SOME FACTORS INFLUENCING THE PRODUCTION OF IgE IN THE RAT

A DISSERTATION SUBMITTED FOR THE DEGREE OF MASTER OF SCIENCE

IN

THE FACULTY OF SCIENCE

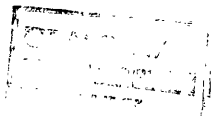
OF

THE UNIVERSITY OF GLASGOW

BY

ELIZABETH P. HALL

JUNE, 1978.



ACKNOWLEDGEMENTS

It gives me pleasure to acknowledge the help and supervision I have received from Dr. Ellen Jarrett, who collaborated in many of the experiments. I acknowledge also the collaboration of David Haig and Bill McDougall in some of the experiments. I would also like to thank Professor W. Mulligan for reading the manuscript, Mrs. E. Webster for typing and Mr. A. Finnie for photography of the figures.

The work was supported by grants from The Medical Research Council and The Wellcome Trust.

TABLE OF CONTENTS

SUMMARY

In Section I various methods employed for the quantification of IgE are discussed and detailed accounts of two techniques used in this laboratory are presented.

Total serum IgE in the rat is estimated using the radioimmunosorbent test (RIST). This 'sandwich' technique employs anti-IgE coupled to paper discs, which binds to IgE present in samples or standards subsequently added. A final incubation with radiolabelled anti-IgE presents the third layer of the 'sandwich', and radioactive counts in each sample are directly proportional to the concentration of IgE present.

Antigen specific rat IgE is measured in vivo by the Passive Cutaneous Anaphylaxis (PCA) test. Twenty-four hours after an intradermal injection of the diluted test serum an intravenous challenge of antigen and Evan's blue dye is given. Attachment of antigen to the mast cell-bound IgE results in release of vasoactive compounds with ensuing increased vascular permeability which results in the appearance of a blue area at the injection site. The titre of the serum under test is taken as the reciprocal of the last dilution giving a reaction of 5 mm in diameter.

In Section II, 1, experiments are described in which primary and secondary IgE antibody responses were elicited in Hooded Lister rats by intradermal injection or oral administration of very small quantities of egg-albumin.

For primary responses the minimum effective dose was found to be 1 μg intradermally and 10 μg orally, both administered with an intraperitoneal injection of B. pertussis adjuvant. In rats immunised with these doses even smaller quantities of antigen evoked booster responses; thus, 1 ng intradermally and 1 μg orally. Large primary doses (> 100 μg) presented by these routes were, on the other hand, found to be inhibitory to the production of booster IgE responses as was reported previously in intraperitoneal immunisation experiments. The results are discussed in relation to IgE production and regulation of antigen absorption through mucosal barriers.

In Section II, 2, experiments are described which show that the adjuvant used in initial immunisation influences the subsequent occurrence of enhanced responses induced by an antigen challenge (booster response) or helminth infection (potentiated response).

Although the level of the primary response was similar following immunisation with egg-albumin (EA) and B. pertussis (Bp), aluminium hydroxide ($Al(OH)_3$) or complete Freund's adjuvant (CFA) as adjuvant, the booster response was inhibited and the potentiated response enhanced when the latter two adjuvants were used. A significant IgE booster response could only be obtained if Bp had been used in initial immunisation.

Concavalin A (Con A) could also act as an adjuvant for the induction of an IgE response to EA. Moreover, a subsequent injection of Con A was found to enhance the EA specific response in a manner analogous to an antigen induced booster response. The Con A enhancing effect occurred only in animals primed with EA together with Bp or Con A and not in animals primed with EA in $A\ell(OH)_3$ or CFA. These results are discussed in relation to the intricacies of IgE production in this model and to more general mechanisms of adjuvant action.

In Section II, 3, the ability of different rat strains to produce IgE antibody responses following large and small doses of antigen and infection with N. brasiliensis has been compared.

Sprague Dawley rats responded poorly to 1 mg and 1 μg EA, but did produce low level secondary responses. Some degree of inhibition was evident in CFHB and Hooded Lister strains following immunisation with 1 mg EA, since secondary responses were higher following immunisation with 1 μg of EA. Following antigen challenge, CFY produced similar but low level secondary responses whether they had been initially immunised with a high or low dose of EA, even although a primary response was not detected after immunisation with 1 μg EA.

Elevation of total serum IgE was less marked following N. brasiliensis infection in the 'poor' IgE responding strains, and the total serum IgE response was in some cases unaccompanied by potentiation of the EA IgE response.

The findings are discussed in relation to the function of regulatory mechanisms known to be operative in the control of IgE responses.

GENERAL INTRODUCTION

IgE is the fifth and the most recently discovered immunoglobulin, forming a distinct antibody class in man and a variety of animals including dog, rat, mouse, rabbit, monkey and guinea-pig. This immunoglobulin mediates Type I anaphylactic or immediate hypersensitivity reactions which, in man, cause allergic disorders such as hay fever and atopic eczema. In these conditions and in helminth infections, IgE is present in elevated levels.

The fundamental event in immediate hypersensitivity is the binding of IgE molecules to target cells which have the appropriate receptors on their surfaces; such target cells have been recognised as circulating basophils and tissue mast cells. The molecule binds by way of the Fc portion leaving 2 Fab portions free to combine with allergen molecules. The mechanism of mediator release requires that adjacent IgE molecules are linked together with antigen forming a bridge. Following attachment of antigen a sequence of biochemical events is triggered off, culminating in release of active compounds such as histamine, serotonin, slow reacting substance of anaphylaxis (SRS-A) and eosinophil chemotactic factor of anaphylaxis (ECF-A). These chemicals exert effects on surrounding tissues including vasodilation and smooth muscle contraction, which are in turn manifested in the clinical symptoms of allergy.

Discovery and Identification of IgE antibodies

Early investigators included Blackley (1893), himself a hay fever sufferer, who showed that the attacks could be provoked

by the deliberate inhalation of grass pollen and also that localised reactions could be elicited by the contact of pollen grains on to abraded areas on his forearms. This was obviously in contrast to the slower development of delayed type reactions which were cell-mediated. Ramirez in 1919 observed there must be a humoral factor involved in allergies, following the accidental transfer of allergic sensitivity by blood transfusion between normal and allergic individuals. This was confirmed by the demonstration of passive transfer of allergic reactions to a normal individual by Prausnitz and Kustner (1921). Prausnitz sensitised sites on his forearms by an intradermal injection of serum from Kustner who was hypersensitive to fish. Twenty four hours later he challenged the same site with an intradermal injection of fish extract and this caused an erythma and weal reaction similar to that evoked directly on Kustner's arm by an intradermal injection of fish extract. This finding was subsequently extended, it being shown that sensitivity to other allergens could be passively transferred in a similar way. The 'P-K' test was used in the diagnosis of hay fever and allergic asthma and remained the only method of assay of reaginic antibodies for 40 years.

The serum constituent was investigated and although it was found to be similar to antibodies produced in response to conventional immunisation, it did not appear to form a precipitate nor fix complement when combined with specific

antigens *in vitro*. Coca and Grove (1925) coined the term 'atopic reagin' to describe the skin sensitising factor in allergic serum since it was not decided at that stage to confer antibody status.

The general features of antibodies remained unknown for 45 years thereafter. Eventually it became clear they could be divided into classes depending on their immunological properties (Franklin, 1964). Each immunoglobulin molecule consisted of a 4-chain polypeptide structure, 2 heavy chains and 2 light chains. IgG, IgM and IgA had been discovered by 1960, and initially it was thought that IgA might be associated with reaginic activity (Hereman and Vaerman, 1962). However, further studies showed that reaginic activity was due to 'impurities' in the IgA rich serum. In 1964 a myeloma carrying a fourth antigenic determinant was found and designated IgD. Ishizaka and co-workers (Ishizaka *et al.*, 1966a) presented convincing evidence against any association between IgA or IgD with reaginic activity. Rabbits were immunised with a reagin rich fraction from a ragweed sensitive serum and the antiserum obtained was absorbed with normal IgG and myeloma proteins of known immunoglobulin classes. The absorbed antiserum did not give a precipitin band with IgG, IgM, IgA or IgD, but a γ_1-precipitin band on immunoelectrophoresis with reagin rich fraction. Moreover the precipitin band specifically combined ^{131}I-ragweed antigen (Ag.E) indicating definite antibody activity and so reagin was designated γE (Ishizaka *et al.*, 1966b).

Correlation between reaginic activity and γE was investigated more fully and allergic serum was fractionated by ion exchange

chromatography, gel filtration, zone elctrophoresis and sucrose density gradient ultracentrifugation. The distribution of reaginic activity, i.e. skin sensitising activity as measured by the P-K reaction, paralleled that of γE antibody as measured by radioimmunodiffusion (Ishizaka et al., 1966c). Subsequent results demonstrated that both reaginic activity and γE antibody were completely precipitated by anti-E, (Ishizaka et al., 1967c) and so γE was identified as the carrier of reaginic activity. The association of reaginic activity and γE was subsequently demonstrated in other reagin-allergen systems (Ishizaka and Ishizaka, 1968c).

IgND

Meanwhile, in Sweden in 1965, the discovery of an atypical myeloma ND also established the existence of a unique immunoglobulin. A 50 year old farmer from Uppsala was admitted to hospital complaining of pain in the lower back and chest. A high red blood cell count, immature plasma cells in bone marrow smears, Bence-Jones protein of the lambda type in urine and a serum component (M) migrating in the fast region on zone electrophoresis were features of the analysis. The myelomatosis lacked all known heavy chains. Antiserum directed against the Fc portion of myeloma ND and antiserum to γE reagin preparation gave a single precipitin line of identical specificity against γE and this line bound radiolabelled ragweed allergen (E), (Bennich et al., 1968). Furthermore, anti-E antiserum precipitated myeloma ND. Specific blocking of the P-K reaction with myeloma ND established a structural relationship between IgND and γE, and a new class of

human serum immunoglobulin designated IgE was proposed by WHO in Laussane in 1968 (Bennich et al., 1968). Subsequently, another case PS from Hanover, N. Eng., was discovered (Ogawa, et.al., 1969), from which IgE myeloma was isolated. Since then a number of other cases have been reported - a total of 8 to date.

Properties of IgE

Physico-chemical

The physico-chemical properties were investigated after the discovery of the myeloma cases, IgND being the most extensively studied. Its sedimentation coefficient by sucrose density gradient ultracentrifugation was found to be 8s and molecular weight 190,000. It travels in the fast γ_1 mobility range in electrophoretic analysis. A glycoprotein with a carbohydrate content of about 12%, it consists of approximately 60 residues of hexose, 20 of N-acetylglucosamine, 6 of N-acetyl-neuramic acid and 6 of fucose (Bennich and Johansson, 1968).

Reduction with 2-mercaptoethanol and alkylation by iodo-acetamide, followed by fractionation through a G100 column reveals 2 types of polypeptide chains; 2 light and 2 heavy. The light chains have a molecular weight of 22,500 and constitute about 22% of the molecule; the heavy chains have a molecular weight of 72,300 all the carbohydrate moeities being associated with them.

