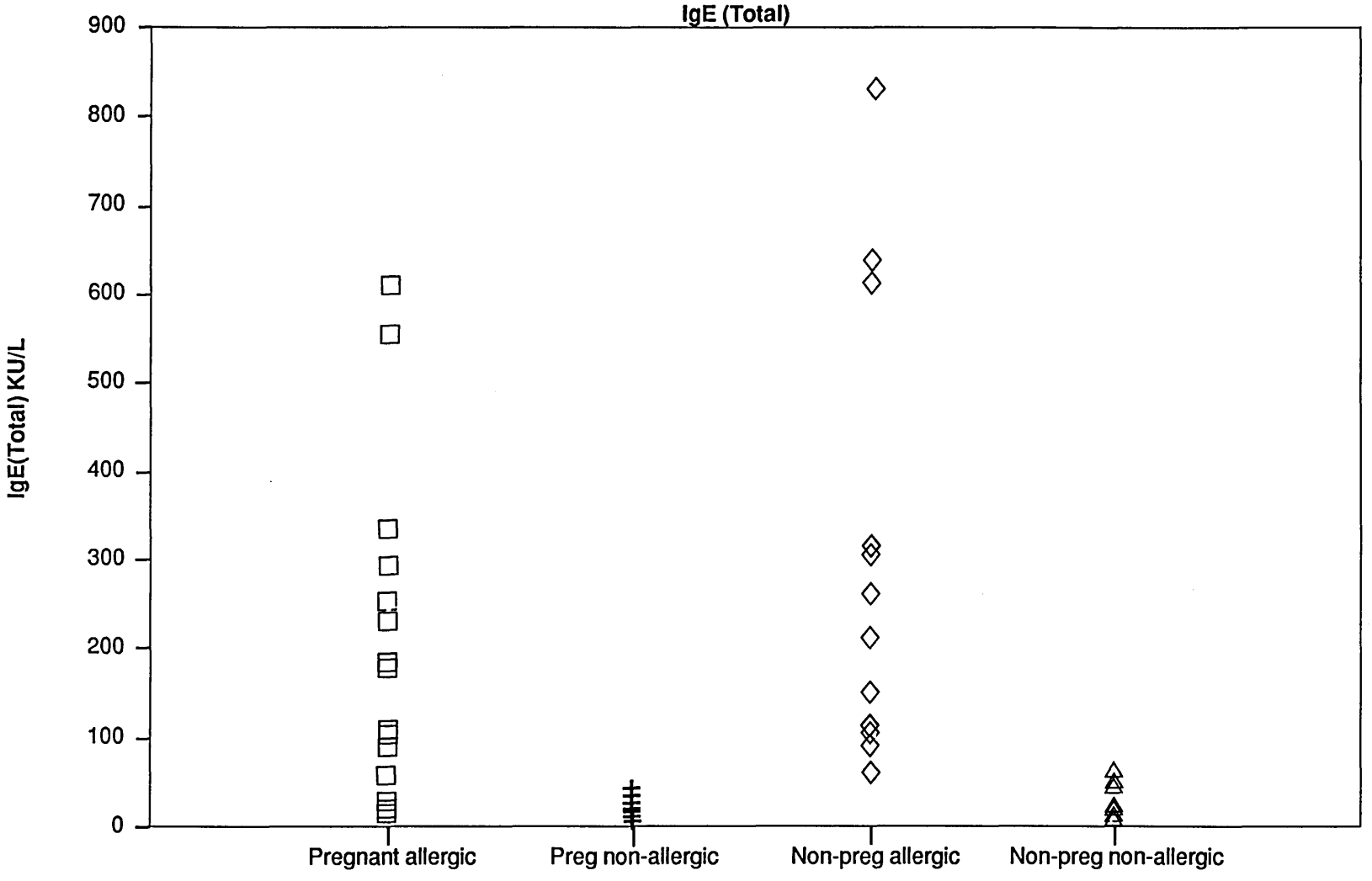

IgE (Total)
IgE(Total) KU/L
900
800
700
600
500
400
300
200
100
0
Pregnant allergic
Preg non-allergic
Non-preg allergic
Non-preg non-allergic

Fig .7

Because blood concentrations of IgE (total) have been reported to vary on a seasonal basis in response to the pollen count (Borg & Johanson, 1971), samples from the non-pregnant women were collected within a one month period. However, this was not possible for the pregnant women. Seasonal differences were noted (Table 14, Fig.8), although there were no significant differences at p=0.05 between any of the four seasons.

The serum concentration of IgE (total) was investigated at three stages during pregnancy (see above). The results (Table 15, Fig. 9) indicate a progressive fall in IgE concentration in the serum of pregnant allergic women as the pregnancy progresses. Six weeks after parturition (Stage C) the concentration of IgE (total) was significantly lower than at both Stage A (10 +/- 3 weeks, $p<0.001$) and at Stage B (33 +/- 3 weeks, $p=0.002$). The concentration of IgE appeared to return to non-pregnant levels by 6 months post partum, although an insufficient number of samples were obtained to test the significance of this observation.

No such differences in serum IgE (total) were seen in non-allergic pregnant women during pregnancy (Table 12b).

There were no differences in serum IgE (total) in pregnant allergic women of different parities (Fig 10).

Antigen-specific IgE was not detected (< 0.35 PRU/ml) in 16 of the 18 non-pregnant non-allergic women (Table 12d), and only a single serum sample from the pregnant non-allergic women had a detectable level of this antibody (Table 12b). Measurable levels were found in all of the non-pregnant allergic women (mean 17.48 PRU/ml, range 0.65 - 214.3). Significantly lower levels were found in the group of pregnant allergic women (mean 1.22 PRU/ml, range <0.35 - 27.3, $p<0.001$). These results are illustrated in Figure 11.

Table 14. The effect of season on the serum concentration of total and antigen-specific IgE in pregnant allergic women

Season	Mean conc IgE (a-s)	(n=)	Mean conc of total IgE (kU/L)	(n=)
Feb-Apr	1.31	7	137.7	11
May-July	0.81	7	185.1	7
Aug-Oct	4.24	9	204.3	13
Nov-Jan	1.02	6	213.8	12

in this case related to the pregnant state. The group of pregnant allergic women had serum levels of IgG4 (total) (396 +/- 219) which were not significantly different from either the non-pregnant allergic women or the pregnant non-allergic group. These data are illustrated in Figure 5.

Timothy grass allergen-specific IgG4, IgG4 (a-s), was present in the sera from women in all of the four groups investigated (Fig 6). Although the highest concentration of such antibodies were found in the non-pregnant non-allergic women (17.7 per cent) there was no significant difference in the concentrations found in any of the groups (Table 13E).

6.1.6 Serum concentrations of total and antigen-specific IgE during pregnancy in allergic and non-allergic women.

The mean serum concentration of IgE (total) in the group of normal non-pregnant women was 23.5 kU/l (range <5 - 63.0). As expected, the mean concentration of this immunoglobulin in serum from the allergic group of non-pregnant women was very much higher than this (mean 200.3 kU/l, range 63-834, $p<0.001$).

In the group of pregnant non-allergic women the mean serum level of IgE (total) was significantly lower (12.6 kU/l, range <5 - 63.1, $p=0.012$) than in the normal non-pregnant group of women. Concentrations of IgE (total) in the sera from the pregnant allergic group of women were significantly higher (103.2 kU/l, range 11 - 700, $p<0.001$) than in the non-allergic pregnant group. Although this is only about half the level found in the non-pregnant allergic group, the difference between the two allergic groups (pregnant and non-pregnant) was not significant ($p=0.074$).

These results are illustrated in Figure 7.

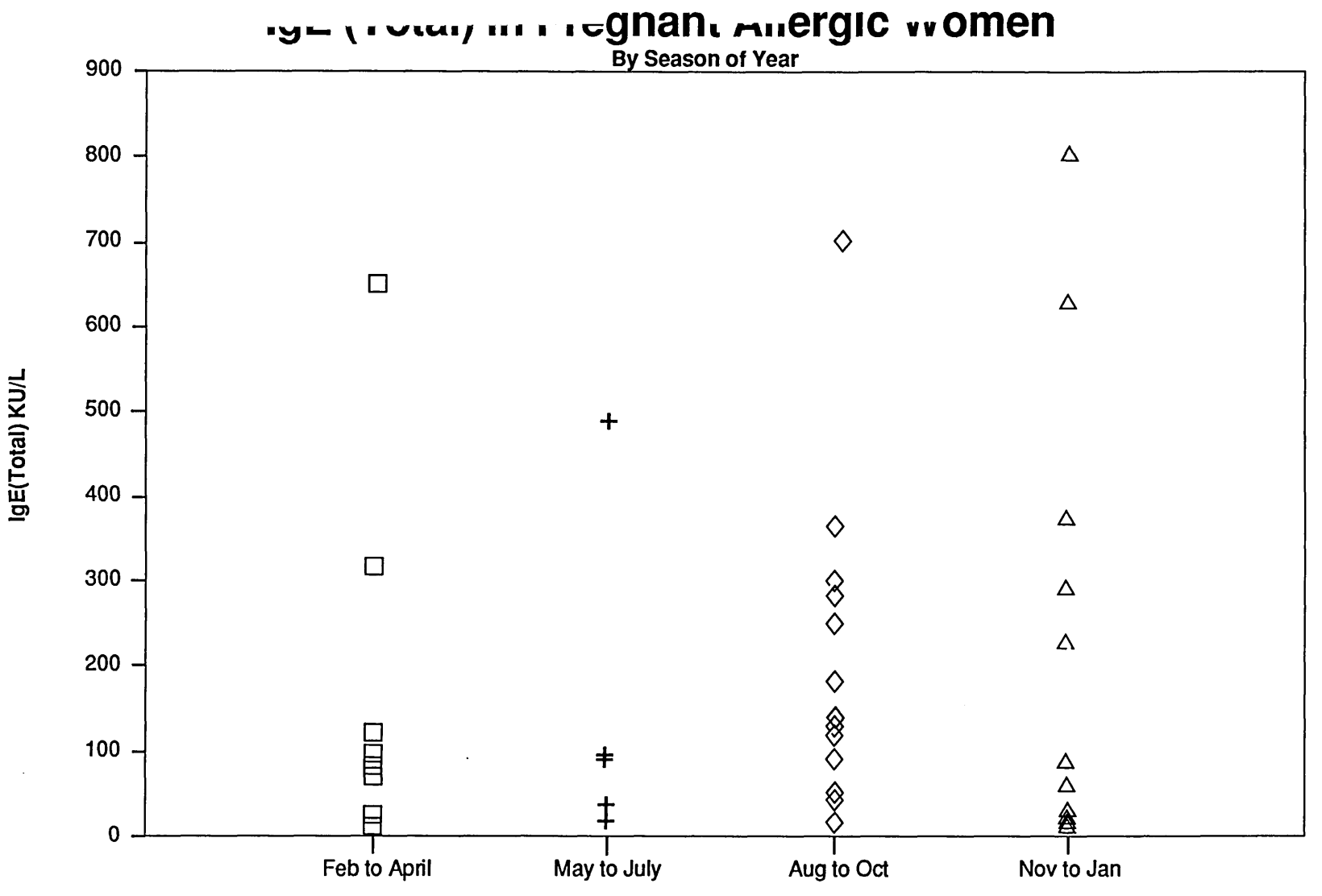

IgE (Total) in Pregnant Allergic Women
By Season of Year
IgE(Total) KU/L
900
800
700
600
500
400
300
200
100
0
Feb to April
May to July
Aug to Oct
Nov to Jan

Fig 8

Table 15. The serum concentration of total and antigen-specific IgE in pregnant allergic women during and after pregnancy

Stage of Pregnancy	IgE (a-s)	(n=)	IgE (Total)	(n=)
A	3.39	(13)	210.7	(18)
B	0.93	(9)	180.4	(14)
C	0.98	(7)	113.4	(14)
D	1.03	(2)	320.93	(3)

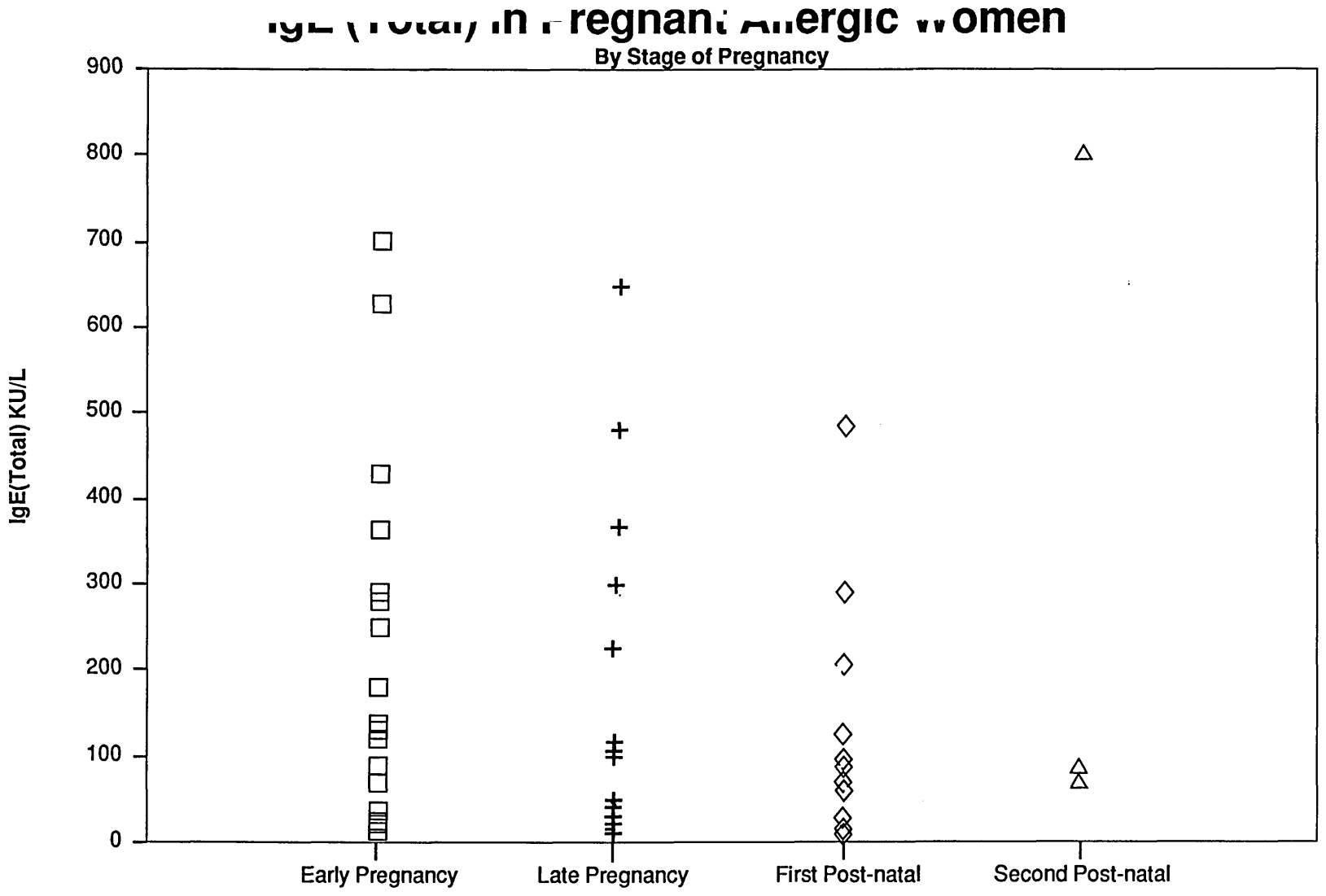
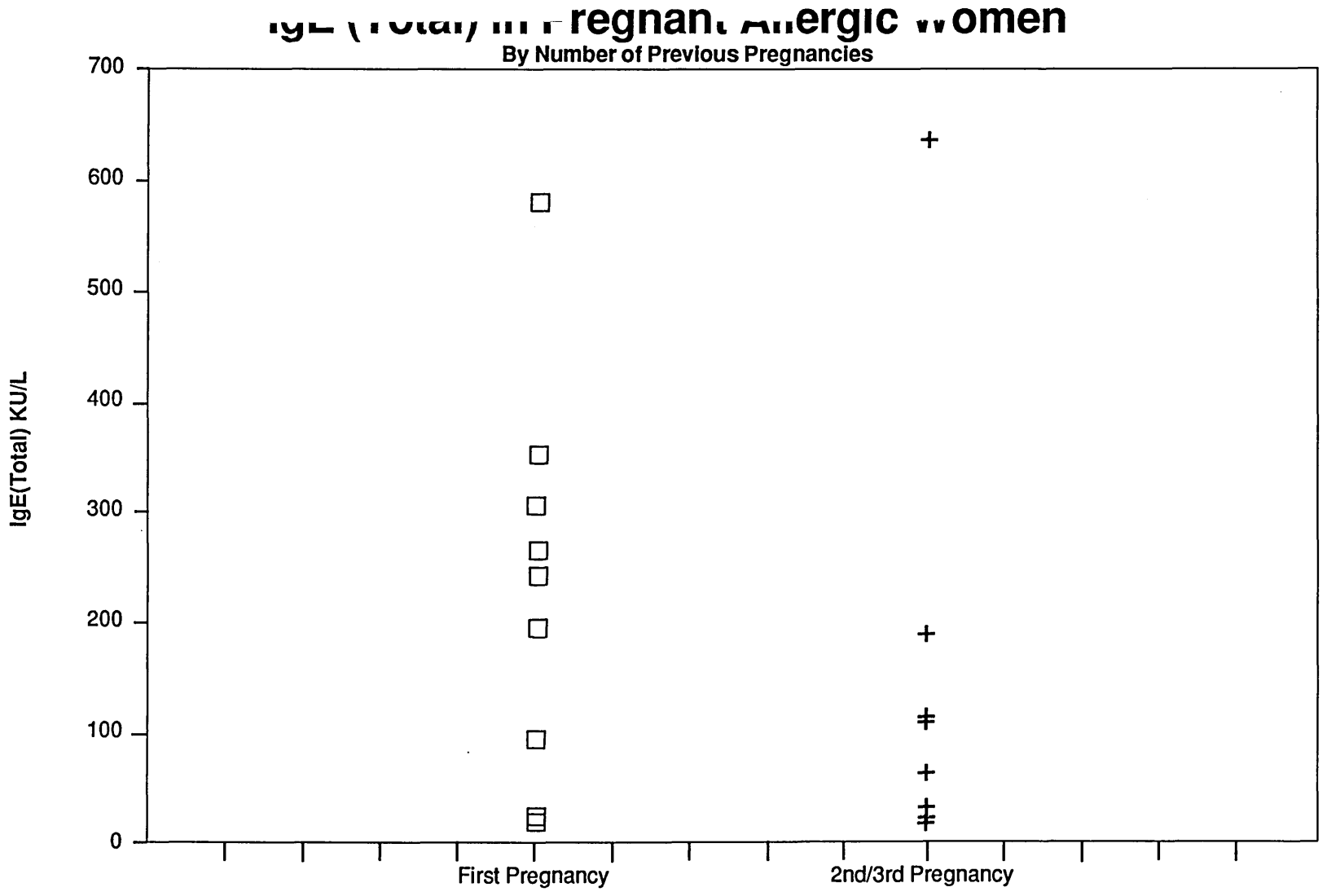