Following papain digestion, the IgE molecule is split into Fc and Fab fragments, both considerably larger than IgG fragments (molecular weight 95,000 and 52,000 respectively). Antigenic

analysis of the fragments of reduced IgE reveals 2 antigenic determinants (D) located on the heavy chain, D_E1 and D_E2. The Fc fragment carries both determinants, D_E1 at the amino terminal portion, and D_E2 at the carboxy end (Bennich and Johansson, 1971).

The Fc fragment can block the P-K reaction as effectively as intact IgE (ND) and it appears that the skin binding site is contained in the carboxy end of Fc, although the amino terminal end is also required in some way.

Extensive work has been carried out on the chemical structure of IgE (ND) by von Bahr Lindstrom and Bennich (1974). They employed various chemical reagents and proceeded to elucidate the peptide content of the heavy (E) and light (λ) chains. Using cyanogen bromide to split at methionine residues, they deduced that the heavy chains had 7 methionine residues and the light chains had 1. Proceeding from the carboxy end, fragments were released for subsequent structural analysis enabling the composition of the chains to be described.

Twenty intra-chain and inter-chain disulphide bonds are present in the IgE molecule and on subjection to various reducing agents, these can be broken. Reductions at pH 7 in the absence of an unfolding agent, cleaves 8 of the 20; final reduction takes 20 hours in 0.1 M 2-mercaptoethanol or 4 hours in 10 mM dithiothreitol (DTT). One disulphide bond links the light to the heavy chain and between 4 and 6 link the heavy chains (Bennich and Dorrington, 1973).

The antigenic characteristics of the IgE molecule were studied at different stages of cleavages of the disulphide bonds. After the reduction of 8, the decrease in antigenicity was 85% - as measured by the RIST, (Bennich and Johansson, 1971), although with different techniques employed, the percentage reduction in antigenicity varied. Changes in tissue binding activity occurred after reduction, and at least 2 of the 8 accessible disulphide bonds within the carboxy half of the heavy chain are critical for tissue binding (Stanworth et al.,1968).

IgE is heat labile and its activity is destroyed after heating at 56°C for one hour. Presumably conformational changes are induced and the disulphide bonds in the Fc are disrupted (Stanworth et al., 1968). A loss in specific antigenic determinants was also observed although binding to allergen was not affected (Ishizaka et al., 1967).

Biologic and Immunologic Properties

IgE can agglutinate red cells coated with antigen and on the basis of antigen binding capacity, IgG and IgE have comparable haemagglutinating activities (Ishizaka et al., 1968c). Detection of complement fixation by IgE has not been found by the classical pathway, but non-specifically aggregated IgE molecules do fix complement by the alternative pathway involving factors C3 to C9. The Fc portion had the highest complement fixing activity and it appears that the structures necessary are contained in the amino portion of Fc, although the carboxy end plays an additional part (Ishizaka et al., 1972b). The importance of this late fixation of complement by aggregated IgE is not known.

IgE does not cross the placenta so any IgE present in the foetus has been synthesised endogenously. This protects the offsprings of allergic mothers from becoming passively sensitised in utero.

Persistence and Turnover

The most characteristic feature of reaginic antibodies is their affinity for homologous tissue. They become attached to mast cell receptors via the Fc portion and remain there for a considerable time. The half-life of IgE in human skin is around 13 days (Augustin, 1967) and its presence can be detected for up to 90 days. By contrast, Waldman (1969) found the half-life of IgE myeloma in the circulation to be 2-3 days, as compared to 25 days for IgG. When ^{131}I-myeloma IgE was injected into human and monkey skin, the protein rapidly decreased to about 5% of the original dose within 3-4 days, as did IgG2a. However, a second phase was entered and the half-life of the remaining IgE fell to 8.5-14 days, while IgG continued to disappear rapidly (Ishizaka and Ishizaka, 1971).

This indicates that two different processes are involved in decay of the antibodies. The initial loss phase involves diffusion away into the surrounding fluid and circulation. The second slow phase denotes the firm attachment of the IgE molecules on to the mast cells, which process renders them less susceptible to catabolism.

Origin and Target Cells

Since circulating IgE has such a short half-life it suggests that it is being continuously synthesised in atopic individuals and that production would be greater than expected from normal serum levels. Tada and Ishizaka (1970) investigated the distribution of IgE forming cells in lymphoid tissue by the fluorescent antibody technique. They found tonsils and adenoids (removed after recurrent infection) possessed the most IgE-forming cells. IgE plasma cells are also predominant in bronchial and peritoneal lymph nodes and respiratory and gastrointestinal mucosae. They were scarce in spleen and subcutaneous lymph nodes and none were found in the lung, blood or bone marrow. From these investigations they put forward a possible role of locally synthesised IgE in allergies.

Sensitisation at the cellular level was studied by assaying histamine release from human leukocytes following incubation with anti-IgE. Normal and atopic sera were used and it was concluded that histamine release was a consequence of combination of anti-IgE with IgE on the leukocytes. A series of experiments provided evidence that basophil leukocytes were the target cells for IgE antibodies (Ishizaka et al., 1970), and it was estimated that each cell had in the region of 10,000-40,000 receptors (Ishizaka et al., 1973). Subsequent work with rat mast cells has given an estimate of 300,000-400,000 receptors per cell (Conrad et al., 1975).

IgE Levels in Normal and Disease States

The IgE level in healthy adults has been investigated in many laboratories (Johansson, 1968a; Gleich, Averbeck and Swedlund, 1971), and has been found to be present in very small quantities in normal serum. The mean is in the order of 100-200 ng/ml, but was found to range from 100-800 ng/ml. A research standard supplied by WHO (Rowe et al.,1970) was designated to contain 10,000 units where 1 unit is equivalent to 2 ngs.

IgE content of cord serum has been found to be very low, about 36 ng/ml (Johansson, 1968a), and even this value, on subsequent study, has been found to be an overestimate. This was probably due to a non-specific inhibiting effect of the high protein concentration of cord serum which had to be tested neat. Recent detection by Johansson and colleagues (not published) indicate cord serum has levels between 0 and a few ng/ml depending on the method used. The development of the immunoglobulin levels during childhood have been studied by many authors. Fig. 1 shows the development patterns for IgG, IgA, IgM, IgD and IgE. The slow rise of IgA is in sharp contrast to the fast increase of IgM. The pattern of IgD is not so well traced, but will be easy to investigate by the new highly sensitive methods. In a preliminary study of IgE development in 50 healthy children, 6 weeks to 5 years of age, a rather slow increase with age was found (Johansson, 1968a). The difference in IgG and IgM concentrations which was found in boys and girls could not be shown for IgE (Berg and Johansson, 1969b).

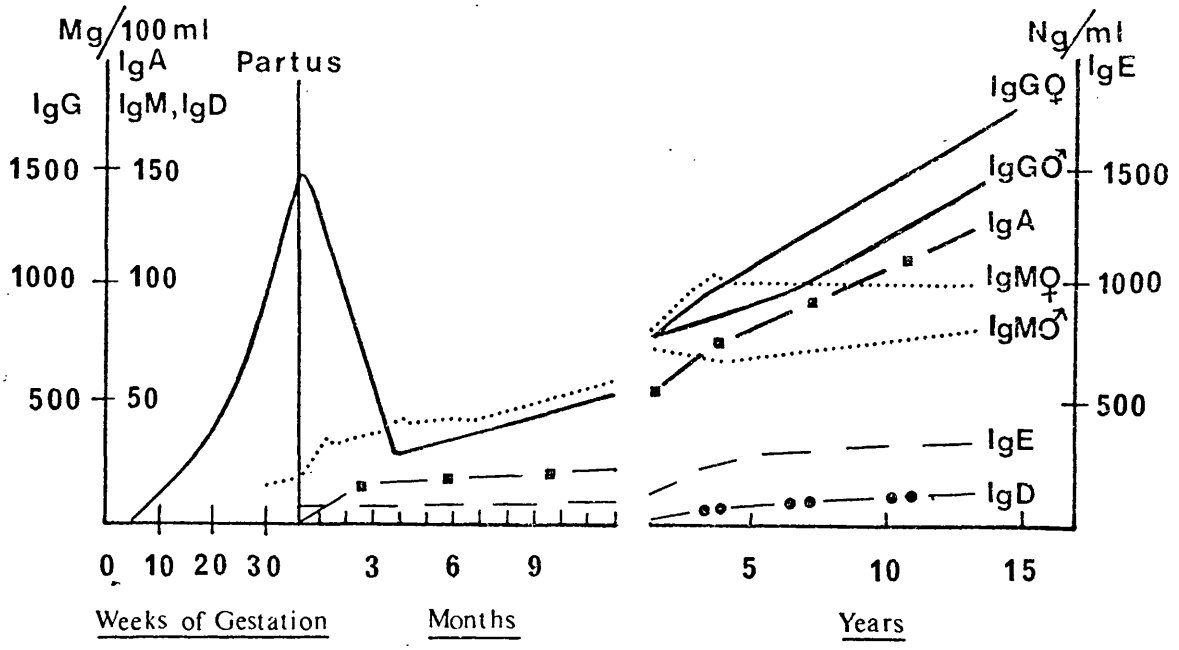


Fig. 1 The development during childhood of the serum concentrations of the five immunoglobulins. Data from Bennich and Johansson (1971) by permission of H. Bennich.

This is of interest, since it is well known that there are twice as many boys as girls suffering from asthma. Adult levels are reached at about 10 years of age and levels exceeding 800 units are considered pathologically elevated.

Atopic Conditions

It has been established that the 10-20% of the population who have allergies, generally tend to produce more IgE than the normal non-allergic individuals. Significantly raised levels of serum IgE were found in 63% of the patients with extrinsic allergic asthma, and 5% of those with intrinsic non-allergic asthma (Johansson, 1967). The former had mean levels of 1,589 ng/ml, six times higher than in the intrinsic group who had levels no higher than normal people. Elevated levels of IgE are also observed in other conditions, e.g. hay fever and atopic eczema (Bennich and Johansson, 1971).

The question arose whether this elevation of IgE was due to a high content of specific reaginic antibodies or the formation of non-specific IgE protein. Studies on atopic eczema have indicated that much of the increased IgE represents specific antibodies which can combine with common antigens as measured by the RAST (Hoffman et al., 1975; Gleich and Jacob, 1975).

The level in an atopic person may fluctuate: the IgE level increases following exposure to allergen and pollen allergic patients had much higher levels of IgE during the pollen season (Berg and Johansson, 1969a). Also, the more allergens a person is hypersensitive to the higher is the serum level of IgE.

Non-Allergic Diseases

Increased production of IgE in helminth infection is now a feature of great interest. It has been found that virtually all helminth infections give rise to elevated levels of IgE.

Studies on Ethiopian children revealed high IgE levels (Jchansson et al., 1968c). Those with stools positive for Ascaris lumbricoides had a mean serum IgE level of 4,400 ng/ml, those that were negative had 860 ng/ml. Similar increased production of IgE has been reported in infestation caused by Toxocara canis (Hogarth-Scott et al., 1969) and the roundworm C. philippinensis (Rosenberg et al., 1971). Other helminth infections inducing raised IgE levels are schistisomiasis and ancylostomiasis (Bennich and Johansson, 1971) and echinococcosis (Huldt et al., 1973). Live worm parasites appear to provide a strong stimulus for the increased biosynthesis of circulating IgE.

Other non-atopic conditions in which IgE levels are raised include Wiskott-Aldrich syndrome (Berglund et al., 1968). Patients with this disease are very susceptible to infections, thrombocytopenia and eczema. The IgE level correlates with the state of eczema; when the latter heals spontaneously, the IgE level decreases.

The Role of Reagin

The beneficial role of IgE is not known. There is a possibility that it may have a protective role in helminth immunity. Clearly, its production in allergic individuals is not beneficial.

In Haemonchus contortus infection of sheep, Stewart (1953) observed that adult worms were frequently expelled (self-cure) from the host after reinfection with larvae. Various physical effects,

e.g. rise in blood histamine and abomasal oedema, which occurred following reinfection were proposed as being the cause of adult worm expulsion.

Nippostrongylus brasiliensis infection of laboratory provides a natural host-parasite relationship where a well-defined "self-cure" occurs around day 10 after infection (Jarrett et al., 1968a). The rats become hypersensitive to N. brasiliensis antigen - this being manifest by the occurrence of skin reactions following intradermal injection or anaphylactic shock following intravenous injection of worm antigen (Urquhart et al., 1965). The main shock organ in the rat is the gut (Sanyal and West, 1958) and in experimental infection a lesion occurs at the site of worm accumulation in the gut which resembles that produced by systemic anaphylaxis (Urquhart et al., 1965; Barth et al., 1966). Also mast cells increase in number significantly in the lamina propria (Miller and Jarrett, 1971) and discharge their contents (Murray, 1972).

The inference that has been drawn from these events is that IgE on the mast cells combines with the worm antigen and leads to mast cell degranulation and subsequent leakage of proteins into the gut lumen. A possible useful purpose of this leak lesion would be to facilitate the passage of circulating or locally produced anti-worm antibody into the lumen where it would come into direct contact with the worms (Urquhart et al., 1965; Barth et al., 1966; Murray, 1972). Worm expulsion has been shown to occur earlier if IgG anti-worm antibodies (which damage the worm enzymes) and

the leak lesion are artificially provided in infected rats before they would normally be formed, and so a definite connection can be shown between the lesion formation and the worm expulsion, at least in the experimental situation.

Some Features of Reagin Formation

Application of the RAST has revealed that the reaginic antibody titre in the serum of hay fever patients fluctuated with the changes of the season (Wide and Porath, 1966). The level was constant for most of the year but towards the end and after the pollen season the concentration increased. Inhalation of a minute dose of ragweed pollen gave rise to a secondary response.

In experimental animals, early studies on artificial immunisation of Wistar rats demonstrated reagin formation, but the response was of low titre, transient and could not be boosted (Mota, 1964, 1967). By contrast, in rats infected with the nematode N. brasiliensis it was shown that specific IgE antibodies reached high levels persisting for many weeks and that the response could rapidly be boosted by reinfection (Ogilvie, 1967). Experiments by Tada and Okumura (1971) using standard immunisation procedures, evoked responses of fairly high titres, but in contrast to those occurring in parasite infections, these responses were transient and could not be boosted. Vaz and Levine (1970) and Levine and Vaz (1970) succeeded in producing a pattern of persistent and boosterable results in certain inbred strains of mice by repeated small doses of antigen in aluminium hydroxide gel. Jarrett and Stewart (1974)

demonstrated a long-lived primary IgE response and this response, provided the rats had been immunised with a small dose of antigen, could be boosted to high levels following challenge.

Such results suggest that in different strains of rats and mice, and following different doses of antigen, different populations of lymphocytes become activated. It has now become clear that there are at least two types of IgE suppressor T cells. There are T cells, the suppressive effect of which is antigen non-specific but selective for IgE responses as a class (Watanabe et al., 1976; Chiorazzi et al., 1977), and there are T cells which suppress IgE antibody responses to specific antigens (Tada, 1976; Ishizaka, 1976).

It is now thought that variation among strains of mice and rats in the capacity to form IgE responses may reflect the presence or absence of one or both of these suppressor mechanisms. Whether these regulatory effects are present in man remains to be determined. If they are, it could be that allergies arise through defective regulatory mechanisms. More detailed knowledge of the immunoregulatory phenomena may provide better insight into treatment for such disorders. More detailed discussion on regulation will be give in Section II 'Control of IgE Production'.

MATERIALS AND METHODS

MATERIALS AND METHODS

Most of the materials and methods are described in the appropriate sections. General animal handling methods were as follows:

Animal Maintenance

The rats were kept in plastic cages with wire tops and floors, suspended above sawdust-containing trays. The cages were stacked in racks and were washed regularly.

The temperature in the animal house was constant at 22°C and a change of air took place 12 to 15 times per hour.

The rats were fed a pelleted diet (Primrose diet 41) and this was used exclusively.

Anaesthetic

Rats were anaesthetised in a large jar containing cotton wool moistened with trichloroethylene.

Collection of Reaginic Sera

Rats were bled from the tail vein by cutting off the tip of the tail and manually 'milking' the tail to deliver the blood into a test tube. Not more than 2 ml was collected except terminally where the heavily anaesthetised rats were bled by cardiac puncture. The blood was allowed to clot in the test tube and serum was collected after centrifugation. The sera obtained from individual rats were stored separately at -20°C.

SECTION I

DETECTION AND MEASUREMENT OF TOTAL SERUM IgE AND ANTIBODY IgE

INTRODUCTION

Tests for the detection and measurement of reaginic antibodies (IgE) are based on one of two principles. The first group of tests makes use of the ability of reagin to sensitise homologous tissue by binding to mast cells and thus to mediate anaphylactic reactions on subsequent encounter with allergen. The second group, more recently developed, makes use of specific antiserum to IgE in *in vitro* radioimmunassays.

1. Detection based on passive sensitisation *in vivo*

From the pioneering studies of Prausnitz and Kustner (1921) emerged the classical test for the detection of allergen specific antibodies. Prausnitz demonstrated that sites on his forearms could be passively sensitised by an intradermal injection of serum from Kustner who was allergic to fish. Subsequent injection of the same sites with fish extract evoked an erythema and weal reaction. Passive transfer of immediate hypersensitivity to other allergens could be achieved in a similar way and this method of detecting reaginic antibodies became known as the Prausnitz and Kustner (P-K) test and was routinely used for many years. Briefly, as described more recently by Stanworth and Kuhns (1965), the method is as follows: 0.1 ml intradermal injections of dilutions of test serum are given into a number of normal skin sites, e.g. back or forearms; a latent period of 24-72 hours is allowed to elapse and then the challenge with antigen is given by pricking in an aliquot of concentrated allergen extract to the same site;

30 minutes later the area of weal evoked is measured. By taking the logarithim of the serum dilution and plotting it against the mean weal area, a linear relationship is obtained. This was a sensitive test, however, with the recognition of the danger of transferring serum hepatitis and the development of *in vitro* methods, the P-K test is now rarely used.

An essentially similar test - the Passive Cutaneous Anaphylaxis (PCA) test (Ovary, 1965) - is the one most commonly employed to titrate reaginic antibodies in experimental animals. Serum is injected intradermally into the shaven skin of normal recipients and 48-72 hours later the appropriate antigen is injected intravenously, together with a dye. If the serum contained IgE antibodies, a local anaphylactic reaction occurs and is delineated by extravasation of the dye at the sites of increased capillary permeability. The PCA titre of a serum is the reciprocal of the greatest dilution giving a reaction size larger than 5 mm. (This test will be described in greater detail below).

2. Detections based on passive sensitisation *in vitro*

A variety of tissues may be sensitised *in vitro*. These include contractile tissues, e.g. guinea-pig ileum or uterus (Dale, 1913), chopped lung fragments from human or monkey (Goodfriend *et al.*, 1966) and peripheral leukocytes (Stanworth, 1973). In these procedures serum is incubated with tissue or cell preparations. After suitable washings, allergen is added

and histamine liberation is estimated by biologic assay, e.g. contraction of guinea-pig ileum in Schulz-Dale bath or by spectrofluorometric methods. These procedures however, tend to have certain disadvantages such as non-specific histamine release which reduce their reproducibility.

A technique based on the degranulation of rat mast cells by human IgE has been described by Korotzer, Haddad and Lopapa (1971) and Perelmutter et al., (1973). Equal volumes of allergic serum, allergen, normal rat serum and rat peritoneal mast cells are incubated at 37°C for 3 minutes. After washing, the preparation is spun and the resultant pellet stained and microscopically examined for degranulation. Results are expressed as percent of morphologically altered cells. Alternatively, histamine liberation may be measured and the results expressed in terms of the minimum concentration of allergen eliciting a response.

3. Tests using specific antiserum to IgE

The discovery and subsequent purification of the human IgE myeloma (ND), (Johansson and Bennich, 1967) led to the production of antiserum specific for IgE which made this second category of tests possible. They may be used to detect total IgE in serum or other body fluids, or allergen specific IgE.

a) Detection of total serum IgE

The radioimmunosorbent technique (RIST) was introduced by Wide and Porath (1966). It enables the detection of immunoglobulins at concentrations of 0.01 μg/ml or 1/1000 of that

detected by the single radial diffusion method (Mancini et al., 1965). This assay was modified for IgE by Johansson, Bennich and Wide (1968), incorporating anti-myeloma ND as solid phase antibodies. Antibodies specific to Fc fragment of myeloma ND are coupled covalently to an insoluble dextran derivative. The serum to be tested is applied to this and also an aliquot of radiolabelled myeloma ND:

Sephadex particle + Anti-IgE + Sample or standard containing IgE + ^{125}I myeloma ND

The concentration in a sample or standard is measured by its capacity to inhibit binding of ^{125}I ND to the anti-ND on the sephadex particle and the gamma count varies inversely with the concentration of IgE in the sample.

This test is commercially available as the 'Phadebas' IgE kit by Pharmacia and is frequently used in clinical application.

With the introduction of solid phase antibodies, came the development of systems other than those based on competition and 'sandwich' techniques were devised using such solid phase reagents as fine particles of Sephadex (Johansson et al., 1968), insides of test tubes (Salmon et al., 1969), cellulose carbonate (McLaughlin et al., 1971) and filter paper discs (Ceska and Lundkvist, 1972). In the 'sandwich' method, anti-IgE or allergen is coupled to the solid phase, e.g. a paper disc, and

the sample containing IgE is presented to it. IgE present in the sample binds to the anti-IgE on the disc. Highly purified anti-IgE is radiolabelled and when added, forms a third layer in the system. The gamma count then varies directly with the concentration of IgE present in the sample or standard. This test is used in this laboratory for the measurement of IgE in experimental animals, and will be described in detail later.