IgE (Total) in Pregnant Allergic Women
By Stage of Pregnancy
IgE(Total) KU/L
900
800
700
600
500
400
300
200
100
0
Early Pregnancy
Late Pregnancy
First Post-natal
Second Post-natal

Fig 9


IgE (Total) in Pregnant Allergic Women
By Number of Previous Pregnancies
IgE(Total) KU/L
700
600
500
400
300
200
100
0
First Pregnancy
2nd/3rd Pregnancy

102

Fig 10

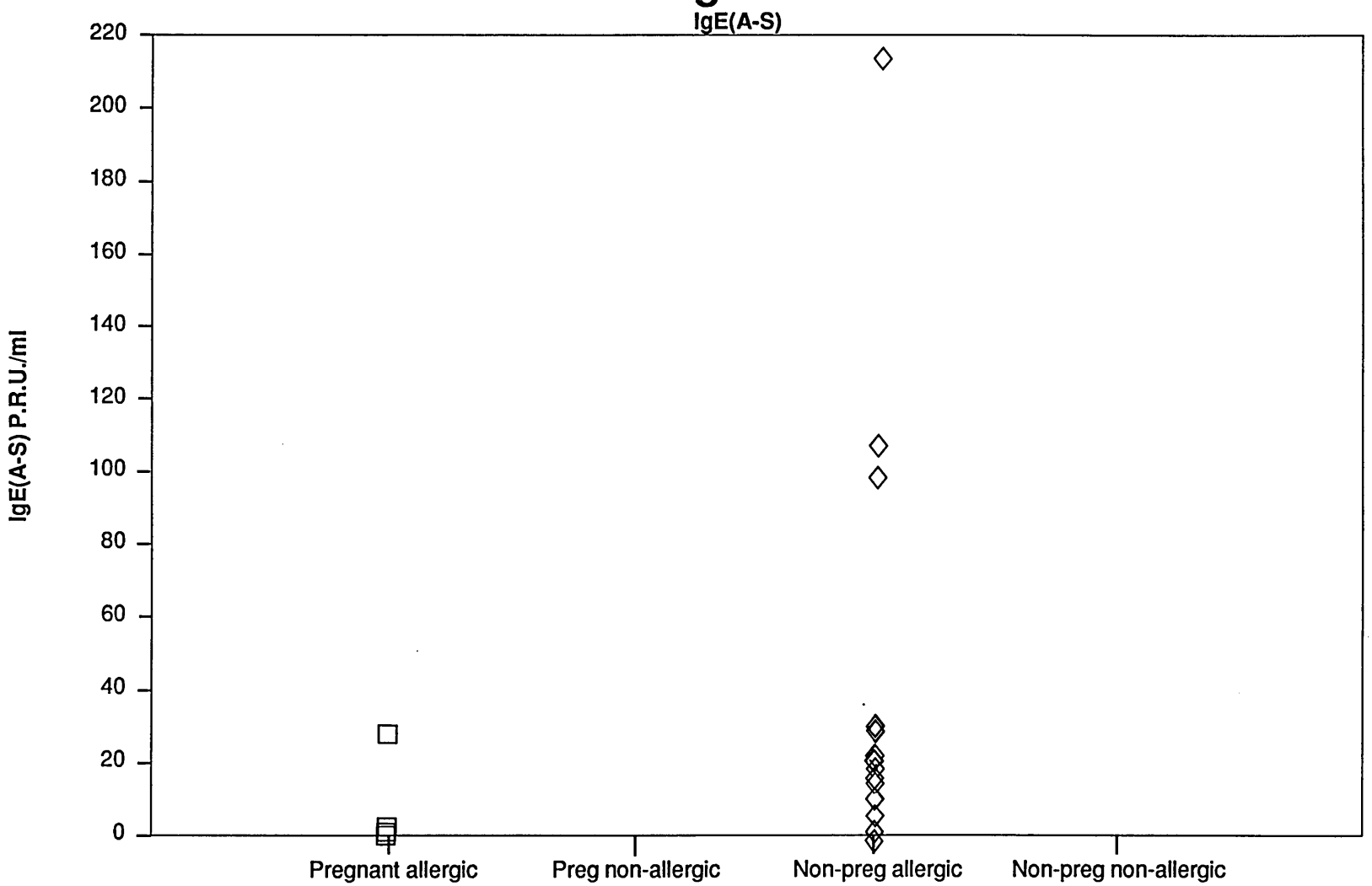

Level of Immunoglobulin Present
IgE(A-S)
IgE(A-S) P.R.U./ml
220
200
180
160
140
120
100
80
60
40
20
0
Pregnant allergic
Preg non-allergic
Non-preg allergic
Non-preg non-allergic

Fig 11

6.2 Distribution of Total IgG, Total IgG4 and Allergen-Specific IgG4

Statistical analysis of total IgG and total and antigen-specific IgG4 is described in Chapter 6.1 above. Total IgG remained unchanged in both test groups during pregnancy and remained unaffected by the period of gestation. There was no significant variation in the levels of total IgG between pregnant allergic and pregnant non-allergic women and were similar to the non-pregnant control groups 3 and 4 (Fig. 4).

Total IgG4 levels were found to be high among non-pregnant allergic women when compared with non-pregnant non-allergics. Such antibody levels were nearly twice as high in Group 3 non-pregnant allergics when compared to Group 4 non-pregnant non-allergics (Fig. 5). During pregnancy, total IgG4 levels were found to be similar in Group 1 pregnant allergics and Group 2 pregnant non-allergics and Group 3 non-pregnant allergics. Group 4 non-pregnant non-allergics showed nearly half the level of such immunoglobulins compared with Groups 1, 2, and 3 (Fig. 5). Pregnancy appears to increase the levels of such antibodies among pregnant non-allergic women.

Timothy grass allergen-specific IgG4 was present in all four groups, Group I allergic pregnant, Group 2 allergic non-pregnant, Group 3 allergic non-pregnant women and Group 4 non-pregnant non-allergic women. The highest concentration of such antibodies were found among Group 4 non-pregnant non-allergic women and were almost twice the level to those of Group I and Group 2 (Fig. 6).

Statistical analyses show that the total serum IgG concentration was not different in any of the groups of women investigated. There was no significant difference in the serum concentration of IgG subclass 4 between pregnant

allergic women and non-pregnant allergic women. Levels of IgG4 were approximately twice as high ($p < 0.01$) in pregnant women with no allergic symptoms than in the non-allergic non-pregnant group. Total IgG4 concentrations were not different in allergy sufferers and non-allergic women during pregnancy; however, among the non-pregnant group of women those with allergies had significantly ($p = 0.017$) higher levels of this IgG subclass than those without. Summary of serum concentrations of various immunoglobulins in pregnant and non-pregnant women with and without symptoms of allergy shown in Table 16.

6.2.1 Distribution of IgE Levels

In both the pregnant and the non-pregnant women there was a higher ($p<0.001$) concentration of total IgE in serum from the allergic than the non-allergic subjects. Although the level of IgE was significantly ($p=0.004$) lower in the allergic pregnant group, the differences were relatively small (Table 16) and seem unlikely to be of great physiological significance.

In both pregnant and non-pregnant women the serum concentration of antigen-specific IgE was higher in the allergic than the non-allergic subjects. However, among the allergy sufferers, the concentration of antigen-specific IgE was very much lower in the pregnant women, at about 6% of the non-pregnant level ($p<0.001$). The concentration of antigen-specific IgG4 was also lower in the pregnant allergy sufferers, being about half of the level found in the non-pregnant individuals ($p<0.002$).

The clinical symptoms were determined for this group of allergic women during the first trimester of pregnancy. Ten women reported an improvement in symptoms during the first trimester, the symptoms became more severe in

Table 16

Ig	Serum concentration in first trimester of pregnancy		Serum concentration in non-pregnant women	
	allergic	non-allergic	allergic	non-allergic
IgG (total) (g/l)	9.38+/-2.19 (n=18)	9.49+/-2.22 (n=16)	9.36+/-1.74 (n=15)	9.06+/-229 (n=18)
IgG4 (total) (g/l)	0.40+/-0.22 (n=14)	0.47+/-0.21 (n=12)	0.35+/-0.18 (n=15)	0.22+/-0.04 (n=18)
IgG4 (a-s) (%)	15.9+/-19.5 (n=18)	13.4+/-7.1 (n=16)	33.2+/-53.5 (n=15)	20.7+/-11.6 (n=18)
IgE (total) (KU/l)	180+/-176 (n=18)	17.7+/-17.1 (n=16)	272+/-229 (n=15)	25.1+/-16.4 (n=18)
IgE (a-s) (PRU/ml)	2.6+/-6.1 (n=18)	nd (<0.35) (n=16)	40.5+/-55.6 (n=15)	nd (<0.35) (n=18)

six patients and two reported no change. There was no correlation between serum levels of total IgE, allergen-specific IgE or allergen-specific IgG4 and symptomatology in these individuals.

Timothy grass pollen is a well known seasonal allergen and the levels of IgE, particularly antigen-specific IgE, might be expected to vary with the season. Analysis of the data from the allergic women during pregnancy failed to show any significant differences (at $p=0.05$) (Fig. 12) in allergen-specific IgE levels in three of the four seasons (February-April; May-July; August-October). There was only a single pregnancy in which the first trimester fell within the winter season (November-January). However, the total serum IgE concentration was significantly greater (mean 224 KU/l) in pregnancies during the August-January period than in February-July (mean 90 KU/l, $p<0.001$).

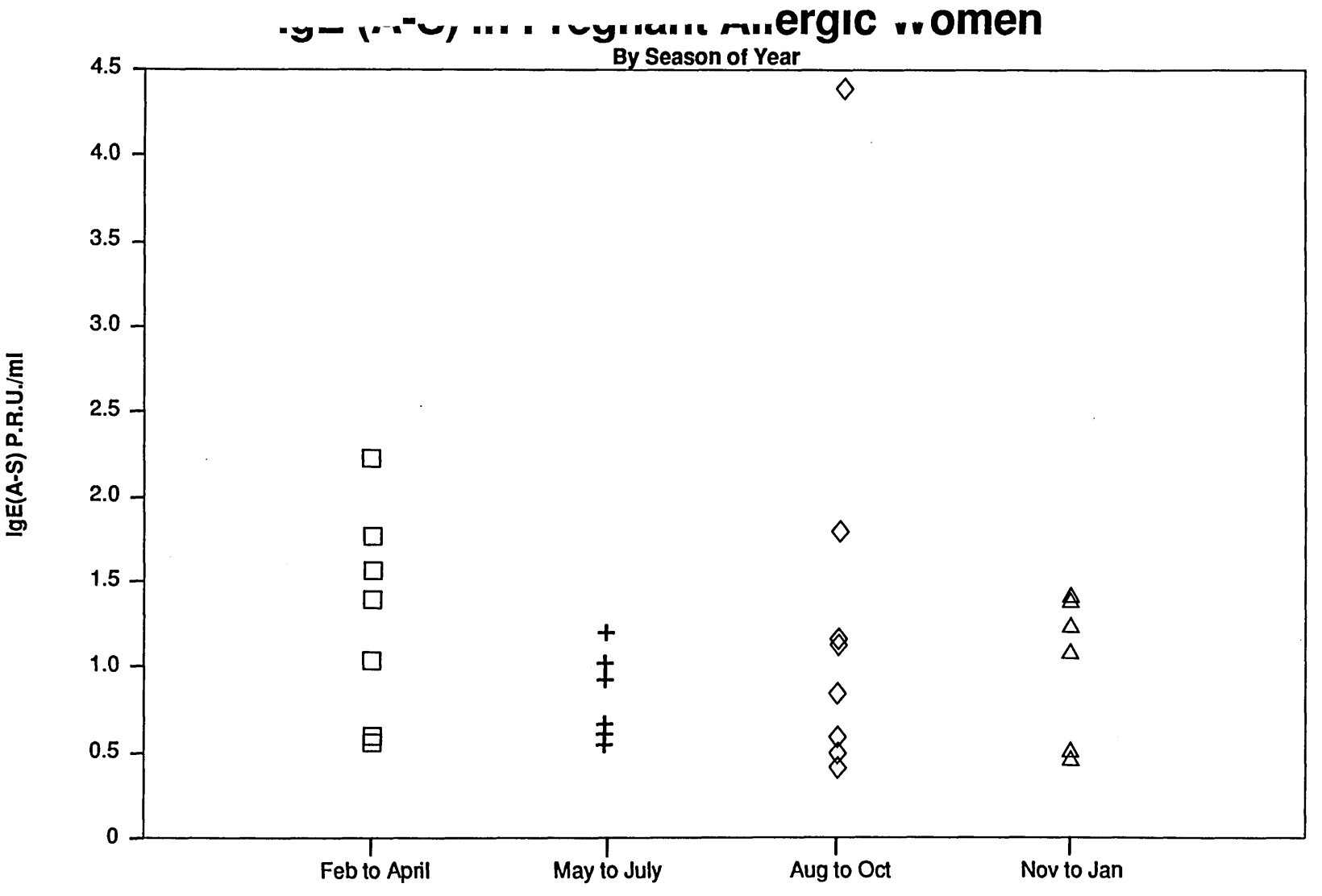

IgE (A-S) in Pregnant Allergic Women
By Season of Year
IgE(A-S) P.R.U./ml
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
Feb to April
May to July
Aug to Oct
Nov to Jan

Fig 12

6.3 Discussion

The present study was undertaken to elucidate the relationship between pregnancy and the Type I hypersensitivity immune response among allergic women. It has been suggested by Katz (1985) that raised IgE levels are caused by T lymphocyte imbalance and polyclonal activation. Pregnancy requires modulation of the maternal immune system for the survival of the fetal semi-allograft. Stites et al., (1979) showed that cellular immunity is depressed during pregnancy though conflicting views have been reported regarding the effect of pregnancy on humoral immunity. Merritt et al. (1969) found that humoral immunity remained unchanged, Ostensen (1983) noted a decrease in serum IgG and IgA while IgM and alloantibodies remained stable. Other observers noted a slight increase in immunoglobulin levels during pregnancy (Kenny et al., 1977).