Diffusion in gel reactions have been used since 1905, and are relatively simple to perform. Their use in modern immunochemistry began with the work of Oudin, who introduced the technique of antigen diffusing into agar which contained antibodies (Oudin, 1946). This single linear diffusion was extended by Mancini, Carbonara and Heremans (1965) to single radial diffusion. They incorporated antiserum into the agar layer and cut wells into which the antigen was placed, subsequently diffusing radially into the agar to form a ring of precipitate at the point of equivalence. A relationship is described between the diameter of the precipitin ring and the concentration of antigen in the well. A calibration curve may be constructed using standard concentrations of antigen so that unknown concentrations in the samples can be read off.

Rowe (1969) described a modified version of Mancini's single diffusion which increased the sensitivity of the test

and so the lower levels of IgE in normal serum could be detected. This technique was subsequently used to assay IgE in the rat (Jarrett and Bazin, 1974). Briefly, the procedure is as follows; goat anti-rat IgE is incorporated into agar using dilutions of 1:2000, the mixture is poured on to a glass plate. Wells are punched in the agar and dilutions of serum samples and test samples are dispensed into the holes. The plate is incubated at 37^{o}C for 48 hours, after which it is washed in buffer. It is subsequently incubated with radiolabelled rabbit anti-goat IgG and after further washings is dried and placed in contact with a photographic film for 2-3 days. When developed, the precipitin rings are visible on the film. The radiolabelled stage is essential as the precipitin rings are otherwise invisible due to the small quantity of IgE in normal serum. The ring diameters are proportional to the concentration of IgE in the samples.

With the development of the rat PRIST, the Rowe-Mancini method has largely been superseded, but it may still be useful in laboratories where a gamma counter or the purified reagents necessary for the PRIST are unavailable.

A number of other radioimmunoassays for the measurement of serum IgE have been developed. A sensitive double antibody radioimmunoassay has been described by Gleich, Averbeck

and Swedlund (1971), who studied sera from normal individuals and those with hay fever and asthma. The technique used was similar to that employed by Morgan and Lazerow (1963). Rabbit anti-IgE (0.1 ml) and an aliquot of radiolabelled ^{131}I IgE (0.1 ml containing approximately 0.1 ng of protein) is added to standard or test samples (0.1 ml) in tubes. The system is incubated for 24-96 hours at $4^{o}C$, after which goat anti-rabbit IgG (0.2 ml) is added, and lastly some normal rabbit serum. Following a further incubation period, the tubes are spun at 3,500 revs for 30 minutes, the supernatant decanted and the precipitate counted. Counts in tubes containing IgE standard or test samples are divided by the total radioactive count and are expressed as a percentage. Standard samples are run and a curve constructed to include a range of 1 ng - 14 ng of IgE. The percentage of IgE in the sample is read off and the concentration of IgE obtained.

A comparison of three radioimmunoassays for the measurement of total serum IgE was conducted by Polmar, Waldman and Terry (1973); the double antibody technique (Gleich et al., 1971), sepharose-bound antibody (Johansson et al., 1968) and bromoacetylcellulose (BAC)-bound antibody (Mann et al., 1969). They detected IgE in patients with schistosomiasis having very high levels, as well as cases

of ataxia telangiectasia - an immunodeficient condition in which IgE levels are very low. All three assays gave similar readings in the high cases, but distinctive differences were apparent with the lower levels. The double antibody technique confirmed its sensitivity rating and gave the low readings expected for immunodeficient sera, the BAC and, to a lesser extent, the sepharose technique, gave levels which would otherwise be considered normal rather than actually immunodeficient.

b) Detection and measurement of allergen specific IgE using anti-IgE

A sandwich technique similar to the RIST, the radio-allergosorbent test (RAST), is used as an *in vitro* radioimmuno-assay, for the detection and measurement of allergen specific IgE. This test, introduced by Wide and colleagues (Wide *et al.*, 1967), has been used in the diagnosis of allergies, for which purpose a commercial kit has been recently developed by Pharmacia. The RAST is based on the anti-globulin principle of Coombs and colleagues (1953). Relevant antigen is coupled to paper discs (Ceska and Lundkvist, 1972) which are then incubated with test serum. Excess serum is washed away and the discs are further incubated with radiolabelled anti-IgE. The counts on each disc are then directly proportional to the IgE antibody content present in the test serum. A variety of allergen extracts are available commercially, e.g. ragweed and

timothy grass, and may be coupled to the paper discs. However, the potency or allergenic activity of such extracts may vary between different companies, and until standards are available, results from different laboratories cannot be compared.

The Red Cell Linked Antigen-Antiglobulin Technique (RCLAAR), an immunoglobulin specific technique based on agglutination, was introducted by Coombs et al., (1953). Allergen is coupled by photo-oxidation to rabbit antihuman red cell antibodies. Such rabbit antibodies will no longer agglutinate human red cells, but instead when mixed with red cells will combine with them and link the coupled antigen to the cells. On incubation with test sera any allergen specific IgE will be adsorbed on to the cell surface. Specific antiserum to IgE is then added to cause agglutination of the sensitised cell suspension; this is subsequently read by the pattern of sedimentation of the cells. Good correlation has been found between the Red Cell Linked Antigen-Antiglobulin Reaction measuring reagin and the results of provocation testing in atopic patients (Coombs et al., 1968).

Some of the techniques described have been in common use in other laboratories, but others, particularly the RIST as applied to rat IgE detection, have been developed here with the collaboration of Dr. H. Bennich and his colleague Dr. T. Karlsson of Aarhus University, Denmark and Uppsala University, Sweden, and Dr. H. Bazin of the University of Louvain, Brussels. There follows in this section a detailed description of this technique which is extensively used in this laboratory.

DETECTION OF IgE IN THE RAT

The use of the rat as a model for IgE studies gained greatly in potential as a result of the discovery of immunocytomas occurring in, and peculiar to, the Lou/Wistar strain of rat (Bazin et al., 1973). These tumours originate in the ileocaecal lymph node and are transplantable in histocompatible animals, retaining their capacity for immunoglobulin secretion during successive transplantations. The monoclonal immunoglobulins synthesised by these tumours have been purified and used as antigens for the production of antisera. In the test described here, specific antiserum to the IgE myelomas IR2 or IR162 is used in the detection of total circulating IgE by the 'sandwich' radioimmunoassay designed by Ceska and Lundkvist (1972).

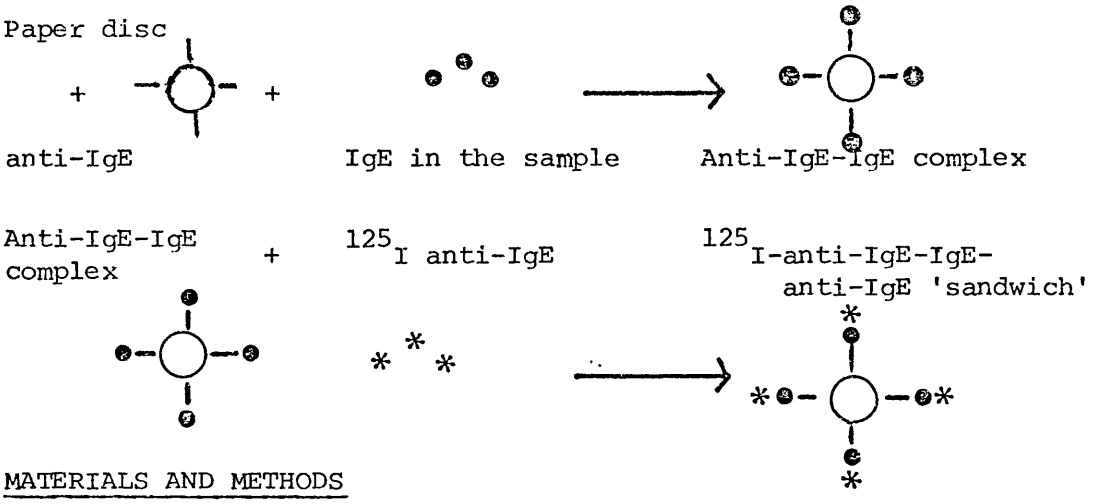
Detection of allergen specific IgE by Passive Cutaneous Anaphylaxis is also described.

1. Detection and Measurement of Total IgE in the Rat by the Radioimmunosorbent Technique (RIST)

The principle of the test

The test involves a three-layered system with a solid phase immunosorbent taking the form of anti-IgE antibodies coupled to paper discs. These are dispensed into small plastic tubes and incubated with standard or serum samples containing IgE. Any IgE present binds to the anti-IgE on the disc; unbound protein is washed away. A second incubation presents radiolabelled anti-IgE antibodies to the system which then binds to the anti-IgE-IgE complex, forming the third layer of the 'sandwich'. Unbound protein is washed out and each tube is counted on the gamma counter.

The numbers of counts varies directly with the IgE present in the sample.



MATERIALS AND METHODS

Paper discs

Filter paper discs with a low ash content were used. We have used Whatman No. 1 with success. Discs 6 mm in diameter were initially punched with an office paper punching machine, but subsequently were purchased as a special order from Whatman Ltd. 5 gms were soaked in distilled water with several changes to remove suspended particles.

Activation of discs

This process was carried out in a fume cupboard.

The discs were activated using Cyanogen bromide according to the method of Ceska and Lundkvist (1972) with some modifications. Cyanogen bromide crystals were weighed out in a stoppered container and added to 100 ml of distilled water to form a 3-5% solution. The crystals were completely

dissolved using a magnetic stirrer. The discs were then emptied into the cyanogen bromide mixture and 1M NaOH solution added dropwise to maintain the pH between 10-11 until about 20 ml of the base had been consumed. The solution was aspirated and the discs washed several times in ice-cold 0.005 M $NaHCO_3$ buffer to a total of 5 litres, followed by similar washings in 2 litres of ice-cold distilled water.

The discs could then be coupled immediately or could be dried by successive washings in 25%, 50%, 75% and 100% acetone and stored in an air-tight container at $4^{o}C$ for several months.

Antiserum

The antiserum was raised in rabbits by immunising them with 0.5 mg of purified myeloma IR162, injected intramuscularly in Freund's complete adjuvant. A booster dose of 0.5 mg was given two weeks later in Freund's incomplete adjuvant, and the rabbits bled two weeks after this. Goats were also used for this purpose.

The rabbit anti-rat IgE thus obtained was rendered epsilon (E) chain specific by absorption with gluteraldehyde cross linked (Avrameas and Ternynck 1969), normal Sprague Dawley rat serum, which has a very low content of IgE. The antibodies were then purified by adsorption to an immunosorbent

column of purified myeloma IR2 coupled to Sepharose 4B, with subsequent elution. IgE of a different myeloma was used (IR2) to cause elimination of IR162 idiotype specific antibodies.

Coupling procedure

As the first layer of the 'sandwich', either purified antibodies or a gammaglobulin fraction of an anti-IgE serum was coupled to the discs. Where the latter was used, it was assumed that 1% of the protein was specific for IgE. 100-200 ng of pure anti-IgE antibodies or anti-IgE globulin containing the equivalent amount of antibodies, were incubated with activated discs (about 200/universal in 20 ml 0.1 M $NaHCO_3$ pH9) for 4 hours at $4^{o}C$ on a vertical rotator. After use, the coupling solution could be frozen and re-used several times by adding 20-30 ng of fresh IgE antibodies. After incubation, the solution was decanted and kept, and unbound protein was washed away from the discs by three washes in 0.5 M $NaHCO_3$ pH9. Remaining active sites on the discs were blocked by incubation with 0.1 M TRIS buffer pH8, overnight in the refrigerator or 3 x 1 hour washes at room temperature. After this the discs were washed 3 times alternately in 0.5 M $NaHCO_3$ buffer and acetate buffer pH4, each wash lasting for 30 minutes to ensure that any non-specifically bound protein was washed away. Finally, following three washes in incubation buffer, the discs were

ready for use. They could also be stored in incubation buffer at 4°C for up to 3 months. Before use, they were washed twice in incubation buffer to remove any anti-IgE which had become unbound.

Incubation buffer

This consisted of 0.05 M phosphate buffer pH8 made up as follows: 7.3 gm NaCl, 0.75 gm B.S.A. and 2.3 ml Tween in 2.5 litres. A stock 0.5 M PO_4 buffer was usually made up and diluted 1:10 when required. This could be stored at 4°C with the addition of a few drops of Sodium azide (10%).

Standard IgE

Standards of purified myeloma IR162 or IR2 were prepared by Dr. H. Bennich. The standards were diluted in incubation buffer to concentrations ranging from 1 ng - 20 ng/50 µl, were stored at -20°C and used repeatedly.

Labelling of anti-IgE antibodies

The procedure followed was the chloramine T method of Greenwood, Hunter and Glover (1963).

25 µl of 0.5 M PO_4 buffer pH7.5 was added to 1 mCi of ^{125}I in a bijou bottle. 25 µg of purified anti-IgE in 0.5 M PO_4 buffer was added to the above, followed by 25 µl of a 1 mg/ml solution of chloramine T. The latter acts as

an oxidising agent and starts the binding of ^{125}I to the protein. The mixture was rotated continuously for 30-90 seconds. At the end of this time 25 µl of a 2 mg/ml solution of Sodium metabisulphite was added to stop the binding reaction.

Some crystals of Potassium iodide were added to make the mixture 'heavy', i.e. excess iodine to facilitate better separation. The labelled protein was then placed on a G200 column (50 x 1 cm) equilibrated with 0.05 M PO_4 + 3% BSA. The eluate was collected in 0.5 ml fractions. Two peaks were observed when the tubes were counted on the gamma counter, the first was labelled protein and the second was unbound ^{125}I (see Fig. 2).

The few fractions forming the tip of the first peak were taken and pooled for use in the test. Fractions from the leading and declining portions of the first peak were discarded, as the former contain aggregates and the latter breakdown products and some free ^{125}I. An aliquot (100 µl) of the pool was counted at 1:100 dilution for 1 minute to estimate the total count. The pooled eluate was then stored at -20°C and kept for not longer than 4-5 weeks. Subsequently, before each test, radioactivity per 100 µl aliquot was adjusted to 20,000-30,000 counts per minute using incubation buffer as diluent.

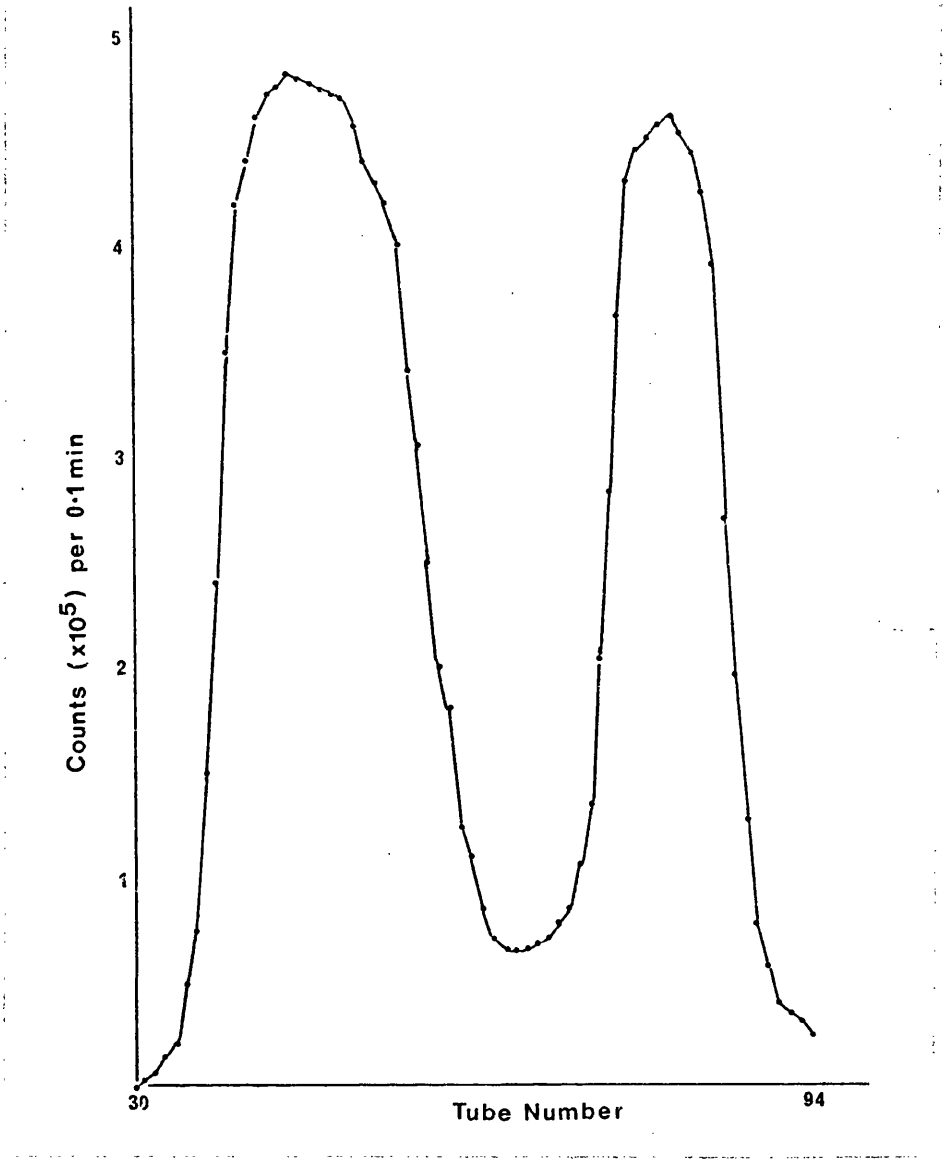


Fig. 2 Separation of ^{125}I-labelled anti-IgE antibodies from unbound ^{125}I. Fractions collected from a G200 column eluate. Six fractions from the top of the first peak representing labelled protein were pooled and used in the RIST.

Test procedure

Each antibody coated disc was dispensed into a Luckham plastic disposable tube (LP3) using a pasteur pipette attached to a vacuum pump. The tubes were numbered and arranged in racks.

Automatic pipettes and disposable plastic tips were used throughout the test procedure. 50 µl of incubation buffer was pipetted into each tube. 50 µl of the standard at the known concentrations and the same of samples were added. Samples were diluted according to the expected values of IgE present; high IgE sera, i.e. from parasite-infected animals, were diluted at 1:1000 or 1:2000, those with expected low levels were diluted 1:10. At these dilutions the IgE content usually fell within the range of the standard curve.

Each assay was done in duplicate and a buffer control without standard or sample was included at the beginning. The tubes were incubated for 3-4 hours at room temperature with agitation, at the end of which unbound protein was washed away with 3 washes in incubation buffer. 100 µl of ^{125}I-anti-IgE was dispensed into each tube. An additional tube was added to measure the total count of the radioactive aliquot, previously set to approximately 20,000 counts per minute. Following overnight incubation, the tubes were washed four times in incubation buffer and then counted on the gamma counter for a minute.

Results and Discussion

The results of a rat RIST are shown in Table 1 and Figure 3.

The mean was calculated for each set of tubes and from the standards a curve was then constructed on lin-log graph paper (3 cycles log x ½ mm 1 cm). The sample counts could then be read off and their total IgE content computed by multiplying by 20 and by the dilution factor.

This direct radioimmunoassay is sensitive and easy to perform, the best results being obtained when unknown sample counts fall on the straight portion of the standard curve (Fig. 3). Levels as low as 50 ng/ml could be detected and sample with very high concentrations were diluted appropriately until they fell within the straight portion of the standard curve. A normal rat serum sample was run in every test to check reproducibility.

TABLE 1. Results of a rat RIST performed on sera from an immunosuppression experiment.

Tube No.	Contents	Dilution	Mean Counts per minute	IgE concentration
1	Buffer control	-	327	-
3	Standard IgE	-	363	0.05 ng/50 µl
5	"	-	401	0.1 "
7	"	-	453	0.2 "
9	"	-	687	0.5 "
11	"	-	947	1.0 "
13	"	-	1523	2.0 "
15	"	-	4475	5.0 "
17	"	-	6940	10.0 "
19	"	-	8966	20.0 "
21	"	-	10000	50.0 "
23	Normal rat serum	1:10	5218	1.30 µg/ml
25	Before immunisation	1. "	2664	0.66 "
27	"	2. "	818	0.15 "
29	"	3. "	743	0.12 "
31	"	4. "	686	0.11 "
33	"	5. "	1140	0.27 "
35	"	6. "	1276	0.31 "
37	"	7. "	942	0.20 "
39	D.14 after	1. "	1377	0.35 "
41	immunisation	2. "	1163	0.28 "
43	"	3. 1:50	682	0.23 "
45	"	4. 1:10	883	0.18 "
47	"	5. "	1250	0.30 "
49	"	6. "	1277	0.31 "
51	Controls	1. "	1065	0.24 "
53	"	2. "	3661	0.84 "
55	"	3. "	5568	1.40 "
57	"	4. "	3900	0.90 "
59	"	5. "	6993	2.04 "

Cont'd...

TABLE 1 (Cont'd.)