In the present study there appeared to be a gradual fall in IgE concentration among allergic women during pregnancy (Fig. 9). There is a significant fall of allergen specific IgE in this group over the same period (Table 15). A fall of total IgE following bone marrow transplantation (BMT) was noted by Walker et al. (1986) in two patients where there was a "down regulation" of total IgE synthesis without loss of allergen specific IgE. This was thought to be due to a genetic influence regulated largely by a single autosomal gene R/r with the low IgE level allele (R) being dominant over the high level allele (r).

Zetterstrom et al., (1981) used the Phadebas IgE (PRIST) method to determine the total IgE in the serum of 412 adult patients with respiratory symptoms, of which 160 were classified as non-atopics and 252 as having atopic diseases. Results showed that total IgE levels below 24 KU/L were present in

84% of the non-atopic patients where as 78% of the atopic patients had levels greater than 100 KU/L. We were unable to define clearly any such high or low responder groups in our study.

It is of interest that during a study of the relevance of milk and egg specific IgG4 in atopic eczema Shakib (1984) noted total serum IgE levels greater than 100 IU/ml in 20 out of 22 eczema patients and 76.9% of those with raised specific IgG4 had no demonstrable specific IgE. Borg and Johanson (1971) found that, in the pollen season, changes in antigen-specific IgE were more pronounced than those of total IgE with rapid increases in levels averaging more than 438% of the initial values. A high level of total serum IgE is also well documented in parasitic disease.

CHAPTER SEVEN

CLINICAL ASPECTS OF ALLERGIC DISEASE IN PREGNANCY

7. Clinical Observations

In the immunoglobulin study a total of 18 pregnant allergic women and 16 pregnant non-allergic women were enlisted. Clinical details are shown in Tables 17 and 18 (Appendix V). We monitored the clinical changes of 18 allergic pregnant women during pregnancy and the post-natal period. The age distribution of the test population in both groups was between 19 and 41 years with the average age of both groups being 28.5 years. Nine of the allergics were primigravida, four were pregnant for the second time and four had more than three pregnancies.

Among the non-allergics five were primigravida, five were pregnant for the second time and the rest had more than three pregnancies.

Of the 18 pregnant allergic women, 9 patients had hay fever associated with summer wheezing requiring pharmacological intervention in the past. Seven or 41 per cent of the group had dual symptoms, e.g. eczema/hay fever together and one was known to be a chronic asthmatic with seasonal exacerbation of asthma. Among other allergic symptoms two were allergic to penicillin and one also reported allergy to nettle.

Tonsil and adenoid problems were reported by 8 patients among the allergic group, of which 4 patients had had surgical treatment in the past. We also noted a relatively high level of renal glycosuria among the allergics when urine tests repeatedly confirmed presence of glucose in 4 patients. Glucose tolerance tests failed to confirm diabetes in these women.

High blood pressure was also noted in 4 allergic patients during pregnancy.

Psychiatric problems, e.g. depression and anxiety were present in 4 patients in allergic group.

One of the interesting observations in the obstetric history revealed that 4 allergic women or 22.2 per cent of the test population had a previous incidence of miscarriage/threatened abortion. While such incidence were reviewed among non-allergics, only one reported history of two previous miscarriages. Non-allergics reported similar levels of tonsillitis/hypertension and anxiety related morbidity.

The effect of pregnancy on the clinical symptomatology of the allergy remained unclear. Seven of the allergic patients did not notice any significant change of symptoms of their pre-existent illness. Four reported worsening of symptoms and six improved. These findings are in line with previously published data.

7.1 Atopy and Autoimmune Disease in Pregnancy

The observation discussed in Section 7 above, relating to the apparently higher prevalence of threatened abortion/miscarriage in the group of pregnant women with allergic/atopic symptoms, indicated the need for further investigations in both high risk pregnancy with immunological disorders and normal patients. A literature review on the subject did not provide any previously published useful information on such a relationship. Attempts made to analyze computerized obstetrics records both in Sheffield and Buffalo were unsuccessful due to inadequacy of recording allergic/atopic disease in obstetric clinics. We were, however, able to identify and review the case notes of a group of high risk women with a history of repeated abortion. These women

had history of autoimmune disease/abnormal antibodies with or without active immunological disease during pregnancy.

7.1.2 Observation and Management of the Antiphospholipid Syndrome in Pregnancy: The Buffalo Experience

The Department of Medicine at The Children's Hospital of Buffalo State University of New York, Buffalo, USA, has screened approximately 40 patients since 1986. Almost all these patients were referred by a single obstetric unit of the hospital and one particular internal medicine group. Preliminary immune screening confirmed single or multiple immunological abnormalities at the time of referral to the Medicine Department. Almost all of the women attending the unit were white and had miscarriage associated immunological disease, were found to be positive on autoantibody screening without overt symptoms, or were referred purely for abnormal antibody screening. Case histories of a group of seven patients are described in Appendix V.

Table 19 shows the clinical summary of SLE patients observed in this study.

7.2 Observation and Outcome of Pregnancies in Women Attending the Langold Health Centre between September 1986 and December 1990.

We retrospectively reviewed all the pregnancies of women attending the ante-natal clinic at Langold Health Centre between September 1986 to December 1990.

A total of 187 women attended for ante-natal care during the specified period. Ante-natal care was shared between the attendant general practitioners and hospital obstetricians at Bassetlaw General Hospital, Worksop and followed the standard nationally agreed ante-natal care protocol. Review of

clinical notes and personal interviews (as necessary) based on the allergy screening protocol (Form II, Appendix II) confirmed that 104 of these patients had no significant history of allergic disease whereas 83 women had confirmed allergic symptoms. Table 20, page 117 shows the data from women attending the Health Centre.

TABLE 19. Clinical Summary of Seven Patients with SLE Referred because of a Pregnancy.

No.	Allergy	ANA* titre	SLE symptoms?	Obstetric History
1	none	1/160	-	2 normal deliveries
2	none	-	+	1 normal delivery
3	allergic asthma hayfever	1/320	-	1 normal delivery 1 miscarriage
4	allergic dermatitis	1/2560	-	1 premature delivery
5	allergic asthma hayfever allergic dermatitis	-	-	5 normal deliveries
6	allergic asthma hayfever	1/80	-	2 normal deliveries 4 miscarriages
7	allergic eczema	-	-	4 normal deliveries 1 miscarriage

* ANA - anti-nuclear antibody

Details of each patient are shown in Appendix V.

Table 20.

Data from women attending The Health Centre, Langold, ante-natal clinic from 1986-1990.

	Allergic	Non-allergic
Number of women	83	104
Conceptions	193	228
Terminations	11	8
Pregnancies analysed	182	208
Threatened miscarriages (% of pregnancies)	4 (2.2)	0 (0)
Miscarriages (% of pregnancies)	33 (18.1)	11 (5.0)
Premature delivery (% of pregnancies)	5 (2.7)	1 (0.4)
Women with 2+ miscarriges	7	1
Women with problem pregnancies (% of women in group)	30 (36.1)	10 (9.6)

Details of individual patients included factors, e.g. parity, miscarriages, period of gestation, known allergies, blood group and associated medical history (Table 21, Appendix V). Nineteen had legal pregnancy terminations. Available information confirmed a total 421 pregnancies. Allergic women (83) had 193 conceptions and non-allergic women (104) had a total of 228 conceptions. Among the allergic group, 33 pregnancy losses (18.1%) were reported whereas the non-allergic group had 11 pregnancy losses (5.0%) during the observed period. Five of the allergic women (6.2%) attended an infertility clinic and 3 (1.9%) non-allergics attended an infertility clinic during this period. Other factors, e.g. blood group, medical history, etc. did not provide any significant information relating to high pregnancy loss prevalence among allergic women.

Of the 403 pregnancies considered in the retrospective study, 44 ended in first trimester miscarriage, a rate of loss of 10.9%. This is not dissimilar to the miscarriage rate found in previously published prospective studies (Regan, et al., 1989). Pregnancies of women with symptoms of allergy, had a 3.5-fold greater rate of first trimester miscarriage than those of non-allergic women. Although this difference could reflect an increase in miscarriages among women who have previously miscarried (Regan et al., 1989) both the number of women with symptoms of allergy who experienced a miscarriage and the number of miscarriages in such women was over 3 times greater than in the non-allergic group. Statistical analysis by analysis of the binomial distribution of allergic reactions and problem pregnancies showed that allergic women have a significantly greater ($p < 0.001$) chance of having a problem pregnancy than non-allergic women.

The observed value of miscarriage was also statistically significantly higher among allergic women ($p < 0.001$). However, there was no difference in rate of conception between the two groups.

These observed differences could be the result of a secondary effect of factors such as particular drug use by the allergic patients; however these women were specifically advised against continuing drug treatment for their allergy as soon as a pregnancy was suspected. Rubin et al., (1986) reported that over 93% of pregnant women avoid exposure to drugs in the first trimester and it seems unlikely that the observed difference in miscarriage rate between the two groups reported here is drug induced. Although it is possible that there might have been other undetected differences in the two groups of patients investigated, given the manner in which the sample was selected (all women attending an ante-natal clinic over a 52 month period) and the size of the sample (187 women, 402 pregnancies) this is unlikely. Furthermore, the data from the small group of women suffering from SLE tended to confirm the observations from the ante-natal clinical study. Taken overall, the results suggest that there may be an underlying abnormality in the allergic women predisposing to miscarriage.

7.2.1. Discussion

Little has been published on the immunological responses to allergens during pregnancy, studies in this area being confined to late pregnancy relating the sensitisation of the fetus in utero to the development of allergy in early infancy (Iikura et al., 1989). One factor that may link allergic symptoms and miscarriage could be a defect in the production of antigen-specific

immunoglobulin IgG4 during early pregnancy in the allergic group. Such a breakdown in the production of "blocking" antibodies during pregnancy has been linked to recurrent abortion (Mowbray et al., 1984) and may also be important in the manifestation of allergic symptoms (Heinen et al., 1984). Alternatively, the high levels of histamine released as a mediator of type 1 hypersensitivity reactions may be an agent in this increased rate of pregnancy loss.

In the investigation of the serum concentration of certain immunoglobulins in a subgroup of these women (Chapter 6) it was revealed that, as expected, IgE levels were around tenfold higher in the allergic than the non-allergic pregnant women, the levels being similar in the relevant non-pregnant control groups. Whereas antigen-specific IgE was also raised in the allergic group compared to the non-allergic group during pregnancy, the antigen-specific IgE response was very much lower in the pregnant than in the non-pregnant allergic group. This suggests that, during the first trimester of pregnancy, there may be an inhibition of the antigen-specific IgE response. Similarly, although less dramatically, there appears to be an inhibition of the allergen-specific IgG4 response during pregnancy.

It is tempting to postulate an immunological basis for the observed increase in the rate of early miscarriage in the pregnant allergic women and to relate this to the immunoglobulin findings. One factor could be the apparent block in the production of antigen-specific immunoglobulins (IgG4 and IgE) during early pregnancy in this group. This may reflect a breakdown in the production of "blocking" antibodies during pregnancy, a deficiency of which is linked to recurrent abortion (Mowbray, et al., 1984). Although it has been

proposed recently that IgE is important in antigen capture and presentation (Mudde et al., 1990) the role of this immunoglobulin has not been investigated in pregnancy. An additional factor involved in the higher rate of pregnancy loss in the pregnant allergic group of women could be a general stimulation of the maternal immune system consequent upon the high level of IgE in these individuals. This could result in an increase in the production of a range of cytokines including IL-2 which has been shown to prevent fetal development in mice if administered after mating (Tazabwala, et al., 1989).

CHAPTER EIGHT

FINAL DISCUSSION AND CONCLUDING REMARKS

8. Final Discussion and Concluding Remarks

The immunology of human pregnancy has been investigated widely, particularly relating to the survival of the semi-allogeneic feto-placental unit in the potentially hostile immunological environment of the female reproductive tract. This survival has been related to a number of factors including the secretion of blocking antibodies (of the IgG4 subclass) by the maternal immune system (Rocklin, et al., 1979), presumably directed against paternal antigens of the fetus, and the production of localised immunosuppressive molecules such as the protein Placental Protein 14 (Bolton, et al., 1987). A defect in any of these could result in threatened miscarriage, actual miscarriage or, if manifested later in gestation, possibly as premature delivery. Indeed, a deficiency of blocking antibodies has been associated with recurrent spontaneous abortion, leading to immunisation with paternal cells as a treatment for this condition (Mowbray, et al., 1984).

Despite extensive studies on the maternal immune system, little is known of the role of IgE during pregnancy. Investigations carried out in this area have concentrated on late pregnancy in the context of intrauterine sensitisation and the development of allergy during early infancy (Iikura et al., 1989).

8.1 The Immunoglobulins

The serum levels of IgG, total IgE, total IgG4, allergen specific IgE and allergen specific IgG4 were measured in non-allergic and allergic pregnant women during early and late pregnancy and the post partum period. These levels were compared with non-pregnant controls. All allergic women had a demonstrated allergy to Timothy grass pollen.

8.1.1 IgG and the Sub-Classes

The levels of total IgG remained unchanged during pregnancy and there was no significant difference noted among test groups. Total IgG4 increased significantly during pregnancy among non-allergic women. Allergic women demonstrated a higher level of total IgG4 in both the pregnant and non-pregnant states.

The level of total IgG in the present study remained unchanged during pregnancy, a finding that differed from some previously published reports, e.g. Gudson et al. (1969) Amino et al. (1978). However, these findings are in agreement with those of Miller et al. (1984), Ostensen and Merritt et al. (1969), and Kenny et al. (1976) where no significant changes of IgG were noted.

A high level of IgG4 (total) was noted in both allergic and non-allergic women during pregnancy. Such an observation is of interest as IgG4 is thought to be a 'blocking' antibody both during pregnancy and also in allergic diseases.

The role of allergen-specific IgG4 in the present study is unclear. All four test groups demonstrated the presence of this sub-class of antibody in their sera with the highest concentration among non-pregnant, non-allergic women. These data suggest that in allergic women the high levels of IgG4 are not further increased during pregnancy. Such an observation indicates that IgG4 secretion by these women may have already reached maximal levels.

Another interesting clinical observation in the present study is that allergic women appear to have a higher tendency to miscarry when compared with non-allergic women. Whether such a finding can be attributed to "failure" of the IgG4 antibody production to enhance the existing IgG4 antibody repertoire due to increasing demand caused by pregnancy is not known.

Among the non-allergic women IgG4 levels were increased during pregnancy. It is possible that such newly synthesised antibodies have a "blocking" role during pregnancy and thereby contributed to a relatively lower incidence of miscarriage among these women. Rocklin et al. (1979) claimed that maternal blocking antibodies are absent from the serum of spontaneous aborters.

In Group 3 (non-pregnant allergic women) a significantly higher level of IgG4 and IgE was noted when compared with Group 4 non-pregnant non-allergic patients. Such findings conform with the currently held views which suggest that chronic antigenic exposure produces a predominant IgG4 response. The phenomenon is likely to be the result of a sequential "down-stream" switch of genes along the CH gene locus (Shakib, 1988). Class switching from IgM to IgE is also postulated among atopic individuals.

The findings reported here may suggest that such allergen specific Ig4 antibodies are part of a normal general immune response and may have a blocking role among non-allergics against local environmental factors. These findings are contradictory to the previously reported survey where inhalant antigen-specific IgG4 was rarely detectable in 156 normal individuals taking part in a community survey (Merrett, 1983). These contradictory findings are probably due to environmental factors, as the study population in this project is from a rural area with extensive grasslands and an abundance of airborne pollen (Appendix I).

8.1.2 IgE and Allergen-Specific IgE

The results showed that total IgE decreased during pregnancy and that the decrease is greater as pregnancy advances. Allergen-specific IgE demonstrated a highly significant decrease in pregnancy among pregnant

allergic women but remained the same as pregnancy proceeded. No such antibodies were found among non-pregnant non-allergic women and pregnant non-allergic women.