Tube No.	Contents		Dilution	Mean Counts per minute	IgE concentration
61	D.12 after infection	1.	1:100	9539	59.00 µg/ml
63	"	2.	"	8214	30.40 "
65	"	3.	"	9371	52.00 "
67	"	4.	"	8887	39.00 "
69	"	5.	"	7636	25.50 "
71	Controls	1.	1:1000	7696	260.00 "
73	"	2.	"	8103	296.00 "
75	"	3.	"	7103	215.00 "
77	"	4.	"	7113	217.00 "
79	"	5.	"	9064	425.00 "
81	Total count	-	-	20770	-

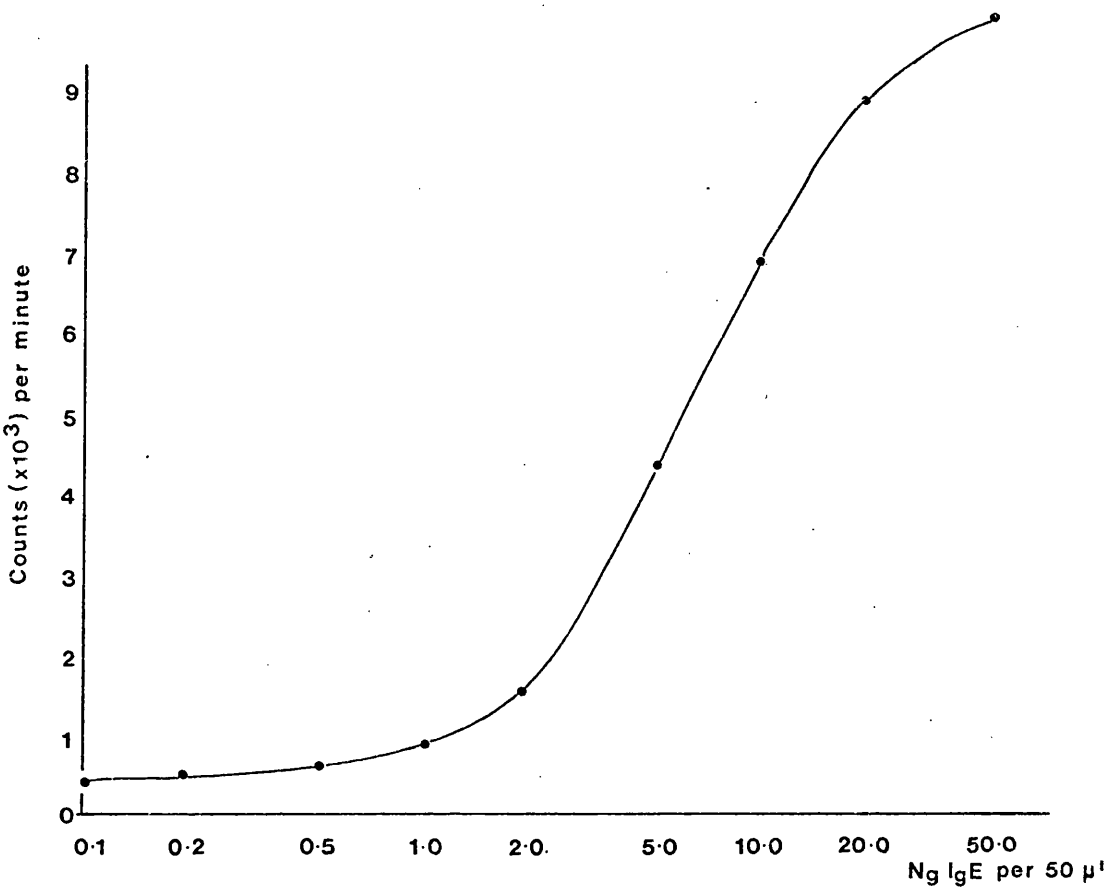


Fig. 3 A typical standard curve obtained by plotting radioactive counts against the concentration of IgE in standards. See p.40

2. Detection and Measurement of Specific Antibodies in the Rat by PCA

The method most commonly used for assessment of specific IgE in the rat is Passive Cutaneous Anaphylaxis (PCA). This *in vivo* assay was developed by Ovary (1952) and illustrates the observations originally made by Prausnitz and Kustner (1921) that skin sensitising ability of reagins could be transferred to normal individuals. The receptors on mast cells/basophils are specific for IgE of the homologous species and so PCA reactions for the detection of IgE antibodies must be performed on the same species or occasionally closely related species, e.g. human reagins can be detected by PCA on primates and mouse IgE by PCA in the rat.

The injected IgE antibody in the test serum sensitises the tissues by attaching to the mast cells in the skin. It remains for a considerable period of time - at least 3 weeks (Brocklehurst, 1973). On intravenous challenge with the appropriate antigen and a dye (such as Evan's blue which attaches to the plasma albumin), the antigen attaches to the cell bound antibody and this union triggers off a series of biochemical events, culminating in the release of vasoactive compounds from the mast cell. These substances cause an increase in vascular permeability which results in leakage of the dye at the site of the injection. Elsewhere the dye is retained in the blood. The titre of the serum under test is taken as the reciprocal of the last dilution giving a reaction of 5 mm in diameter.

The PCA is not inhibited by antigen excess, therefore it is advisable to use large excesses of antigen, otherwise the resultant PCA titre might not truly be a function of the amount of antibody present (Ovary, 1964).

The number of mast cell receptors available for passive sensitisation will depend not only on the number of mast cells actually present, but also on the total serum IgE level of the recipient animal. It has been shown that helminth infected rats become almost completely refractory to passive sensitisation for the production of PCA or systemic anaphylaxis (Jarrett et al., 1971). A comparable effect has been reported in human patients with IgE myelomas (Ogawa et al., 1971) and subsequently in parasite infected human patients (Bazaral et al., 1973). The mechanism of this phenomenon is that in animals producing very high levels of serum IgE, the mast cell receptors for this immunoglobulin become saturated and hence unavailable for passive sensitisation.

Care should be taken when administering PCA injections to ensure that the serum is not injected subcutaneously, as this gives diffuse reactions if any. Proper intradermal injections result in superficial blebs which remain visible for at least 30 minutes.

IgG2a antibodies also elicit PCA reactions, but do not remain fixed to the skin sites for long periods. A 4 hour PCA reaction for the detection of these antibodies is as follows: an aliquot of test serum is heat inactivated at 56°C for 1-3 hours; this inactivates IgE but not IgG2a. Four normal rats are used and each of the recipients are given two sets of injections intradermally; one of heat inactivated serum and one of normal serum. One pair of rats receive the intravenous challenge 4 hours later for detection of IgG2a antibodies, the other pair 48 hours later to check for the complete inactivation of IgE antibodies. Four hour PCA reactions with adequately heat inactivated serum are mediated by IgG2a antibodies. If the 48 hour PCA is still positive with the heat inactivated serum, it means that IgE antibodies have been incompletely inactivated and therefore require further inactivation followed by a retest.

Method

Sera were diluted in saline by serial, doubling dilutions. 0.1 ml aliquots were injected intradermally with 26 gauge x 3/8" needles, and 1 ml plastic disposable syringes. Each sample was done in duplicate or triplicate in different rats to control for individual variation.

The recipients were anaesthetised with trichloroethylene and their backs shaved with small animal clippers. Usually ten intradermal injections could be done on one rat, three down either side and four down the mid-line. The rats were then left for a period of 24-72 hours to allow for fixation of IgE to the tissues and for the diffusion away of any IgG reaginic antibody.

Following this, the rats were challenged with antigen (e.g. 2.5 mg egg-albumin or 0.5 ml whole worm extract), together with 0.5 ml Evan's blue dye; both administered intravenously using a 25 gauge 1" needle attached to a 1 ml syringe. Intravenous challenge was by injection into the tail vein which had been dilated by brief immersion of the tail into warm water. The reactions were read after 20 minutes and scored as positive or negative. It was sometimes necessary to study the reactions on the inside surface of the skin where their borders were often more clearly delineated.

Some sera with particularly high titres of IgE, e.g. parasite potentiated responses, if done at too low a dilution gave very large reactions which could cover a whole back and mask the results of other injections. To avoid this, it was important to dilute high titre sera appropriately, e.g. starting at a dilution of 1/256. Sera with unknown content of IgE antibody were usually started at a lower dilution.

At the intravenous challenge, the rats must not come in contact with the trichloroethylene in the anaesthetic jar, as this is an irritant to the skin causing non-specific release of the vasoactive compounds.

SECTION II

IgE PRODUCTION IN THE RAT

GENERAL INTRODUCTION

Mota (1964) found that two distinct antibodies were evoked in the rat following immunisation with antigen and adjuvant. In addition to the usual precipitating antibody a small quantity of 'mast cell sensitising' antibody was detected in the early post-immunisation period. This antibody sensitised the animal for cutaneous and systemic anaphylaxis. It was found to sensitise mast cells *in vivo* and *in vitro* causing damage to the cells on further encounter with specific antigen and was therefore called 'mast cell sensitising' antibody.

Properties

Rat 'mast cell sensitising' antibody was discovered to be quite independent of the precipitating antibody. It was found to be thermolabile being inactivated at 56°C. On zone electrophoresis the anaphylactic antibody migrated as a β-globulin ahead of the main γ_2 antibody; ultracentrifugation demonstrated a sedimentation coefficient intermediate between 7s and 9s; immunoelectrophoresis however did not reveal any precipitin line that could be ascribed to anaphylactic antibody (Binaghi *et al.*, 1964). Quantitation was by PCA and the characteristics observed exhibited a similarity to human reagins as measured by the P-K test, i.e. long incubation required for maximal sensitisation and persistence of antibody at the skin site for many days (Binaghi and Benaceraff, 1964).

An antiserum was prepared to rat homocytotropic antibody which failed to recognise rat IgGa, IgGb, IgA, IgM or γ_1 but precipitated only with an electrophoretically fast immunoglobulin with antigen binding capacity. This antiserum used as an immunosorbent could remove the PCA activity attributed to rat homocytotropic antibody from rat antiserum. This unique class of rat homocytotropic antibody was designated rat IgE (Stechschulte et al., 1970).

The rat has latterly been used by several workers as a model for the study of production of IgE despite some obvious disadvantages, namely the apparent requirement for large doses of antigen, the transient and quantitatively poor nature of the IgE response and the inability to evoke significant booster responses. These features originally described by Mota (1964) and Binaghi and Benaceraff (1964) were the general experience of subsequent workers.

Regulation and Production of IgE in the Rat

Tada and Okumura (1971) have studied the control of IgE production in Wistar rats, using as standard immunisation technique, a footpad injection of Ascaris suum - DNP and an intraperitoneal injection of Bordetella pertussis (killed) organisms as adjuvant, followed 5 days later by a booster of antigen alone. Specific IgE antibody to the hapten was found to disappear rapidly (35-40 days) and could not be boosted by a subsequent dose of antigen. A series of experiments were

conducted by Tada and colleagues, in which enhancement or inhibition of reagin formation in the rats was effected by various immunologic manipulations. It was found that passively administered antigen specific IgG antibody suppressed the IgE response (Tada and Okumura, 1971) as had also been found in rabbits (Strannegard and Belin, 1970) and mice (Ishizaka and Okudaira, 1972). It was suggested that a similar mechanism could operate naturally. Enhancement of IgE antibody production was reported to occur after splenectomy and/or thymectomy (in adults) after a lethal dose of X-irradiation, and following administration of a variety of immunosuppressive drugs such as cyclophosphamide, cortisone or actinomycin D, or a small dose of anti-lymphocyte serum (reviewed by Tada 1975). The broad interpretation of these enhancing effects was that as a result of these various treatments there occurred a selective depletion of certain populations of lymphoid cells, probably of thymic origin, which regulated reagin production. Confirmation of this was obtained when T cells primed with carrier, passively transferred to hapten-carrier primed recipients, were observed to terminate the IgE response (Tada and Okumura, 1971). Similarly, it was found that cell free extracts from thymocytes and spleen cells primed with hapten-carrier, or just carrier, showed suppressive activity (Okumura and Tada, 1974).

Later a helper cell was also described when the same crude extract from thymocytes restored ability to produce

reagin in neonatally thymectomised rats (Okumura and Tada, 1974) and it was suggested that the inductive and suppressive effect was associated with the same molecule. However, further studies showed that they were different molecules and that the helper component had a molecular weight comparable to 7s IgG and contained Fab and μ determinants. Unlike the suppressor activity it could not be absorbed by ALS (anti-lymphocyte serum) and its properties were similar to IgT, the monomeric form of IgM from T cells in mice.

The conclusion drawn from these observations was that the control of reagin production was exerted by a combination of the effects of the two populations of antigen specific T cells as well as (possibly only under some conditions) the antibody mediated "feedback" mechanism.

Other investigations in IgE production suggested there were strain differences in ability to mount a good IgE response. For example, when subjected to similar immunisation procedures SJL inbred mice and ACI and Wistar rats were poor responders, whereas SW55 inbred mice and Brown Norway and Hooded Lister rats were good responders (Levine and Vaz, 1970; Murphey et al., 1974; Jarrett and Stewart, 1974).

The recent discovery of non-antigen specific T suppressor lymphocytes has been reported. Watanabe et al.,(1976) and Chiorazzi et al.,(1977) using different immunisation schedules employing DNP conjugates in SJL mice, demonstrated that there normally

exists in this strain a non-antigen specific suppressor cell which selectively suppresses both primary and secondary IgE responses. The subjection of poor IgE responders to X-irradiation, appropriate doses of cyclophosphamide or anti-lymphocyte serum enhanced IgE production. The mice can be restored to their former state by the transfer of syngeneic cells from unimmunised donor animals and this restoration is inhibited by pre-transfer treatment of spleen cells with anti-θ serum and complement. This showed that the cells involved were T cells (Chiorazzi et al., 1977). These suppressor T cells appear to act selectively on IgE antibody response with no effect on IgG.

These findings have shed a new light on the strain differences mentioned above. Poor IgE responsiveness is now seen as a genetic capability, inherited as an active inhibition of IgE responses, rather than a genetic inability to form IgE antibodies. Also supporting this theory, is the identification of the level of action of these suppressor cells. By different combinations of immunisation and irradiation times, the site of action was indicated to be at the helper T cell induction, as this suppression was alleviated by exposure to X-irradiation at the time of initial carrier priming rather than at any other point (Chiorazzi et al., 1977).

Production of IgE responses in the rat basically depends on the dosage, the manipulation of which reveals the strains capacity to respond. Animals of poor responder strains require a large dose of antigen, probably to "overcome" the non-specific suppressor mechanisms, but at the same time the large dose activates specific suppressor mechanisms which abrogates the response and inhibits booster responses. By initially immunising with small doses (1 μg) of antigen, Jarrett and Stewart (1974) obtained a good primary IgE response in Hooded Lister rats, which could be boosted. A large primary dose, however, inhibited the formation of a secondary response.

Hooded Lister rats are good IgE responders and have been used for a long time in this laboratory for the study of IgE production. By contrast to poor IgE responders it would seem that these animals are deficient in their non-antigen specific suppression or at least defective in its expression, since IgE responses can be formed in them on exposure to minute doses of antigen. The administration of large doses of antigen, on the other hand, activates other (antigen specific) regulatory mechanisms which eventually terminate the response and booster responses.

1. REAGINIC ANTIBODY RESPONSES TO INTRADERMAL AND ORAL ADMINISTRATION OF EGG-ALBUMIN IN THE RAT

Introduction

The optimal conditions for the induction of reaginic antibodies have yet to be defined with the objective of gaining a better understanding of the development of IgE-mediated allergic diseases.

It had previously been found (Jarrett and Stewart, 1974) that reagin responses were evoked in Hooded Lister rats by intraperitoneal immunisation with any one of a range of doses from 1 mg - 1 μg egg-albumin. Booster responses could be evoked but only in animals initially immunised with the smaller doses of antigen. Large immunising doses of antigen, i.e. excess of 100 μg had an inhibitory effect on the development of challenge responses.

Access by antigen to the peritoneal cavity, however, is not a method whereby sensitisation is likely to occur naturally. Here experiments are described in which egg-albumin was administered orally or by intradermal injection in order to discover whether IgE production could be induced by these means, whether dose effects similar to those obtained by intraperitoneal inoculation could be observed, and whether immunisation by these routes would have any special effects on the IgE response.

In preliminary experiments a booster response was evoked in rats which were immunised with egg-albumin intraperitoneally and challenged with 10 ng intradermally (Jarrett and Stewart, unpublished). McDougall (1974) found that both primary and secondary IgE responses could be evoked by the ingestion of egg-albumin dissolved in the drinking water. The rats were given water, in which egg-albumin was dissolved at a concentration of 100 µg/ml, ad libitum for periods between 24-48 hours during which time it was estimated that the quantity consumed by each rat was between 2 mg - 5 mg of egg-albumin. This however was probably an overestimate as allowances were not made for spillage and denaturation of the protein.

The experiments described below were designed to find the minimum dose of antigen necessary to evoke primary and secondary IgE responses by the intradermal and oral routes.

Materials and Methods

Animals

The animals used were Outbred female Hooded Lister rats weighing 150-200 g obtained from Animal Suppliers (London) Ltd. They were fed a diet free of egg proteins.

Antigen administration

Sigma egg albumin (EA) Grade V, freshly prepared to a concentration 10 mg/ml in saline was diluted so that the

appropriate dose was contained in 0.5 ml. Intradermal injections were performed on the shaven back with a 26-gauge needle so that a superficial pale bleb appeared at each injection site. 0.1 ml of fluid was injected into each of five sites. For oral sensitisation the dose was administered by stomach tube, the latter consisting of a 3 cm length of nylon intravenous cannula, diameter 1.65 mm, (Portex Ltd., Hythe, Kent) attached to a 1 ml syringe. On some occasions (as indicated in the Results section) the animals were deprived of food but not water for 24 hours preceding intubation.

Bordetella pertussis

A suspension (Wellcome Biological Reagents) containing 10^{10} organisms was injected intraperitoneally at the same time as the administration of the immunising dose. Subsequent doses of antigen were given without adjuvant.

Statistical analysis

Results were compared where appropriate by Student's t-test using logarithmically transformed antibody titres. Animals that did not produce detectable antibody were not included in the calculations. The number of non-reacting animals in each group is shown in the Results section.

Results

Reaginic antibody response after intradermal injection of antigen

Table 2 shows the individual reaginic antibody titres of three groups of animals immunised with 1 mg, 100 μg and 10 μg EA respectively, and then challenged after 30 days with 0.1 μg EA. Both doses were given intradermally and *B. pertussis* was administered with the first dose but not with the second. Clearly, even the smallest sensitising dose of 10 μg administered by this route was sufficient to induce an IgE antibody response. Moreover, 0.1 μg EA induced secondary responses which were significantly greater than the primary response in Group 2 ($p < 0.05$) and Group 3 ($p < 0.01$) although not in Group 1.

In order to test the efficacy of even smaller doses of EA for both primary and secondary IgE responses, the experiment set out below was performed.

Group	No. of rats	Amount of EA injected intradermally			
		First dose (Day 0)		Second dose (Day 31)	Third dose (Day 60)
1	16	10 μg	1a	10 ng	1 ng
			1b	1 ng	1 ng
2	15	1 μg	2a	10 ng	1 ng
			2b	1 ng	1 ng

TABLE 2. Reaginic antibody response after intradermal injection of egg-albumin (EA) sensitising doses ranging from 1 mg to 10 µg, challenge dose 0.1 µg.

Rat No.	Group 1		Group 2		Group 3	
	1st dose 1 mg	2nd dose 0.1 µg	1st dose 100 µg	2nd dose 0.1 µg	1st dose 10 µg	2nd dose 0.1 µg
1	0	8	256	512	64	64
2	128	512	64	128	64	2048
3	256	128	32	128	32	512
4	32	256	32	128	16	128
5	8	64	0	0	256	2048
6	128	512	128	512	4	512

EA PCA titres 13 days after 1st dose and 4 days after 2nd dose of EA.

The results are shown in Table 3.

The most important feature of the primary IgE response was that it was detectable in twelve of the sixteen animals in Group 1 immunised with 10 μg but in only five of fifteen animals of Group 2 immunised with 1 μg. Second doses of 10 ng or of 1 ng, however, evoked secondary responses not only in the rats which had previously produced reagins, but also in the majority of those which had not. By 26 days after challenge the IgE levels had declined again or had disappeared so that the overall picture in each group resembled that of the primary response. At this point the animals of all groups were challenged again with 1 ng EA given intradermally, and were exsanguinated 4 days later. The figures in Table 3 show that the IgE antibody titres were re-elevated in some rats of each group but this was most striking in Groups 1b and 2b which had been challenged with 1 ng on both occasions. It is only in the latter two groups that the mean titres for day 26 after the second dose and day 4 after the third dose are significantly different ($p < 0.025$ for both).

Reaginic antibody response after orally administered antigen

In order to discover the most effective and the lowest effective dose for the stimulation of IgE antibody production following immunisation by the oral route, five groups of

TABLE 3. Reaginic antibody response after intradermal injection of EA sensitising doses of 10 or 1 µg, challenge doses of 10 or 1 ng.

Group	Treatment of rats: Ag doses injected*		Days bled after each dose	EA PCA titres of 8 rats: 1	2	3	4	5	6	7	8
1a	1st	10 µg	12	8	128	8	0	4	32	8	0
	2nd	10 ng	4	512	2048	64	4	256	512	512	128
			26	32	64	0	0	32	32	8	2
	3rd	1 ng	4	512	128	0	1	64	16	8	64
1b	1st	10 µg	12	8	1	32	128	0	8	0	16
	2nd	1 ng	4	512	16	512	512	8	512	64	128
			26	32	1	128	128	2	128	2	16
	3rd	1 ng	4	512	8	512	256	8	256	512	256
2a	1st	1 µg	12	0	8	0	0	4	0	4	
	2nd	10 ng	4	0	512	128	2	512	64	1024	
			26	0	16	8	0	16	2	8	
	3rd	1 ng	4	0	Died	256	2	4	128	64	
2b	1st	1 µg	12	8	0	0	1	0	0	0	0
	2nd	1 ng	4	512	32	128	128	0	8	0	4
			26	2	0	0	2	0	0	0	4
	3rd	1 ng	4	1024	2	512	256	8	64	0	64

*Antigen injected on days 0, 31 and 60.

eight rats were given doses of EA increasing by ten-fold increments from 10 μg to 100 mg inclusive, by stomach tube. B. pertussis was injected intraperitoneally. Food but not water had been withheld from the animals for 24 hours beforehand. The results in Table 4 show that each dose, even the smallest of 10 μg was effective in inducing an IgE response in some animals.

All the rats were challenged 31 days later with 100 μg EA given by stomach tube. No adjuvant was given and the rats were not fasted on this occasion . The inhibitory effect of the larger primary doses of antigen on secondary IgE responses which was demonstrated in our intraperitoneal immunisation experiments is again clearly evident (Table 4). Again, many rats which did not produce reagins after the first dose were found to have been primed, since a secondary response occurred after challenge.

The results of another similar experiment are shown in Table 5 in order to demonstrate that in some experiments using oral antigen we have been able to evoke a further booster response by giving a third dose of antigen after the decline of the secondary response, as in the experiments involving antigen given intradermally. In this experiment the rats were not fasted. The dose effects are again clearly evident.