Previous reports on the level of IgE during pregnancy is controversial. The continuous variability of serum IgE level concentrations in health and the allergic state has been well documented. So far four studies have been reported in the literature relating to the changes in serum IgE levels during pregnancy. Knoblock et al. (1974) reported no statistical difference between serum IgE levels in different subjects during the first trimester of pregnancy compared with the levels in the third trimester. Amino et al. (1978) serially measured serum IgE levels during early, mid- and late pregnancy in the normal pregnant women and found no significant change in mean levels and no consistent trend towards increasing or decreasing levels. Gluck and Gluck (1976) studied the serum IgE during pregnancy in 17 women with asthma and six controls. They reported that those with an increased or unchanged IgE had a tendency for their asthma to worsen during pregnancy, whereas decreasing levels of IgE were seen in normal controls and in asthmatic women whose asthma improved or remained unchanged during pregnancy. In contrast, Schatz et al. (1985) were unable to find any difference in serum IgE levels during early, mid- and late pregnancy or post partum in 18 women whose asthma had improved during pregnancy compared with 16 women whose asthma had worsened. No significant relationship was observed between the IgE levels and symptomatology during pregnancy among the allergic group in this study.

8.1.3 Mast cell mediators

Products of arachidonic acid metabolism which are implicated in IgE-dependent inflammation and are newly generated by mast cells via the cyclooxygenase and lypoxygenase pathways, such as prostaglandin E, prostacyclin and thromboxane A2, are increased during pregnancy. In contrast, levels of the pre-formed mediator histamine decrease.

Observations suggest that prostaglandins are necessary for pregnancy to continue. Reports published on animal studies (Lewis et al. 1982, Hyland et al. 1982, Hwang et al. 1988, Watson et al. 1989) indicate that fetal and maternal prostaglandins are important in pregnancy maintenance. Prostaglandins are known to regulate T-cell lymphoproliferative responses by modulating the development of receptors for transferrin (Chaouat et al., 1985). In humans, the use of drugs such as prostaglandin inhibitors during pregnancy is contraindicated (British National Formulary 1990). Aspirin in high dose was reported to cause congenital malformation, perinatal mortality and low birth weight, whereas low doses of aspirin given to selected groups of pregnant women with associated alloimmune diseases are beneficial for both mother and the baby. Imbalance between thromboxane and prostacyclin is reported during pre-eclampsia and related complications (Walsh, 1990). Prostaglandin release can be pharmacologically modulated by aspirin and indomethacin by inhibiting the cyclooxygenase pathway in a time-dependent, concentration-dependent manner (Smith and Lands, 1971).

In the present study, attempts have been made to determine the nature and effect of pregnancy on inflammation induced by natural allergens such as grass pollen. It is known that serum factors in pregnancy can markedly

decrease lymphocyte proliferation to antigen and mitogen in vitro, although the physiological significance of these observations is not well-established. All biological studies by their very nature, have a high degree of variability. The present study is influenced by additional factors: (1) stability of serum Ig levels; (2) admixture of maternal blood with fetal blood; (3) genetic regulation of total and allergen-specific immunoglobulins; (4) endocrine events that are controlled by both maternal and embryonal mechanism at different stages of pregnancy; (5) problems of reproducibility in the laboratory environment and finally, (6) the effect of environmental factors such as pollen count and seasonal variation. In spite of such difficulties the results showed that the levels of total and allergen specific IgE decrease in both allergic and non-allergic women as pregnancy advances. The levels of these immunoglobulins in early pregnancy were higher than in later pregnancy suggesting that late pregnancy factors could play a significant role in suppressing the production of such immunoglobulins.

Evaluation of disease activities assessed from parameters made on the basis of clinical assessment and personal questionnaire did not confirm a uniform relationship between serum immunoglobulin levels and clinical symptomatology.

8.2 Hypersensitivity Reactions and the Fetal Semi-Allograft

In the introductory Chapters IgE mediated inflammatory events resulting in infiltration of leucocytes, eosinophils, neutrophils and also the generation of substantial amounts of LTB_4 and PAF acether were discussed. These mediators promote adherence of eosinophils and neutrophils to endothelial cells of post-capillary venules. Late phase IgE mediated events result in oedema, exudation

of proteins and hypersecretion of other inflammatory products. Extensive epithelial denudation and eosinophil infiltration is noted during such events.

In the human reproduction process, destruction of maternal epithelium following implantation of the fertilized egg is noted and is thought to be trophoblast initiated. Trophoblast, exhibiting foreign paternal antigens may be regarded as a semi-foreign allograft and is likely to initiate a classical graft rejection reaction.

In the graft rejection process, foreign MHC. Class II antigens on the graft stimulate host T-helper cells (Th) and cytotoxic T-cells (Tcyt) to destroy the target graft cell. Tcyt cells recognise the graft cell via the foreign MHC class 1 antigen. The cells reacting to the graft release lymphokines which stimulate macrophages to enter the graft and destroy it. Although, in pregnancy, MHC antigens are absent (or perhaps expressed at extremely low density) on trophoblast tissues of the fetal semi-allograft in direct contact with maternal blood (Billington and Bell, 1983 a,b), other major trophoblast populations opposing maternal decidual tissue have been shown to express Class I MHC antigens. Paternally encoded Class 1 MHC antigens have been described on the spongy zone trophoblast layer of the placenta in the rat. MHC Class II (HLA-DR) positive cells found in human placenta are thought to be macrophages (Sutton et al., 1983). Starkey (1987) reported that cytotrophoblast cells can bind antibody specific for MHC Class II HLA-DP antigen. TLX (trophoblast lymphocyte cross reactive) antigens are also reported in early pregnancy (Faulk and McIntyre, 1983).

Delayed hypersensitivity reactions are implicated in graft rejection mechanisms. An initial IgE mediated immediate hypersensitivity reaction

followed by a late phase delayed hyersensitivity reaction after challenge by antigens of the fetal semi-allograft could possibly occur at about 4 days following fertilisation, coinciding with the arrival of the morula at the uterus through the tubal isthmus. Such a hypothesis is compatible with our present state of knowledge. The proposed mechanisms for graft rejection are, therefore, possibly applicable in the context of pregnancy. A vigorous IgE-mediated delayed hypersensitivity reaction may result in an immune reaction resulting in fetal expulsion in a similar manner to graft rejection. While alternative/associated immunosuppressive mechanisms of pregnancy are already geared to counteract such a rejection reaction, in a genetically pre-disposed select group of women such as atopics, such events may contribute to an increased risk of spontaneous abortion.

Atopic individuals are known to mount an immune response to environmental antigens with excessive production of IgE due to T-cell abnormalities. It is, therefore, possible that these women have an inherent, inbuilt tendency for a vigorous IgE mediated, delayed hypersensitivity (IL4/CD23/IgER11 dependent) reaction during pregnancy, thus increasing the prevalence of threatened abortion. In a normal healthy pregnancy initial membrane disturbances involved in implantation may be initiated by IgE mediated events. Failure to mount such a reaction or sensitisation phase may inhibit or impair further pregnancy processes and thereby cause infertility in some women. On the other hand, inadequate, late phase (challenge phase) events, such as a distorted release mechanism of secondary inflammatory mediators may result in spontaneous abortion (50% of <u>in vivo</u> fertilised human

eggs are aborted with a substantial proportion being due to lethal genes and chromosome anomalies (Clark et al., 1986a).

The enhanced effector functions of IgE antibodies are well documented in live targets, e.g. in helminthic diseases, whereas microbial or tumoral targets do not evoke IgE responses. The late appearance of IgE in phylogeny as well as the high rate of evolution of the E chain in comparison with other immunoglobulin heavy chains (Ishida et al., 1985) suggest that nature has selected for the continuous existence of this class of antibodies. There is therefore the possibility that IgE is an essential participant in the complex immune responses in pregnancy as well as parasitic infections and other clinically significant allergic responses to environmental antigens.

It has been shown that IL4 stimulates and IFNgamma inhibits lipopolysaccharide (LPS) activated murine B cells to secrete IgE and IgG1 in vitro. IL4 produced by T cells appears to determine whether IgM production switches to either IgE or IgG. The stimulatory role of IL4 is of particular interest in allergy as the over-production of IgE antibodies is the major cause of allergic diseases. Delespesse et al., (1989) reported that IL4 is the "binding-factor" in enhancing IgE synthesis.

Finkleman et al., (1986) investigated the T-help requirement for the generation of an in vivo IgE response in mice and reported that the IgE response is blocked by anti IL4 antibody. However, such an effect appeared to be short-lasting.

8.3 Miscarriage, Allergy and Effects on Seasonality of Human Birth

Miscarriage is defined as "the expulsion of a fetus from the womb before 28 weeks of pregnancy". It is now estimated that up to 50 per cent of in vivo

fertilized human eggs are aborted (Clark et al., 1986a). The causes of such pregnancy loss are varied and various physiological and social factors are attributed by the medical profession and by the public at large. These include: (1) abnormal fetus; (2) associated illness; (3) hormonal imbalance; (4) age; and (5) psychological factors. Seasonal variation in the human birth rate is an interesting and yet unresolved area. It is, however, well documented that the pattern of birth shows a major peak in spring and a minor peak in autumn (James, 1990). There is substantial literature on the seasonality of human births (Cowgill, 1966a, 1966b). Although attempts have been made by the social scientists to explain such a variation, no satisfactory explanation is yet available. Paraskevaides et al. (1988) studied the seasonal distribution in conceptions achieved by artificial insemination by donor. This study of 259 conceptions at an artificial insemination clinic confirmed that conception is influenced by the season of the year. Conception was not influenced by the number of donors or patients attending the clinic, the frequency of inseminations, or medical skill. Conception was found to be more common from early winter until early spring (October to March) with a peak in November. Variables such as frequency of intercourse and ovulation were excluded in these women.

Such a finding may suggest that seasonal variation may affect the quality of the ovulated egg or the maternal receptibility to the fertilized egg. In this group of pre-selected patients other known contributory factors, such as chromosomal or hormonal impairment, were excluded and the pattern and distribution of conception was found to be similar to natural conception. Such a finding is of interest in the context of the seasonal distribution of pollen count (it is well documented that the pollen count is highest from April to August)

and is inversely proportional to the rate of conception. Such an observation may suggest that during the pollen season, the immune system is stimulated by natural allergens to that of increased "alertness" whereby any "foreign" antigen is likely to precipitate a hostile reaction. As an invading sperm or a fertilized ovum may produce a similar immune response (the pregnant mother is competent to respond immunologically to the fetus with the production of an immunological reaction against the conceptus) the degree of such a reaction is of vital importance. An exaggerated or inappropriate adaptive host response (defined as a hypersensitivity reaction) is likely to cause tissue damage both to invading sperm and also to the host endometrium during such an "altered" immune state.

As immunoglobulins of various types are responsible for the expression of antibody-antigen immune response in such types of reactions and subsequent inflammatory processes, involvement of IgE antibodies at the earliest stage of pregnancy processes makes it a possible hypothesis. However, as the immunoglobulins of various types do not function in isolation, other types of immunoglobulin are also likely to play respective roles in such a phenomenon. IgG antibodies are probably of critical importance in such a situation whereby the "blocking effect" of such antibodies will affect the ultimate fate of the invading sperm or the immune response generated by the fertilized ovum. In this study an increased prevalence of miscarriage among the allergic women was observed. Pollen induced saturation of IgG4 antibody production in these women, with a subsequent failure to secrete pregnancy associated IgG4, presumably directed against paternal antigens may increase the chances of a "blighted fertilized egg" or hostile endometrium, and could result in an

increased number of miscarriages. A quantitatively greater response could lead to effects earlier in the pregnancy and manifest as reduced fertility. Theresulting rate of conception during the pollen season may be responsible for the observed seasonal variation of human births. If the findings reported here can be repeated elsewhere there could be significant implications for the management of the allergic woman who is or wishes to become pregnant. It would be of great interest to determine whether approaches such as desensitisation to specific allergens or the use of peptide vaccines (Stanworth et al., 1990) might overcome the problems described here.

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APPENDIX I

ENVIRONMENTAL FACTORS

1. Pollen count from 31 May to July 1984
   21 May to August 1985
   30 May to 27 July 1986

2. Distribution of local agricultural products.

3. Distribution of local forestry.

ROTHERHAM METROPOLITAN BOROUGH COUNCIL

DEPARTMENT OF ENVIRONMENTAL HEALTH

POLLEN COUNT FOR 1984

Date	Pollen Count	Date	Pollen Count
May 31st	103	July 13th	8
June 1st	176	14th	29
2nd	7	15th	31
3rd	36	16th	33
4th	35	17th	72
5th	22	18th	64
6th	3	19th	14
7th	8	20th	23
8th	34	21st	136
9th	34	22nd	20
10th	-	23rd	97
11th	-	24th	43
12th	31	25th	13
13th	58	26th	17
14th	48	27th	9
15th	222	28th	3
16th	161	29th	25
17th	195	30th	17
18th	301	31st	24
19th	182		
20th	156		
21st	168		
22nd	58		
23rd	59		
24th	25		
25th	220		
26th	174		
27th	230		
28th	146		
29th	63		
30th	34		
July 1st	162		
2nd	89		
3rd	65		
4th	252		
5th	337		
6th	143		
7th	358		
8th	263		
9th	173		
10th	102		
11th	116		
12th	54		

ROTHERHAM METROPOLITAN BOROUGH COUNCIL

POLLEN COUNTS 1985

May		July	
20th	2	1st	190
21st	3	2nd	310
22nd	4	3rd	386
23rd	13	4th	251
24th	38	5th	175
25th	57	6th	148
26th	No count	7th	217
27th	No count	8th	191
28th	19	9th	114
29th	31	10th	174
30th	33	11th	128
31st	20	12th	113
		13th	242
June		14th	88
		15th	60
1st	47	16th	34
2nd	38	17th	52
3rd	26	18th	39
4th	25	19th	34
5th	5	20th	38
6th	1	21st	30
7th	2	22nd	23
8th	10	23rd	176
9th	11	24th	83
10th	19	25th	80
11th	8	26th	28
12th	13	27th	42
13th	11	28th	7
14th	11	29th	3
15th	20	30th	25
16th	45	31st	10
17th	34		
18th	55	August	
19th	78		
20th	138	1st	8
21st	48	2nd	24
22nd	35	3rd	4
23rd	190	4th	0
24th	154	5th	3
25th	113	6th	0
26th	87	7th	7
27th	40	8th	31
28th	133		
29th	334		
30th	134		

ROTHERHAM METROPOLITAN BOROUGH COUNCIL

DEPARTMENT OF ENVIRONMENTAL HEALTH

POLLEN COUNT FOR 1986

May		July	
30th	14	1st	98
31st	10	2nd	204
		3rd	132
June		4th	36
		5th	20
1st	23	6th	45
2nd	24	7th	69
3rd	23	8th	105
4th	17	9th	88
5th	6	10th	32
6th	11	11th	52
7th	16	12th	6
8th	20	13th	31
9th	16	14th	99
10th	4	15th	130
11th	15	16th	93
12th	20	17th	24
13th	13	18th	9
14th	14	19th	24
15th	28	20th	7
16th	58	21st	17
17th	23	22nd	8
18th	24	23rd	8
19th	53	24th	16
20th	11	25th	1
21st	36	26th	22
22nd	18	27th	15
23rd	8		
24th	91		
25th	126		
26th	102		
27th	79		
28th	97		
29th	76		
30th	44		

11d

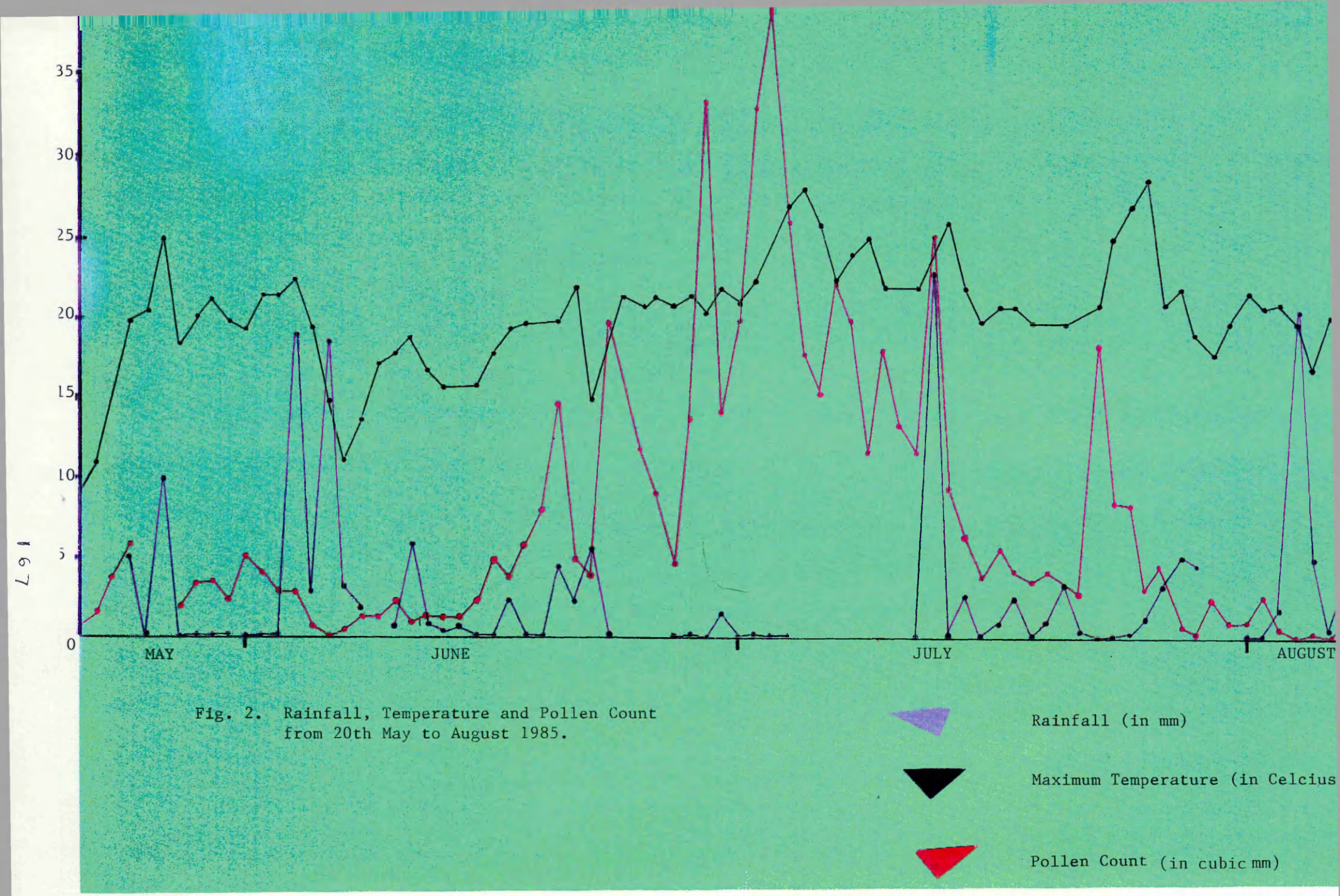

Fig. 2. Rainfall, Temperature and Pollen Count from 20th May to August 1985.

Ministry of Agriculture, Fisheries and Food
Copthall House Potter Street Worksop Notts S80 2ER

Telex Telephone Worksop 475263 ext

Dr Barua
Langold Health Centre
Doncaster Road
Langold
Worksop
Notts

Your reference

Our reference
CLF/JM
Date
25 September 1986

Dear Dr Barua

I am writing in response to your request for information to help you in a study of hay fever during harvest.

CROPS GROWN IN THIS AREA

Tables 1 and 2 show the area of various crops in Nottinghamshire and in the parishes around Langold. The data is taken from the June 1984 census and is the most recent available.

The cropping in particular areas of the county depends largely on the soil type.

Sandland:- Potatoes, sugar beet, barley, peas (and some wheat).

Limestone:- Wheat, barley, oilseed rape, potatoes.

Keuper Marl (in the east):- Wheat, barley, oilseed rape (and some beans).

TRENDS IN CROPPING

Cropping trends are shown in table 3. In addition it is interesting to note that the acreage of grassland has declined noticably.

CONSERVATION

As is typical of most of the UK, there has been a decline in the acreage of hay cut each year. This is due to:-

1. A reduction in livestock numbers.

2. An increase in silage making.

In addition it may be interesting to note an increase in the use of straw choppers (either mounted at the back of the combine or as a separate machine) over the past 2 - 3 years. These chop the straw to approximately 3 - 4" so that it can be easily incorporated into the soil, either as an alternative to burning or to facilitate burning.

ARABLE WEEDS

Common arable weeds in the area are as follows:-

Grasses: Sterile Brome)
Blackgrass) increasing with intensive arable cropping
Wild oats
Couch
Annual meadow grass

TABLE 1

AREA OF ARABLE CROPPING IN NOTTINGHAMSHIRE (JUNE 1984 CENSUS)

Data for all of Nottinghamshire (ha)

Total area	153899
Crops and fallow	113477
Grassland	85325
Rough grazing	1203
Woodland	1698
Other land	2195

Crops and Fallow

Wheat	44980
Winter barley	26114
Spring barley	11204
Oats	794
Mixee corn	27
Rye	154
Maize	91
Potatoes	5232
Sugar beet	8194
Crops for stockfeed:-	
Beans	440
Turnips/swedes	85
Fodder beet/mangels, kale	323
Oilseed rape	12195
Other crops	74
Fallow	404
Horticulture	2437

TABLE 2

ARABLE CROPPING IN SPECIFIED PARISHES - N NOTTINGHAMSHIRE (JUNE 1984)
(ha)

	Styrrup/ Oldcotes	Blyth	Hodsock	Carlton	Worksop	Barnby Moor
Total area	742	1163	964	1065	3671	457
Crops and fallow	678	862	810	868	2726	393
Grassland	52	270	128	159	827	11
Rough grazing				10	53	40
Woodland	9	19	5	19.3	19	4
Other	3	12	21	9	45	9
Crops & Fallow:-						
Wheat	226	194	197	268	594	56
Winter barley	85	149	191	214	568	122
Spring barley	112	215	168	169	478	89
Oats			5		60	
Mixed corn						-
Rye						-
Maize						-
Potatoes	67,6	81	41	37	287	30
Sugar beet	54	103	175	124	259	91
Crops for stockfeed:-						
Beans			1	5	17	
Turnips/swedes					22	
Fodder beet/mangels etc	16				7	
Oilseed rape	18	63	30	50	260	
Other crops						-
Fallow	0.4		2	2	28	
Horticulture	99	57			141	

TABLE 3

TRENDS IN ARABLE CROPPING (NOTTINGHAMSHIRE) 1980 - 85 (ha)

	1980	1984	1985
Wheat	32,000	45,000	45,000
Barley	46,000	37,000	37,000
Potatoes	4,700	5,200	5,000
Sugar beet	9,000	8,000	8,000
Oilseed rape	5,000	12,100	12,300

The Forest District Office
FORESTRY COMMISSION
Edwinstowe Mansfield Notts NG21 9JL

Telephone Mansfield (0623) 822447

Your reference

Dr Barna
Langold Health Centre
Doncaster Road
Langold
Nr Worksop
Notts

Our reference SF17/2

Date 30 September 1986

Dear Dr Barna

I refer to our recent telephone conversation when I promised you information on the relative abundance of broadleaf tree species in the vicinity of Worksop.

I have looked up the recent census of trees in Nottinghamshire and abstracted the following information. Obviously this does not refer to the Worksop area specifically but the data is not broken down on a sub-county basis:-

I trust that this information is of use to you.

Yours sincerely

D A Greig
Forest District Manager

Species	Areas of Woodland (hectares)
Birch	1369
Sycamore	1293
Oak	1178
Beech	600
Ash	589
Sweet Chestnut	393
Poplar	221
Elm	161 (probably less now)
Other broadleaves	139
Mixed "	695

APPENDIX II

CLINICAL DATA RECORD FORMS

1. Form I. Copy of case history form used in the preliminary study.

2. Form II. Copy of ante-natal history form.

3. Form III. Copy of skin test reaction record card.

4. Form IV. Daily Symptom Record Card.

FORM I

Asthma/Atopic Disease Record Chart

Surname ..... Forenames .....
(BLOCK LETTERS)

Date of Birth .....

Address .....

.....

Sex ..... Occupation .....

Name of G.P. .....

Born in the U.K. YES/NO

If the answer to the above question is **NO**, please answer the following:

Place of birth .....

Age at coming to the U.K. .....

Number of years in the U.K. .....

Do you/your child suffer from:

	Yes	No	Not sure
Wheezing/Chronic Cough	☐	☐	☐
Wheezing/Nocturnal Cough	☐	☐	☐
Wheezing/Cough after exercise	☐	☐	☐
Asthma	☐	☐	☐
Hay Fever	☐	☐	☐
Perennial Rhinitis	☐	☐	☐
Sneezing	☐	☐	☐
Eczema	☐	☐	☐

Others, e.g. nasal polyp., urticaria .....

Age of onset of symptoms .....

Severity of symptoms Mild ☐ Moderate ☐ Severe ☐

Are symptoms All year round ☐ Seasonal ☐

If seasonal:

Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec

Perennial Constant ☐ Intermittent ☐

Affected by Wet ☐ Dry ☐ Hot ☐ Cold ☐

Summer ☐ Winter ☐ Indoor ☐ Outdoor ☐

Animal Contacts:

Cat ☐ Dog ☐ Gerbil ☐ Hamster etc. ☐ Budgies and cage birds ☐

Rabbit ☐ Horse ☐ Cow ☐ Sheep ☐ Poultry ☐

House Dust ☐ Grass Pollen ☐ Other .....

Does anyone smoke in the house? .....

If YES 1. How many per day .....

2. How long have you smoked .....

Information relating to early feeding:

Was the child bottle fed? YES ☐ NO ☐ .

Breast fed? YES ☐ NO ☐ If YES, to what age? .....

History of allergy to:

	YES	NO
Egg	☐	☐
Wheat	☐	☐
Milk	☐	☐
Other food products e.g. prawns	☐	☐
Any plants or flowers	☐	☐

Family History

	Asthma	Hay Fever	Allergy		
Father	☐	☐	☐		
Mother	☐	☐	☐		
Brother/Sister				Age	Sex
1)	☐	☐	☐	☐	☐
2)	☐	☐	☐	☐	☐
3)	☐	☐	☐	☐	☐
4)	☐	☐	☐	☐	☐
5)	☐	☐	☐	☐	☐

Are you a vegetarian? YES ☐ NO ☐

Is there any significant dietary difference YES ☐ NO ☐

Present medication .....

.....

.....

Do you think your present treatment is satisfactory? YES ☐ NO ☐

P.E.F.R. (P.E.F.R. to be recorded three times at four monthly intervals, should include early May)

Please record the best of three readings on each occassion

Date	Morning	Afternoon	Night
1st	_____	_____	_____
2nd	_____	_____	_____
3rd	_____	_____	_____

Plan of action:

Initial Assessment and Comment: Recorded by

Intermediate Assessment and comment: Recorded by

Final Assessment and Comment: Recorded by

NOTE:

Diagnostic criteria of severity of symptoms.

Mild - minor symptoms and signs suffered to warrant treatment, but insufficient to greatly alter daily life style.

Moderate - more marked symptoms and signs sufficient to alter life style, e.g. confined to home, time off school/work.

Severe - marked symptoms and signs requiring frequent medical care, sufficient to alter normal life style and activities.

FORM II

ANTE-NATAL ALLERGY/ATOPY RECORD CHART

CONSULTANT:

SURNAME: FORENAMES:

DATE OF BIRTH:

CAUCASIAN ☐

ASIAN ☐

AFRO-CARIBBEAN ☐

NAME OF GENERAL PRACTITIONER:

DO YOU SUFFER FROM: ASTHMA ☐ ECZEMA ☐ HAY FEVER ☐ ALLERGY ☐

If yes: Age of onset _____

Severity of Symptom: Mild (1) Moderate (2) Severe (3)

Are Symptoms All year round ☐ Seasonal ☐

If SEASONAL - PLEASE INDICATE:

Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec

PLEASE RECORD:

SINCE PREGNANCY - SYMPTOMS Less ☐ Increased ☐ No change ☐

If increased - Severe ☐ Moderate ☐ Mild ☐

PLEASE INDICATE - Months when symptoms present since pregnancy

Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Period of Pregnancy (in months)

Severity of Symptom

Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec

NOTE:

Diagnostic criteria of severity of symptoms.

MILD - Minor symptoms and signs suffered to warrant treatment, but insufficient to greatly alter daily lifestyle.

MODERATE - More marked symptoms and signs sufficient to alter lifestyle, eg confined to home, time off work.

SEVERE - Marked symptoms and signs requiring frequent medical care, sufficient to alter normal lifestyle and activities.

PC1AAJ

FORM III

NORISEN SKIN TEST REACTION AND ORDER FORM
(PLEASE COMPLETE EVERY SECTION)

Patient's Surname _____ Date of Birth _____ Age _____
Forename(s) _____ Name of Hospital _____ Dept. _____
Address _____ Merck Ref. No. _____ Hosp Ref. No. _____
Patient's GP _____ Tel. No. _____
Surgery Address _____ Doctor's signature _____ Date _____

B Prescribing Doctor

COURSE(S) REQUIRED: (Seasonal and perennial allergens should not be mixed) C
SEASONAL ☐ (Complete section 1)
PERENNIAL ☐ (Complete section 2)

SKIN TEST REACTIONS D
Negative ☐ Positive ☐

SEASONAL VACCINE

N.B. NO MORE THAN 6 ALLERGENS PER VACCINE

TREATMENT COURSE REQUIRED:
INITIAL TREATMENT ☐
STOCK COMBINATION (see letter) ☐
Treatment to commence .....
(Treatment should commence 10-13 weeks before allergen(s) become borne)

DOSAGE SCHEDULE REQUIRED:
Extremely sensitive (a special dilution vial will be supplied free of charge) ☐
Very sensitive ☐
Mildly sensitive ☐

E

F

	POLLENS	Result	Treat
2067	GRASS MIX		
	(Where only grass mix is required a Norisen Grass Set should be ordered See Note opposite*)		
1214	BARLEY		
1479	MAIZE		
1263	OAT (cult.)		
1586	RYE		
1735	WHEAT		
	WEEDS		
2075	WEED MIX		
1438	DANDELION		
1065	MUGWORT		
1099	NETTLE		
1230	PELLITORY		
1693	PLANTAIN		
	FLOWERS		
2091	FLOWER MIX		
1040	ASTER		
1123	CHRYSANTHEMUM		
1131	DAHLIA		
1255	GOLDEN ROD		
1487	MARGUERITE (daisy)		

	TREES	Result	Treat
0125	TREE MIX ♦ (early blossoming)		
0133	TREE MIX ♦♦ (mid blossoming)		
1156	ALDER ♦		
1164	ASH		
1107	BEECH ♦♦		
1081	BIRCH ♦♦		
1327	ELDER		
1685	ELM ♦		
1016	FALSE ACACIA		
1297	HAZEL ♦		
1149	OAK ♦♦		
1537	PLANE ♦♦		
1529	POPLAR ♦		
1008	SYCAMORE		
1701	WILLOW ♦		
	ADDITIONAL EXTRACTS		

G

PERENNIAL VACCINE (Maintenance treatment recommended)

N.B. NO MORE THAN 6 ALLERGENS PER VACCINE

TREATMENT COURSE REQUIRED:
INITIAL TREATMENT ☐
(Maintenance set will be included with initial treatment unless otherwise indicated by doctor's signature below)
Do not require a maintenance set on this order (signed) .....
MAINTENANCE TREATMENT ☐
(A continuation of previous treatment only)
STOCK COMBINATION (see letter) ☐
(see opposite)
Maintenance set only being ordered please give planned date of final injection from previous set .....

DOSAGE SCHEDULE REQUIRED:
(for initial treatment)
Extremely sensitive (a special dilution vial will be supplied free of charge) ☐
Very sensitive ☐
Mildly sensitive ☐

* Due to the high allergenicity of the house dust mite, mixtures requesting this extract will contain no more than 25% of the total volume as house dust mite. If required as a single extract 100% house dust mite will be provided with a very sensitive dosage schedule.

H

	INHALANTS	Result	Treat
7252	D. PTERONYSSINUS * (house dust mite)		
7070	HOUSE DUST		
7096	HAY DUST		
5967	RYE FLOUR		
5991	WHEAT FLOUR		
	EPITHELIA		
3220	FEATHER MIX		
3210	BUDGERIGAR		
3095	CAT		
3178	COW		
3061	DOG		
3046	GOLDEN HAMSTER		
3111	GUINEA PIG		
3145	HORSE		
3087	RABBIT		
3186	SHEEP'S WOOL		
	STINGING INSECTS		
6510	BEE ▲		
6551	HORNET ▲		
6643	WASP ▲		

▲ Extracts of stinging insects may be mixed with each other, but cannot be mixed with any other Norisen extracts.

I

	MOULDS	Result	Treat
0448	FUNGI MIX I ■		
0455	FUNGI MIX II ■■		
4002	ALTERNARIA ■		
4010	ASPERGILLUS ■■		
4028	BOTRYTIS ■		
4036	CANDIDA		
4044	CHAETOMIUM		
4051	CLADOSPORIUM ■		
4069	CURVULARIA ■		
4077	FUSARIUM ■		
4085	HELMINTHOSPORIUM ■		
4101	MUCOR ■■		
4119	NEUROSPORA		
4127	PENICILLIUM ■■		
4234	PHOMA		
4135	PULLULARIA ■■		
4143	RHIZOPUS ■■		
4168	SERPULA (dry rot) ■■		
4242	SPOROBOLOMYCES		
4192	TRICHOPHYTON		
4218	USTILAGO		
	ADDITIONAL EXTRACTS		
7084	D. FARINAE		

J

MONTH ..... YEAR .....

	DATE																															
	DAY	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
NASAL SYMPTOMS: None ..... 0 Mild ..... 1 Moderate.. 2 Severe ... 3																																
CHEST SYMPTOMS: None ..... 0 Little ... 1 Wheeze - Moderate . 2 Severe ... 3																																
EYE SYMPTOMS: None ..... 0 Mild ..... 1 Moderate . 2 Severe ... 3																																
POLLEN COUNT:																																
TEMPERATURE: (°C)																																
HUMIDITY: (% saturation)																																
NAME OF DRUG / DOSE PRESCRIBED*	Write the number of doses taken day by day in the daily boxes below																															

* NOTE: Put X to indicate if you are taking additional medication.

0935J/jah

APPENDIX III

RESULTS OF INITIAL ALLERGY SCREENING

1. Distribution of skin test reactions to different allergens. Table 8.

2. Age of onset of symptoms. Table 9.

3. Results of preliminary screening. Table 10.

TABLE 8. DISTRIBUTION OF SKIN TEST REACTIONS TO DIFFERENT ALLERGENS.

	++++++++	+++++++	++++++	+++++	++++	+++	++	+	Total
Grass	1	1	1	6	11	18	10	6	54
Barley					9	14	9	3	35
Wheat					1	9	5	4	19
Maize							1		1
Rye							1	1	2
Flower						1	1	8	10
Weed					1	1	2	10	14
Tree					2	1	2	3	8
Birch			1	1			2		4
Ash							1		1
Beech						1			1
Hazel						1			1
H.D. Mite			1	1	6	10	6	8	32
House Dust						3	16	11	30
Hay Dust							1	2	3
Mould					1				1
Aspergillus					1				1
Cat						2	1	3	6
Dog								2	2
Horse								3	3
Rabbit							1		1
Budgies							1		1
Sheep Wool							1	1	2
Attemis					1	1	1	1	4
Botrytis							1		1
Otun								1	1
Leather						1		1	
Candida							1	1	
Tusarium						1		1	
Uadosperium						1		1	
Fungi						1	4	5	
Cereals	3 - but with ill-defined degree of reaction								

Control positive, tests negative 3
All tests negative 3

TABLE 9. Age at onset of symptoms

	PERENNIAL		SEASONAL		SEASONAL AND PERENNIAL	
	MALE	FEMALE	MALE	FEMALE	MALE	FEMALE
Very young	1		1			
6 weeks					1	
2 years		1				
3 years			1			
5 years			1			
6 years			1	1	1	
7 years			1			
8 years			1			
9 years	1		2	1		
10 years			3			
11 years			1	3		
12 years			1	1		
13 years			1	4		
14 years			2	2		
15 years			3			
16 years		2		1	1	
17 years				1		
19 years				1		
20 years				1		
21 years		1				
23 years			1			
26 years			1			
28 years			1			
29 years						1
35 years				1		
46 years	1					
48 years		1				
	11 with uncertain age of onset		10 with uncertain age of onset		5 with uncertain age of onset	

TABLE 10. Results of Preliminary Screening.

A - Asthma B - Hayfever C - Eczema H.D.H. - House Dist Mite Table showing skin test reaction.

NAME	AGE	SEX	SYMPTOMS	AGE AT ONSET	SEASONAL	PERENNIAL	SKIN TEST REACTION				SENSITIVITY
							POLLEN	TREES	INHALENTS	OTHERS	
S.H.	19 yrs	M	A B	9 yrs			Grass ++++ Barley ++++	Birch ++			very sensitive
P.S.	44 yrs	F	A B	35 yrs			Grass ++	Tree ++++ Birch ++++		H.D. Mite H.D. Moulds	Extremely Sensitive
A.G.	16 yrs	M	B	14 yrs			Grass +++ Barley +++ Wheat +			H.D. Mite +	Very sensitive
S.B.	15 yrs	M	B C	10 yrs			Grass +++	Tree mix ++			Extremely sensitive
P.D.	25 yrs	F	A B	14 yrs			Grass				Very sensitive mild 86
F.C.	25 yrs	F	A B	13 yrs			Grass +++++ Barley ++++				Very sensitive
E.S.	38 yrs	M	A	15 yrs			Grass +++++ Barley ++++ Weed ++ Maize ++	Tree Mix ++		Flower ++ H.D.M. ++ H.D. ++	Very sensitive
A.F.	17 yrs	M	B	5 yrs			Grass ++ Barley +	Tree Mix +	1	H.D.M. ++ H.D. ++	Mild sensitive
N.S.	16 yrs	M	A B	3 yrs			Grass mix ++++ Barley +++ Wheat +++			Flower mix + Weed mix + H.D.M. ++++++ H.D. +++	very sensitive
P.D.	22 yrs	M	B	13 yrs			Grass mix ++++ Barley ++ Wheat ++				

TABLE 10. Results of Preliminary Screening. (Cont.)

A - Asthma B - Hayfever C - Eczema H.D.H. - House Dist Mite Table showing skin test reaction.

NAME	AGE	SEX	SYMPTOMS	AGE AT ONSET	SEASONAL	PERENNIAL	SKIN TEST REACTION				SENSITIVITY
							POLLEN	TREES	INHALENTS	OTHERS	
C.S.	38 yrs	F	A B	29 yrs			Grass ++++ Barley ++			H.D.M. ++++ H.D. ++ Flower & weed mix	Mild sensitive
P.M.	31 yrs	M	B	28 yrs			Grass +++++ Barley +++ Rye +			H.D.N.	Very sensitive
D.E.		F	B	9 yrs			Grass +++++ Barley ++++ Wheat +++			Weed mix + fungi ++	Very sensitive
G.M.	32 yrs	M	B	23 yrs			Grass ++++ Barley ++ Wheat +++				Mild sensitive
H.P.	20 yrs	F	B	11 yrs			Grass +++++ Barley +++ Wheat +++				Mild sensitive
S.J.	24 yrs	M	A	very young			Grass ++++ Barley ++++ Wheat ++++	Tree +		Flower mix + H.D.M. + H.D. + Cat +++	Very sensitive
M.C.	23 yrs	M	B	12 yrs			Grass ++++++ Barley ++++ Wheat +++	Tree +		Flower mix + H.D.M. + H.D. + Cat +++	Very sensitive
G.S.	21 yrs	F	B	19 yrs			Grass ++++ Barley +++ Wheat +++			Flower mix + H.D.M. +	Very sensitive
K.C.	19 yrs	M	A	14 yrs			Grass +++++++ Barley +++ Wheat +++			H.D.M. ++++ H.D. ++ Hay dust + Flower mix +	Very sensitive

TABLE 10. Results of Preliminary Screening. (Cont.)

A - Asthma B - Hayfever C - Eczema H.D.H. - House Dist Mite Table showing skin test reaction.

NAME	AGE	SEX	SYMPTOMS	AGE AT ONSET	SEASONAL	PERENNIAL	SKIN TEST REACTION				SENSITIVITY
							POLLEN	TREES	INHALENTS	OTHERS	
A.C.	38 yrs	M	A	6 yrs			Grass Mix ++++ Barley +++			H.D.M. ++++ H.D. ++ Cat & dog + Horse & sheep wool ++	Very sensitive
N.G.	16 yrs	M	A	7 yrs			Grass Mix ++++++++				Very sensitive
T.S.	16 yrs	M	B	15 yrs			Grass				
G.T.	30 yrs	M	A B	9 yrs			Grass Mix + Barley ++++			Weed mix +	Mild sensitive
D.H.	15 yrs	M	B				Grass Mix ++++ Barley +++ Wheat ++	Tree Mix + Ash + Beech ++ Hazel ++		Weed mix	Extremely sensitive
R.D.	28 yrs	M	A B	15 yrs			Grass ++++ Barley +++			Weed +	Very sensitive
M.B.	40 yrs	F	A	13 yrs			Grass +++			Aspergillus +++++ H.D. ++ Fungi mix +++++ Alternia ++++ Cat & horse + Rabbit ++	Very sensitive
E.C.	11 yrs	M	A B	6 yrs 9 yrs 11 yrs			Grass ++++ Barley ++++ Wheat +++			H.D.H. +++ H.D.	Very sensitive

TABLE 10. Results of Preliminary Screening. (Cont.)

A - Asthma B - Hayfever C - Eczema H.D.H. - House Dist Mite Table showing skin test reaction.

NAME	AGE	SEX	SYMPTOMS	AGE AT ONSET	SEASONAL	PERENNIAL	SKIN TEST REACTION				SENSITIVITY
							POLLEN	TREES	INHALENTS	OTHERS	
A.H.	20 yrs	M	B				Grass Pollen +++			Budgies ++	Very sensitive
G.H.	29 yrs	M	A B	26 yrs			Grass Fungi				
T.B.	16 yrs	F	B	11 yrs			Grass +++ Barley ++++			Weeds +++++ Flower mix +	Very sensitive
S.B.	15 yrs	M	B	10 yrs			Grass +++	Tree mix +++		Weed + Flower +	Extremely sensitive
P.G.		M	A							H.D.M. +++ sensitive	Very sensitive
D.J.	25 yrs	M	A	16 yrs						Fungi Alternaria ++ Botrytis ++ Other	Very sensitive
T.T.	22 yrs	F	B	14 yrs			Grass +++ Wheat +			H.D.M. +++ H.D. ++ Cat + Feather ++	Very sensitive
S.N.	22 yrs	F	B	17 yrs			Grass +++++				Very sensitive

TABLE 10. Results of Preliminary Screening. (Cont.)

A - Asthma B - Hayfever C - Eczema H.D.H. - House Dist Mite Table showing skin test reaction.

NAME	AGE	SEX	SYMPTOMS	AGE AT ONSET	SEASONAL	PERENNIAL	SKIN TEST REACTION				SENSITIVITY
							POLLEN	TREES	INHALENTS	OTHERS	
J.L.	17 yrs	F	B	6 yrs			Grass ++++ Wheat +++			Weed + H.D.M. +++ H.D. ++	Very sensitive
H.P.	19 yrs	F	B	13 yrs			Grass +++ Barley ++				Very sensitive
L.B.	50 yrs	F	A B	48 yrs						H.D.M. +++ H.D. ++	Very sensitive
R.A.	22 yrs	F	Rhinitis sneezing	16 yrs						H.D.M. ++++ H.D. ++	Very sensitive
L.A.	10 yrs	M	Rhinitis							H.D.M. +++ H.D. +	Very sensitive
S.H.	19 yrs	M	A	9 yrs			Grass ++++ Barley ++++	Birch ++			Very sensitive
T.S.	44 yrs	F	A B	35 yrs			Grass ++	Tree ++++ Birch Moulds		H.D.M. H.D.	Extremely sensitive
S.G.	16 yrs	M	B	14 yrs			Grass +++ Barley +++ Wheat +			H.D.M. +	Very sensitive
S.B.	15 yrs	M	B C	10 yrs			Grass +++	Tree mix ++			Extremely sensitive
P.D.	25 yrs	F	A B	14 yrs			Grass				Very sensitive 85 mild 86
E.C.	25 yrs	F	A B	13 yrs			Grass +++++ Barley ++++				Very sensitive

TABLE 10. Results of Preliminary Screening. (Cont.)

A - Asthma B - Hayfever C - Eczema H.D.H. - House Dist Mite Table showing skin test reaction.

NAME	AGE	SEX	SYMPTOMS	AGE AT ONSET	SEASONAL	PERENNIAL	SKIN TEST REACTION				SENSITIVITY
							POLLEN	TREES	INHALENTS	OTHERS	
M.S.	38 yrs	M	A	15 yrs			Grass +++++ Barley ++++ Weed ++ Maize ++	Tree mix ++		Flower ++ H.D.M. ++ H.D. ++	Very sensitive
A.F.	17 yrs	M	B	5 yrs			Grass ++ Barley +	Tree mix +	1	H.D.M. ++ H.D. ++	Mild sensitive
N.S.	16 yrs	M	A B	3 yrs			Grass mix +++ Barley +++ Wheat +++			H.D.M. + Hay dust ++	Very sensitive
P.D.	22 yrs	M	B	13 yrs			Grass mix ++++ Barley ++ Wheat ++			Flower mix + Weed mix + H.D.M. ++++++ H.D. +++	Very
L.G.	28 yrs	F	B	20 yrs			Grass +++			H.D. +	Mild sensitive
C.M.	33 yrs	F	B	16 yrs			Grass +++ Barley +++ Wheat ++			H.D. +	Mild sensitive
J.T.	32 yrs	M	A	26 yrs			Grass ++				Mild sensitive
J.M.	11 yrs	M	A	8 yrs			Grass ++++ Barley ++++				Extremely sensitive
L.C.		F	A				Grass +++				Very sensitive

APPENDIX IV

IMMUNOASSAY REAGENTS ALLERGEN-SPECIFIC IMMUNOGLOBULINS

1. Enzymeimmuno reagents For IgE assay.

2. Enzymeimmuno reagents for IgG4 assay.

1. Enzyme Immunoassay Reagents for IgE

(1) Enzyme-Anti-Ige conjugate solution: The anti-IgE is purified from an antiserum raised in rabbits.

(2) Washing Solution: Washing solution additive (16 ml) and NaCl powder (9 g) are made up to 1 litre with redistilled water.

(3) Development Solution: This is reconstituted by adding a to b.

(a) Development reagent, O-nitrophenyl-B-galactoside (substrate) and glutathione (reducing agent). (Lyophilized)

(b) Development solution buffer, 13 ml.

(4) Stop Solution: Dissolve stop substance, (sodium carbonate powder, 4.2 g) in 100 ml redistilled water.

(5) Reference Discs (in buffer solution): Allergen discs impregnated with Timothy grass allergen.

(6) Reference sera (human serum).

Serum A is prepared from a standardized human serum pool with a high content of IgE specific the reference allergen (birch). Sera B, C and D are standardized dilutions (1:5, 1:25, 1:50) of serum A prepared with a buffer solution containing bovine serum.

2. Enzyme Immunoassay Reagents for IgG4

(1) Enzyme-Anti-IgG (mouse monoclonal) conjugate.

(2) Development Solution: reconstitute by adding a to b.

(a) Development reagent, O-nitrophenyl-B-galactoside (substrate) and glutathione (reducing agent). (Lyophilized)

(b) Development solution buffer, 13 ml.

(3) Stop Solution. Stop substance, (sodium carbonate powder 4.2 g) made up to 100 ml with redistilled water.

(4) Washing Solution: Washing solution additive liquid 16 ml + washing solution concentrate liquid (50 ml) + 1 litre, redistilled water.

(5) Reference Reagents

Reference tubes coated with birch allergen.

(7) Reference Sera: Prepared from a standardized human serum pool with a high content of IgG4 antibodies specific for the reference allergen (birch).

(8) Preparation of Samples.The serum sample is diluted 1:50 with sample diluent.

APPENDIX V

Clinical Summary of Allergic and non-allergic women.
Clinical Details of women attending Langold Health Centre Ante-natal Clinic between September 1986 and December 1990.

Table 17. Clinical Summary of Group 1 Allergic Pregnant Women. (Continued)

Name	Age	Parity	Blood Group	Obstetric History	Medical History	Family History of Atopy	Observations of Atopy
JG	23	1	A.Rh.D.P.	1989 elective LSCS delivery/breech. Bartholin's abscess.	Tonsillitis. Adenoidectomy. Allergic to penicillin.	Brother allergic to penicillin.	No significant symptoms.
SH		1	Gone off our list but delivered 1 child 1989. Hayfever improved since pregnancy.				
JW	27	1	O.Rh.D.P.	Normal delivery. Glucose ++.	Enlarged adenoids. Sinusitis. High B.P. Anxiety state. Asthma, dermatitis.	No details.	Asthma increased early pregnancy.
GH	28	1	A.Rh.P.	normal delivery High B.P.	Psoriasis. Dept. of Psychiatry - arachnaphobia.	Mother high B.P. and asthma. Husband allergy related asthma.	No significant symptoms.
JB	39	4	A.Rh.D.P.	normal deliveries	High B.P. Overweight. Warts. Tonsillectomy and adenoidectomy. Polio at 1 year.	Husband and mother high B.P.	No significant symptoms.
SA	19	2	O.Rh.P.	normal delivery	Anxiety state. Overdose 1986. Warts. Allergic to penicillin.	No details.	No significant symptoms.
YB	26	3	O.Rh.P.	normal deliveries	Eczema, hayfever.	Brother hayfever and asthma. Father high B.P.	Symptoms increased up to 35 weeks pregnant then returned to normal.
EW	32	3	A.Rh.P.	miscarriage 1985 2 normal deliveries	High B.P. Overweight. D.V.T. excluded. Eczema, hayfever, bronchitis.	Grandmother and aunt high B.P. Sister and mother eczema	Eczema improved during pregnancy. Asthma slightly better, otherwise no change.

Table 17. Clinical Summary of Group 1 Allergic Pregnant Women. (Continued)

Name	Age	Parity	Blood Group	Obstetric History	Medical History	Family History of Atopy	Observations of Atopy
GB	30	1	B.Rh.P.	emergency LSCS 35 weeks for PM and IUGR	Hayfever. Otitis Media. Appendectomy. Dept. of Psychiatry - sexual problems.	No details.	No symptoms since pregnancy.
JD	25	1	A.Rh.Pos.	Normal delivery. Mastitis.	Tonsillitis. Eczema. Dermatitis.		Symptoms increased to 37 weeks then improved.
GG	32	3	O.Rh.D.Pos.	Emergency LSCS. Utero placental clot.	Hayfever. Eczema. Tonsillitis. Otitis Externa.		Increased to severe up to 18/20 weeks, then improved.
KB	25	2	O.Rh.Pos.	Normal delivery.	Tonsillitis. Hayfever.		No change, wrong time of year for symptoms.
AH	20	1	O.Rh.D.Pos.	Normal delivery.	Hayfever. Anxiety state.		No change.

Table 18. Clinical Summary of Group 2 Pregnant Non-Allergic Women.

Name	Age	Parity	Blood Group	Obstetric History	Medical History
C.H.	31	3	B.Rh.Pos.	Normal deliveries.	Sinusitis. Submandibular salivary tumour removed
L.G.	24	1	O.Rh.Pos.	Normal deliveries. Fainting during early pregnancy.	Contact dermatitis.
W.B.	28	3	Not known.	Faints and migraine during pregnancy. 3 caesareans.	Migraine.
K.B.	38	2	B.Rh.Neg.	Inflammation of cervix.	Fibrofatty breast lump. Bowel irregularity. Allergic rashes. Sinusitis. Psychiatric referrals for anxiety state.
M.B.	21	2	A.Rh.Neg.	2 emergency LSCS.	Tonsillitis.
S.J.R.	36	2	Rh.D.Pos.	Infertility Clinic. EUA. Vicryl suture inserted. Forceps delivery 1988. Emergency LSCS 1987.	Laparoscopy and appendectomy.
K.W.	36	2	A.Rh.D.Pos.	Admitted high B.P. for induction. Emergency LSCS both pregnancies.	Perianal warts.
L.P.	25	1	O.Rh.D.Pos.	Normal delivery.	Eczema, psoriasis.

Table 18. Clinical Summary of Group 2 Pregnant Non-Allergic Women. (Continued)

Name	Age	Parity	Blood Group	Obstetric History	Medical History
P.H.	21	1	O.Rh.D.Pos.	Normal delivery.	Otitis media High B.P.
F.C.	39	3	A.Rh.D.Pos.	Severe constipation during pregnancy. Cervicitis. U.T.I. 2 miscarriages. Third normal delivery.	Anxiety state. Vertigo. Hypochondriac. Appendectomy.
W.E.	24	1	B.Rh.Pos.	Forceps delivery.	Tonsillitis.
T.McS.	22	2	B.Rh.Pos.	Normal deliveries.	Otitis media. Depression. Referred to Department of Psychiatry. Overdose 1987.
A.D.	24	1	Not known.	Forceps delivery. Dyskaryosis. Cdposcopy.	Nose bleeds. Dizzy spells.

Table 21. Clinical Summary of Women Attending Langold Health Centre Ante-natal Clinic, September 1986 to December 1990.

	ALLERGIC	NON ALLERGIC
NORMAL DELIVERIES	149 plus 2 premature deaths 4 premature deliveries 1 cot death	209 including 4 twin births 1 premature delivery
MISCARRIAGES	32 including 2 twin pregnancies plus 4 threatened miscarriages	12

MISCARRIAGES BETWEEN OCTOBER 1986 - DECEMBER 1990. ALLERGIC PATIENTS

NAME & AGE	PARITY	MISCARRIAGE	PERIOD OF GESTATION	ALLERGIES	BLOOD GROUP	OBSTETRIC HISTORY	MEDICAL HISTORY
J.G. 27 yr	3	1 1986	8 weeks	hayfever	AB.Rh.D.Pos.	normal delivery 1987/8	
D.M. 33 yr	3	1	8 weeks	allergic rash	O.Rh.D.Pos.	threatened abortion March 1987 conceived on Clomiphene normal delivery	tonsillitis
J.T. 43 yr	5	1 1	no details 8 weeks	eczema	O.Rh.D.Pos.	1968 abortion an embryonic pregnancy 2 normal deliveries	anxiety state carcinoma cervix 1977
J.H. 37 yr	3	September 1989	12 weeks	dermatitis	O.Rh.D.Pos.	2 normal deliveries 1986/8	scabies
A.A. 37 yr	5	1 1	8 weeks no details	eczema	A.Rh.D.Neg.	2 normal deliveries	alopecia hypertension
E.W. 36 yr	3	1	not known	eczema	A.Rh.D.Pos.	normal deliveries Treated with Clomiphene	allergic dermatitis
L.J.S	1	1990	no details	asthma	no details	no details	no significant details
G.S. 39 yr	2	September 1990	7 weeks	hayfever allergic rhinitis allergic dermatitis	A.Rh.D.Pos.	ventouse delivery January 1988	sinusitis
D.T. 30 yr	2	1 1986	9 weeks	hayfever	A.Rh.D.Pos.	normal delivery May 1988 Infertility Clinic	
A.B. 29 yr	4	3	no details	eczema	B.Rh.D.Pos.	normal delivery	anxiety state
J.G. 27 yr	4	1986	no details	hayfever	A.B.Rh.Pos.	1 abortion 1 premature delivery	no significant illnesses
L.H. 32 yr		October 1987	no details	allergic rash	A.Rh.D.Pos.	normal delivery	tonsillitis mouth ulcers pruritis
D.J. 33 yr	1	September 1988	10 weeks	allergic rash	no details	no details	tonsillitis
F.C. 41 yr	4	2	no details	asthma allergy to plants	A.Rh.D.Pos.	2 normal deliveries	anxiety state hypochondriac

NAME & AGE	PARITY	MISCARRIAGE	PERIOD OF GESTATION	ALLERGIES	BLOOD GROUP	OBSTETRIC HISTORY	MEDICAL HISTORY
L.H.	2	August 1988	no details	asthma	A.Rh.D.Pos.	premature delivery 1988 baby died after 24 hrs	sinusitis
R.R. 36 yr	5	November 1988	10 weeks (twin)	eczema	A.Rh.Pos.	prurigo of pregnancy normal delivery 1988	thyrotoxicosis second child cerebral palsy and hydrocephalus
T.M. 24 yr	3	August 1990	8 weeks	allergic rash	B.Rh.D.Pos.	normal delivery	otitis media psychiatric/ overdose
J.T. 35 yr	3	1986	not known	eczema	not known	Infertility Clinic 2 normal deliveries	acne
C.P. 28 yr	3	June 1988 July 1989	not known not known	allergic rash	O.Rh.D.pos.	treated with Clomiphene normal delivery January 1990	vertigo
D.G. 34 yr	5	January 1989 August 1988 June 1989	6 weeks not known 12 weeks	allergic rash fungal infections	B.Rh.Pos.	LSCS	mastoidectomy vaginal warts otitis media anxiety state
S.M. 34 yr	3	March 1990	not known	allergic rash	O.Rh.D.Pos.	normal deliveries ovarian cystectomy vertigo anxiety state	tonsillitis bronchitis
J.D. 35 yr	3	1 1986	not known	dermatitis	not known	2 normal deliveries hypertension	tonsillitis

MISCARRIAGES BETWEEN OCTOBER 1986 - DECEMBER 1990. NON-ALLERGIC PATIENTS

NAME & AGE	PARITY	MISCARRIAGE	PERIOD OF GESTATION	BLOOD GROUP	OBSTETRIC HISTORY	MEDICAL HISTORY
D.C. 42 yr	4	3	not known	not known	not known	otitis externa
M.C. 31 yr	3	2	not known	O.Rh.D.Neg.	2 normal deliveries	tonsillitis otitis externa
S.N. 31 yr	4	1	not known	O.Rh.Neg.	2 normal births 1 termination 1 twin pregnancy	no significant illnesses
A.K. 26 yr	2	1	12 weeks	not known	1 normal delivery 1 miscarriage	no significant illnesses
L.H.	1	1	not known	not known	not known	otitis media
E.O. 32 yr	5	1	10 weeks	B.Rh.Pos.	4 normal deliveries 1 miscarriage	no significant illnesses
H.P. 33 yr	5	1	5 weeks	O.Rh.Neg.	1 miscarriage 1 termination 3 normal deliveries	anxiety state polyps
M.B. 29 yr	2	1	not known	A.Rh.Pos.	1 miscarriage 1 normal birth	no significant illnesses
P.T. 40 yr	2	1	not known	B.Rh.D.Pos.	1 miscarriage Infertility Clinic normal birth 1989	tonsillitis glandular fever

NORMAL BIRTHS BETWEEN OCTOBER 1986 - DECEMBER 1990. NON-ALLERGIC PATIENTS.

NAME & AGE	PARITY	NORMAL BIRTHS	BLOOD GROUP	OBSTETRIC HISTORY	MEDICAL HISTORY
D.M. 39 yr	4	4	O.Rh.Neg.	normal	no significant illnesses
A.M. 28 yr	3	3	B.Rh.D.Pos.	normal	depression/anxiety state
B.B. 28 yr	2	2	not known	normal	tinnitus
P.S. 38 yr	2	1	O.Rh.D.Pos.	termination normal 1987	otitis media anxiety state peri-anal warts
J.M. 20 yr	1	1	O.Rh.D.Pos.	normal	no significant illnesses
B.S. 46 yr	4	4	A.Rh.Pos.	normal	no significant illnesses
V.M. 25 yr	1	1	O.Rh.D.Pos.	normal	no significant illnesses
M.B. 26 yr	3	2	not known	2 normal births 1 termination	anxiety state vulval warts
A.H. 20 yr	4	3	A.Rh.D.Pos.	3 normal births 1 termination	no significant illnesses
S.H.	2	2	A.Rh.D.Pos.	normal	no significant illnesses
J.S. 28 yr	2	2	A.Rh.D.Pos.	normal	no significant illnesses
C.P. 30 yr	1	1	O.Rh.D.Neg.	normal	Department of Psychiatry
A.K. 30 yr	2	2	A.Rh.D.Pos.	hypertension	tonsillitis moles first child cerebral palsy
K.B. 31 yr	2	2	B.Rh.Pos.	first child premature at 33 weeks	no significant illnesses
M.B. 22 yr	2	1	A.Rh.Pos.	miscarriage 1988	no significant illnesses

NORMAL BIRTHS BETWEEN OCTOBER 1986 - DECEMBER 1990. NON-ALLERGIC PATIENTS. (Continued)

NAME & AGE	PARITY	NORMAL BIRTHS	BLOOD GROUP	OBSTETRIC HISTORY	MEDICAL HISTORY
D.C. 38 yr	4	1	no details	3 miscarriages	olitis externa polyps
M.C. 34 yr	3	2	O.Rh.D.Neg.	1 miscarriage	tonsillitis otitis externa
J.F. 35 yr	2	1	no details	termination	no significant illnesses
P.T. 40 yr	2	1	B.Rh.D.Pos.	miscarriage Infertility Clinic normal birth 1989	tonsillitis glandular fever
A.C. 38 yr	1	1	no details	normal delivery	no significant illnesses
D.H.	2	2	O.Rh.D.Pos.	normal	no significant illnesses
T.C. 20 yr	1	1	A.Rh.D.Pos.	normal	no significant illnesses
J.B. 24 yr	2	2	A.Rh.Pos.	normal	no significant illnesses
C.M. 20 yr	1	1	O.Rh.D.Pos.	normal	no significant illnesses
W.E.	1	1	B.Rh.Pos.	normal	no significant illnesses
H.B. 24 yr	1	1	O.Rh.Pos.	normal	no significant illnesses
E.O. 40 yr	5	4	B.Rh.Pos.	4 normal deliveries 1 miscarriage	no significant illnesses
P.C. 20 yr	2	2	A.Rh.Pos.	normal	no significant illnesses
A.B. 28 yr	1	1	no details	no details	no details
K.W. 23 yr	1	1	O.Rh.Pos.	normal	CIN111
A.B. 23 yr	2	2	AB.Rh.D.Pos.	normal deliveries following Infertility Clinic	no significant illnesses

NORMAL BIRTHS BETWEEN OCTOBER 1986 - DECEMBER 1990. NON-ALLERGIC PATIENTS. (Continued)

NAME & AGE	PARITY	NORMAL BIRTHS	BLOOD GROUP	OBSTETRIC HISTORY	MEDICAL HISTORY
T.R. 26 yr	3	3	A.B.Pos.	normal	Department of Psychiatry/ depression
L.W. 30 yr	3	3	A.Rh.Neg.	normal	no significant illnesses
V.B. 30 yr	2	2	O.Rh.D.Pos.	normal	no significant illnesses
M.B. 22 yr	2	2	A.Rh.Neg.	normal	no significant illnesses
M.M. 20 yr	2	2	O.Rh.Pos.	normal	no significant illnesses
J.E. 29 yr	2	2	O.Rh.D.Neg.	normal	no significant illnesses
M.C. 25 yr	1	1	O.Rh.Pos.	hypertension	no significant illnesses
A.F. S.J. 39 yr	3 2	3 2	A.Rh.D.Pos. no details	normal normal	no significant illnesses no significant illnesses
J.P. 25 yr	3	3	O.Rh.Neg.	normal	no significant illnesses
H.P. 20 yr	5	3	O.Rh.Neg.	miscarriage at 5 weeks termination 3 normal deliveries	anxiety state polyps
C.M. 26 yr	2	2	A.Rh.D.Pos.	normal	anxiety state/depression
K.V. 38 yr	1	1	A.Rh.Pos.	normal	no significant illnesses
R.G. 26 yr	1	1	O.Rh.D.Pos.	normal	no significant illnesses
J.H. 29 yr	2	2	A.D.Pos.	normal	no significant illnesses
M.W. 31 yr	3	3	A.Rh.D.Pos.	normal	tonsillitis

NORMAL BIRTHS BETWEEN OCTOBER 1986 - DECEMBER 1990. NON-ALLERGIC PATIENTS. (Continued)

NAME & AGE	PARITY	NORMAL BIRTHS	BLOOD GROUP	OBSTETRIC HISTORY	MEDICAL HISTORY
P.H. 21 yr	2	2	O.Rh.D.Pos.	normal	no significant illnesses
S.P. 29 yr	2	1	O.Rh.D.Pos.	1 normal delivery	no significant illnesses
J.U. 27 yr	1	1	B.Rh.Pos.	normal	no significant illnesses
L.C.K 40 yr	2	2	not known	LSCS deliveries	no significant illnesses
D.M. 40 yr	3	3	O.Rh.Neg.	normal	sinusitis
K.H. 33 yr	2	2	O.Rh.D.Pos.	normal	pruritis
B.P. 24 yr	2	2	O.Rh.Neg.	normal	glandular fever
H.J.	2	2	A.Rh.D.Pos.	normal	hypothyroidism pruritis
T.K. 24 yr	1	1	A.Rh.D.Pos.	normal	no significant illnesses
T.M. 28 yr	1	1	A.Rh.D.Pos.	normal	no significant illnesses
S.M.	2	2	O.Rh.D.Pos.	normal	no significant illnesses
P.S. 28 yr	2	2	not known	normal	no significant illnesses
S.M. 28 yr	2	2	O.Rh.D.Pos.	hypertension	no significant illnesses
D.A. 29 yr	3	3	O.Rh.D.Pos.	normal	no significant illnesses
D.M. 28 yr	2	2	O.Rh.D.Pos.	normal	no significant illnesses
E.M. 20 yr	2	2	Rh.A.Pos.	hypertension	otitis media Department of Psychiatry/ anxiety state

NORMAL BIRTHS BETWEEN OCTOBER 1986 - DECEMBER 1990. NON-ALLERGIC PATIENTS. (Continued)

NAME & AGE	PARITY	NORMAL BIRTHS	BLOOD GROUP	OBSTETRIC HISTORY	MEDICAL HISTORY
R.C. 25 yr	2	2	A.Rh.D.Pos.	normal	tonsillitis
P.S. 24 yr	2	2	A.Rh.D.Pos.	normal	vulval warts tonsillitis
J.B. 40 yr	4	4	A.Rh.D.Pos.	hypertension	tonsillitis anxiety state psoriasis warts
P.C. 24 yr	3	3	not known	normal	no significant illnesses
J.G. 23 yr	3	3	O.Rh.D.Neg.	normal	no significant illnesses
M.B. 23 yr	2	2	A.Rh.Neg.	LSCS deliveries	tonsillitis
K.W. 38 yr	2	2	A.Rh.D.Pos.	LSCS deliveries	hypertension peri-anal warts
W.B. 20 yr	3	3	A.Rh.Neg.	LSCS deliveries fainting	no significant illnesses
D.W. 27 yr	1	1	A.Rh.Pos.	normal	no significant illnesses
T.W. 36 yr	2	2	B.Rh.Pos.	normal	otitis media tonsillitis
C.H. 39 yr	3	3	not known	normal	sinusitis
L.M. 40 yr	1	1	O.Rh.D.Pos.	LSCS twins following Infertility Clinic	no significant illnesses
T.T. 25 yr	2	2	A.Rh.D.Pod.	normal	no significant illnesses
J.G.	2	2	O.Rh.D.Neg.	normal	no significant illnesses
S.N. 30 yr	5	3	O.Rh.D.Neg.	3 normal deliveries twins miscarriage termination	sinusitis anxiety state

NORMAL BIRTHS BETWEEN OCTOBER 1986 - DECEMBER 1990. NON-ALLERGIC PATIENTS. (Continued)

NAME & AGE	PARITY	NORMAL BIRTHS	BLOOD GROUP	OBSTETRIC HISTORY	MEDICAL HISTORY
M.U. 37 yr	2	2	O.Rh.D.Pos.	normal	no significant illnesses
Y.W. 38 yr	3	3	O.Rh.D.Pos.	normal	no significant illnesses
A.K. 26 yr	2	1	not known	1 normal 1 miscarriage	no significant illnesses
C.F. 24 yr	1	1	not known	normal	fainting anxiety state peri-natal warts
J.S. 32 yr	1	1	not known	normal/twins sterilization	CIN 111. tonsillectomy
E.W. 35 yr	4	3	B.Rh.D.Pos.	3 normal births 1 termination	CIN 111. ovarian cysts
J.W. 33 yr	3	3	A.Rh.D.Pos.	normal	no significant illnesses
J.C. 28 yr	3	3	A.Rh.D.Pos.	normal	no significant illnesses
C.B. 28 yr	2	2	O.Rh.Pos.	normal	tonsillitis
M.C. 34 yr	3	3	A.Rh.Pos	normal	no significant illnesses
J.M. 25 yr	1	1	Rh.D.Pos.	normal	no significant illnesses
S.A. 20 yr	2	2	O.Rh.Pos.	normal	overdose/Department of Psychiatry
G.B. 30 yr	3	3	O.Rh.D.Pos.	normal	tonsillitis
A.M.S 29 yr	2	2	O.Rh.D.Pos.	normal	CIN 111
A.M. 28 yr	2	2	A.Rh.D.Pos.	normal	tonsillitis

NORMAL BIRTHS BETWEEN OCTOBER 1986 - DECEMBER 1990. NON-ALLERGIC PATIENTS. (Continued)

NAME & AGE	PARITY	NORMAL BIRTHS	BLOOD GROUP	OBSTETRIC HISTORY	MEDICAL HISTORY
S.P. 30 yr	2	2	O.Rh.Pos.	2 normal births following Clomiphene/ infertility	no significant illnesses
S.N. 30 yr	4	2	O.Rh.Neg.	May 1987 twins termination 1986	no significant illnesses
C.B. 28 yr	2	2	O.Rh.D.Pos.	normal	no significant illnesses
J.B. 27 yr	1	1	B.Rh.Pos.	normal	otitis media vulval warts Reiters syndrome
A.C. 20 yr	1	1	no details	normal	no significant illnesses
T.P. 24 yr	3	3	A.Rh.D.Pos.	normal	no significant illnesses
T.F.	3	3	no details	severe hypertension	CIN 111 venereal warts
P.S.	2	2	A.Rh.Pos.	LSCS deliveries	overdose November 1990
B.S.	1	1	A.Rh.D.Pos.	normal	no significant illnesses
C.M.	3	3	O.Rh.D.Pos.	normal	no significant illnesses

NORMAL BIRTHS BETWEEN OCTOBER 1986 - DECEMBER 1990. ALLERGIC PATIENTS.

NAME & AGE	PARITY	NORMAL BIRTHS	BLOOD GROUP	ALLERGIES	OBSTETRIC HISTORY	MEDICAL HISTORY
K.B. 27 yr	2	2	O.Rh.Pos.	hayfever	no problems	tonsillitis
V.S. 32 yr	2	2	O.Rh.D.Pos.	eczema	no problems	ear infections vertigo
Y.B. 28 yr	3	3	O.Rh.Pos.	eczema hayfever	no problems	no significant illnesses
D.W. 27 yr	3	3	O.Rh.D.Pos.	eczema	no problems	no significant illnesses
D.T. 30 yr	2	1	O.Rh.D.Pos.	hayfever	miscarriage 9 weeks	no significant problems
J.G. 25 yr	3	2	AB.Rh.D.Pos.	hayfever	miscarriage 8 weeks	no significant illnesses
W.F. 27 yr	2	2	A.Rh.D.Pos.	eczema dermatitis	normal	hayfever in family
C.W. 35 yr	2	2	B.Rh.D.Pos.	eczema	normal delivery emergency LSCS	bronchitis
J.S. 28 yr	2	2	O.Rh.D.Pos.	dermatitis	no problems	high B.P. otitis externa
D.M. 31 yr	3	2	O.Rh.D.Pos.	allergic rash	conceived on Clomiphene/Infertility Clinic threatened abortion March 1987	acne tonsillitis
A.S. 28 yr	1	1	O.Rh.Neg.	dermatitis impetigo allergy to plants	no problems	haemorrhoids
Y.W. 38 yr	3	3	O.Rh.D.Pos.	allergic dermatitis	no problems	no significant history
A.B. 25 yr	2	2	A.B.Pos.	eczema	normal	no significant history
J.P. 27 yr	3	3	O.R.D.Pos.	eczema, dermatitis	hypertension	no significant history

NORMAL BIRTHS BETWEEN OCTOBER 1986 - DECEMBER 1990. ALLERGIC PATIENTS. (Continued)

NAME & AGE	PARITY	NORMAL BIRTHS	BLOOD GROUP	ALLERGIES	OBSTETRIC HISTORY	MEDICAL HISTORY
R.R. 38 yr	5	2	A.Rh.Pos.	eczema	prurigo normal delivery 1988 second child hydrocephalus and cerebral palsy 3 miscarriages	
W.F. 29 yr	2	2	A.Rh.D.Pos.	eczema	normal	no significant illnesses
L.S. 29 yr	1	1	A.Rh.D.Pos.	allergic rash dermatitis	threatened abortion 12 weeks	no significant illnesses
T.M. 24 yr	3	2	B.Rh.D.Pos.	allergic rash	normal miscarriage August 1990	otitis media psychiatric/overdose
T.P. 38 yr	3	3	O.Rh.D.Pos.		normal	otitis media hand warts psoriasis
L.R. 23 yr	3	3	A.Rh.Pos.	eczema	normal deliveries 1988/90 prem. labour 1987	otitis media psoriasis
S.R. 37 yr	2	2	no details	eczema dermatitis	normal	no significant illnesses
J.T. 38 yr	3	2	no details	eczema	miscarriage 1986 Infertility Clinic 2 normal deliveries	acne
A.F. 25 yr	1	1	B.Rh.D.Pos.	eczema dermatitis	normal	otitis media
A.H. 35 yr	2	2	O.Rh.D.Neg.	allergic rhinitis	normal	no significant illnesses
A.E. 26 yr	1	1	no details	allergic rash	threatened abortion 8 weeks	warts scabies
S.J.	2	2	A.Rh.D.Pos.	allergic rash eczema	normal	tonsillitis
B.S. 35 yr	2	2	A.Rh.Pos.	dermatitis	normal	no significant illnesses

NORMAL BIRTHS BETWEEN OCTOBER 1986 - DECEMBER 1990. ALLERGIC PATIENTS. (Continued)

NAME & AGE	PARITY	NORMAL BIRTHS	BLOOD GROUP	ALLERGIES	OBSTETRIC HISTORY	MEDICAL HISTORY
C.D. 22 yr	4	2	A.Rh.D.Pos.	impetigo dermatitis	2 normal deliveries 1 termination	no significant illnesses cot death at 14 months
S.M. 29 yr	2	2	A.Rh.D.Pos.	dermatitis	normal	no significant illnesses
L.H. 33 yr	2	1	A.Rh.D.Pos.	allergic rashes	normal delivery	tonsillitis mouth ulcers pruritis
A.S. 23 yr	4	4	O.Rh.D.Pos.	allergic rashes	normal deliveries	tonsillitis
J.G.	1	1	A.Rh.D.Pos.	eczema	normal	tonsillitis
K.B. 25 yr	2	1	not known	allergic rashes	termination 1 normal delivery	sinusitis Department of Psychiatry
C.C. 30 yr	3	3	A.Rh.D.Pos.	eczema	normal	no significant illnesses
A.D. 24 yr	1	1	not known	dermatitis	normal	no significant illnesses
M.B. 22 yr	3	2	O.Rh.Pos.	dermatitis eczema warts	normal termination 1990	tonsillitis
J.S. 25 yr	1	1	O.Rh.Pos.	rashes	normal	psoriasis
D.W. 26 yr	3	3	O.Rh.D.Pos.	eczema	normal	no significant illnesses
L.W. 20 yr	1	1	no details	dermatitis	normal	athlete's foot
T.P. 24 yr	1	1	A.Rh.D.Pos.		LSCS delivery	psoriasis
F.C. 29 yr	4	2	A.Rh.D.Pos.	asthma allergy to plants	2 normal deliveries 2 miscarriages	anxiety state hypochondriac

NORMAL BIRTHS BETWEEN OCTOBER 1986 - DECEMBER 1990. ALLERGIC PATIENTS. (Continued)

NAME & AGE	PARITY	NORMAL BIRTHS	BLOOD GROUP	ALLERGIES	OBSTETRIC HISTORY	MEDICAL HISTORY
L.H.	2	1	A.Rh.D.Pos.	asthma	premature delivery - March 1988 baby died after 24 hrs miscarriage 1988	sinusitis
J.T. 31 yr	5	2	O.Rh.D.Pos.	eczema	1968 abortion an embryonic pregnancy carcinoma cervix 1977 miscarriages	anxiety state
T.T. 24 yr	3	3	O.Rh.D.Pos.	asthma/hayfever	normal	no significant illnesses
J.H. 35 yr	3	2	O.Rh.D.Pos.	dermatitis	miscarriage September 1989/12 weeks	scabies
G.G. 24 yr	2	1	O.Rh.D.Pos.	asthma/hayfever	forceps delivery 88 termination	tonsillitis
A.H. 25 yr	1	1	O.Rh.D.Pos.	hayfever	normal	anxiety state
L.G. 26 yr	2	2	O.Rh.Pos.	dermatitis	fainting during pregnancy two normal deliveries	no significant ilnesses chesty
A.A.	5	2	A.Rh.D.Neg.	eczema	2 miscarriages	alopecia
J.D. 25 yr	3	3	A.Rh.Pos.	eczema/dermatitis	normal	mastitis
B.W. 37 yr	3	3	O.Rh.Pos.D.	eczema	normal	hypertension
S.V. 36 yr	3	2	not known	eczema/dermatitis	miscarriage 1986 treated with Clomiphene	no significant illnesses
G.S. 37 yr	2	1	A.Rh.D.Pos.	hayfever allergic rhinitis dermatitis	miscarriage 1990 forceps delivery 1988	sinusitis

NORMAL BIRTHS BETWEEN OCTOBER 1986 - DECEMBER 1990. ALLERGIC PATIENTS. (Continued)

NAME & AGE	PARITY	NORMAL BIRTHS	BLOOD GROUP	ALLERGIES	OBSTETRIC HISTORY	MEDICAL HISTORY
L.R. 25 yr	2	2	A.Rh.Pos.	eczema	normal delivery 1990 premature 34 weeks 1988	psoriasis mastoidectomy
D.T. 30 yr	2	1	A.Rh.D.Pos.	hayfever	normal delivery 1988 miscarriage at 9 weeks Infertility Clinic	
J.M. 24 yr	1	1	not known	hayfever	normal	otitis media
E.A. 34 yr	1	1	A.Rh.Pos.	eczema/dermatitis	normal	otitis media
G.B. 29 yr	1	1	B.Rh.D.Pos.	hayfever	LSCS (prem)	sinusitis
J.F. 27 yr	2	2	Rh.B.Pos.	hayfever/eczema	threatened abortion at 10 weeks	no significant illnesses
N.B. 18 yr	2	1	A.Rh.Pos.	asthma	normal delivery 1988 termination 1989 high B.P.	tonsillitis
J.D. 30 yr	2	2	B.Rh.Pos.	allergic rhinitis	premature labour 32 weeks 1986 normal delivery 1989	peri-anal warts
J.D. 35 yr	3	2	not known	dermatitis	2 normal deliveries miscarriage 1986 hypertension	tonsillitis
A.M. 37 yr	1	1	B.Rh.D.Pos.	eczema	normal	no significant illnesses
B.G. 28 yr	2	2	O.R.D.Pos.	eczema	both LSCS	tonsillitis vulval warts
A.B. 29 yr	4	1	B.Rh.D.Pos.	eczema	3 miscarriages 1 normal	anxiety state
C.S. 36 yr	1	1	A.Rh.D.Pos.	allergic rashes	proteinura and pyelonephritis attended Infertility Clinic termination 1973 forceps delivery	tonsillitis sinusitis renal disease

NORMAL BIRTHS BETWEEN OCTOBER 1986 - DECEMBER 1990. ALLERGIC PATIENTS. (Continued)

NAME & AGE	PARITY	NORMAL BIRTHS	BLOOD GROUP	ALLERGIES	OBSTETRIC HISTORY	MEDICAL HISTORY
J.G. 27 yr	4	2	A.B.Rh.Pos.	hayfever	miscarriage 1986 termination 1986 normal delivery 1988 premature delivery 1987	no significant illnesses
C.P. 38 yr	3	1	O.Rh.D.Pos.	allergic rashes	treated with Clomiphene miscarriage normal delivery 1990	vertigo
J.H. 30 yr	3	2	no details	hayfever	termination normal delivery	no significant illnesses impetigo
J.H. 37 yr	1	1	Rh.O.Neg.	dermatitis	normal delivery	no significant illnesses
D.G. 28 yr	5	2	B.Rh.Pos.	allergic rashes fungal infections	LSCS 1986 3 miscarriages miscarriages 1 normal delivery	mastoidectomy vaginal warts otitis media anxiety state
S.M. 32 yr	3	2	O.Rh.D.Pos.	hayfever asthma	1 miscarriage 2 normal ovarian cystectomy	tonsillitis bronchitis vertigo anxiety state
C.A.	1	1	O.Rh.D.Pos.	eczema	normal	tonsillitis
M.L. 22 yr	4	4	A.Rh.D.Pos.	allergic dermatitis	1 LSCS 3 normal	Psychiatric referral for depression
C.G.	2	1	O.Rh.D.Pos.	asthma	termination 1988	no significant illness
C.G. 38 yr	5	5	A.Rh.Pos.	allergic dermatitis	normal	no significant illness
V.C. 20 yr	1	1	O.Rh.Neg.	eczema	normal	no significant illness
F.M.	2	2	O.Rh.D.Pos.	asthma	normal	no significant illness
J.O. 20 yr	1	1	no details	eczema dermatitis	normal	no significant illness psoriasis