TABLE 4. Reaginic antibody response to orally administered egg-albumin: sensitising doses ranging from 100 mg to 10 µg, challenge dose 100 µg

Rat No.	Group 1 100 mg	Group 2 10 mg	Group 3 1 mg	Group 4 100 µg	Group 5 10 µg
	PCA titres 12 days after these doses of EA				
1	8	0	0	8	0
2	0	1	4	1	0
3	32	8	8	0	0
4	0	0	0	1	8
5	0	8	16	0	8
6	0	4	2	0	1
7	2	8	0	0	0
8	16	128	128	0	0
	PCA titre 4 days after challenge of all above rats with 100 µg EA				
1	0	0	0	128	128
2	0	0	8	128	64
3	128	32	512	64	512
4	0	0	2	64	1024
5	0	16	128	0	128
6	0	4	128	16	512
7	0	32	8	0	512
8	256	32	32	512	0

TABLE 5. Reaginic antibody response to orally administered egg-albumin: sensitising doses ranging from 1 mg to 10 µg, challenge dose 10 µg.

PCA titres 12 days after the 1st dose and 4 days after the 2nd and 3rd doses of EA

Group 1 1st dose 1 mg challenge doses 10 µg*			Group 2 1st dose 100 µg challenge doses 10 µg			Group 3 1st dose 10 µg challenge doses 10 µg		
1st response	2nd response	3rd response	1st response	2nd response	3rd response	1st response	2nd response	3rd response
0	1	32	0	0	0	16	128	512
0	2	2	8	128	512	16	32	512
1	0	0	1	128	64	4	128	512
64	32	64	0	32	128	0	128	128
8	4	8	4	128	16	2	64	256
0	2	1	16	128	512	8	512	512

*Rats challenged 30 and 51 days after initial immunisation.

In a final experiment, the results of which are shown in Table 6, ten rats in each of two groups were given 10 µg and 1 µg EA respectively by stomach tube. Each group was then subdivided into two and the rats in each sub-group were challenged with 10 µg or 1 µg respectively 27 days later. An oral dose of 1 µg was found to be insufficient to sensitise rats for IgE production although it could evoke secondary responses in rats previously immunised with 10 µg EA.

Discussion

It has been demonstrated that intradermal or oral administration of small doses of antigen can elicit primary and secondary reaginic antibody responses in Hooded Lister rats. An EA dose as small as 1 µg injected intradermally or 10 µg given orally was found to be effective in stimulating a primary IgE response, if accompanied by an intraperitoneal injection of B. pertussis. In rats immunised with the above doses, secondary responses could be evoked by the administration of even smaller quantities of EA; thus, 1 ng intradermally or 1 µg orally, both without adjuvant.

The primary response was detected by day 10 after immunisation and the peak of the secondary response occurred on day 4 after challenge. The secondary response was manifest by the elevation of existing antibody levels or the rapid appearance (i.e. by day 4) of antibodies in previously negative animals. Intradermal injection was a more relieable immunisation

Table 6. Reaginic antibody response after oral administration of EA sensitising dose 10 µg or 1 µg*, challenge dose 10 µg or 1 µg.

PCA titre 12 days after 1st and 4 days after 2nd dose EA

Rat No.	1st response to 10 µg	2nd response to 10 µg	2nd response to 1 µg
1	0	0	
2	0	0	
3	0	128	
4	8	128	
5	2	128	
6	0		16
7	0		32
8	0		0
9	0		2
10	0		16

*Rats immunised with 1 µg EA orally did not produce detectable reagins either as a primary response or after challenge with the above doses.

procedure than oral administration. Only 50% of the orally administered antigen experiments have been successful. Unsuccessful experiments have been characterised by the failure of the rats to produce IgE responses following immunisation with any of the doses used (100 mg - 10 μg). The reason for this failure is not known but it does not seem to depend on whether or not the rats are fasted beforehand. Other experimental conditions, to the best of our knowledge, have been kept constant.

As with intraperitoneal immunisations (Jarrett and Stewart, 1974) large doses of EA given intradermally or orally (>100 μg) had an inhibitory effect on the development of secondary responses (Tables 4 & 5). It has been proposed that the absence of secondary responses may result from the stimulation of a population of suppressor T cells. Thus, a large amount of antigen presented on immunisation would activate antigen specific suppressor T cells whose effect would be to inhibit the proliferation of those precursor cells which would otherwise effect the next response.

However, by contrast with intraperitoneal immunisation tertiary responses were evoked after intradermal and oral presentation of antigen. This difference may not in fact be a function of the site of antigen presentation but of

the quantity of antigen that reaches the appropriate immunocompetent cells. It is probable that intraperitoneal immunisation results in a relatively greater proportion of the administered dose being made available for stimulation and inhibition of IgE responses than when it is given by the other routes. Orally administered antigen in particular could be expected to be substantially degraded in the intestine. The occurrence of tertiary responses in these experiments raises the proposition that a challenge dose of even as little as 1 ng EA injected intraperitoneally (but not intradermally) is sufficiently large to be inhibitory to the development of the next IgE booster response. Our preliminary (unpublished) results support this proposition.

Numerous reports in the literature, and the success of local prophylactic immunisations, testify to the fact that antigenically intact protein molecules are rapidly absorbed across the intestinal epithelium and may stimulate local or humoral immune responses (Parkins, Dimitriadou and Booth, 1960; Berenstein and Ovary, 1968; Korenblatt, Rothberg, Minden and Farr, 1968; Crabbe, Nash, Bazin, Eyssen and Heremans, 1969; and see review by Bienenstock, 1974). It has been proposed that one of the functions of secretory IgA is to limit such absorption by combination with antigen (Heremans, 1969), a concept which has been extended by the suggestion that the sensitisation of some atopic individuals

to common allergens is the result of a transient period of IgA deficiency or the idiosyncratic possession of low affinity IgA which, by default, allows the absorption via the mucosae of abnormally large quantities of antigen (Taylor, Norman, Orgel, Stokes, Turner and Soothill, 1973).

Paradoxically the results presented here for the rat show that the larger the dose of antigen absorbed by whatever route the less likely it is that persistent IgE responses will result.

The initiation and maintenance of IgE antibody responses in man may, as in the rat, depend on a critical combination of circumstances, prominently including the absorption by genetically predisposed individuals of minute quantities of antigen, initially in the presence of an adjuvant.

2. ADJUVANTS IN THE INDUCTION AND ENHANCEMENT OF RAT IgE RESPONSES

Introduction

The IgE antibody response induced in Hooded Lister rats by administration of antigen and adjuvant may subsequently be enhanced either specifically by further exposure to antigen (Jarrett and Stewart, 1974) or non-specifically by infection with the helminth parasite Nippostrongylus brasiliensis (Orr and Blair, 1969; Jarrett and Stewart, 1972 and reviewed by Jarrett, 1976). In the latter case the enhanced response is associated with a great increase in total serum IgE.

In this laboratory for many years the 'potentiated' reagin response was elicited consistenly by N. brasiliensis infection in rats which had been primed with EA and B. pertussis. Latterly, however, the response had become more elusive and often failed to reach the expected levels although we had not knowingly changed our experimental conditions. When all other components of the system had been examined I considered the possibility that some unknown variation of the B. pertussis adjuvant between batches, while not having an obvious effect on the primary response, might nevertheless influence the process of potentiation. For this reason I tested B. pertussis from different sources and also other kinds of adjuvant for their effect at the level of the enhanced response. It was found

that the adjuvant used to prime rats does indeed determine the occurrence and level of both potentiated and booster IgE responses, and moreover that adjuvants clearly discriminate between the two types of enhanced response.

Additionally, I have examined the effect of Concanavalin A on the IgE response to egg-albumin. Gollapudi and Kind (1975) showed that Concanavalin A could function as an adjuvant for IgE production against egg-albumin in mice if it was injected either in a mixture with, or immediately after, the antigen. We wished to see if it had similar adjuvant properties in the rat and also whether it subsequently augmented the response in the same way as N. brasiliensis infection if given alone some time after initial immunisation.

Materials and Methods

Antigen/Adjuvant preparations for initial immunisation

Solutions of egg-albumin (EA) (Sigma Grade V) or keyhole limpet haemocyanin (KLH) (Calbiochem A grade) were prepared in 0.15 M saline. The antigen dose was injected with one of the following adjuvants:

B. pertussis (preparations of phase 1 heat killed organisms)

A. From the Institute Pasteur (Production), 'Perthydral' vaccine, alum absorbed.

B. From the Wellcome Research Laboratories, Beckenham, Kent, a preparation of alum absorbed B. pertussis, and

C. the same batch in simple suspension (experimental batch VL4769) both kindly donated by Dr. W.H. Foster.

D. From Wellcome Biological Reagents Ltd., standard commercial preparation of B. pertussis in simple suspension (batch KO173).

E. From the Department of Microbiology, the University of Glasgow, a simple suspension of heat killed bacteria kindly provided by Professor A. Wardlaw.

EA in saline was mixed with B. pertussis suspension immediately before use, and each animal was injected intraperitoneally with 0.6 ml of the mixture containing the antigen dose and 10^{10} B. pertussis.

Aluminium hydroxide adjuvant ($Al(OH)_3$) was prepared from aluminium sulphate and sodium hydroxide according to the method of Herbert (1973). 250 ml of 5 per cent aluminium sulphate was added to 100 ml of 5 per cent sodium hydroxide with vigorous stirring. The resultant precipitate was washed twice by centrifugation and decantation and then resuspended in 0.15 M saline to the original volume.

The antigen was absorbed by mixing equal volumes of antigen solution and suspension of aluminium salt so that

the antigen dose was contained in 0.2 ml of the mixture. The mixture was incubated at room temperature for approximately 1 hour, and a dose of 0.2 ml was then administered to rats by intraperitoneal injection.

Complete Freund's Adjuvant (CFA) was purchased from Miles Laboratories. EA in saline and CFA were mixed in the proportions 1:2 and an emulsion was prepared with a Silverson homogeniser. Rats were immunised by intramuscular injection of 0.2 ml of the emulsion into the hind leg.

Concanavalin A (Con A) was purchased from Pharmacia (Great Britain) Ltd. The antigen solution was mixed in equal volumes with an 8 mg/ml solution of Con A in 2.5 M saline and the mixture was incubated at 37°C for 30 minutes. Rats were immunised by the intraperitoneal injection of 0.1 ml of the mixture containing 400 µg Con A and the desired antigen dose.

Procedures for enhancing the primary IgE responses

a) Booster dose of antigen - in the experiments described here, this was always 1 µg EA injected intraperitoneally without adjuvant.

b) N. brasiliensis infection - rats were infected by subcutaneous injection of 4000 N. brasiliensis larvae. A culture of N. brasiliensis is maintained in this laboratory by repeated sub-inoculation in Hooded Lister

rats. The method of culture and infection of rats was as follows. Faecal pellets (containing parasite eggs) were collected from rats with a 7-10 day old infection. The pellets were moistened and spread on to filter paper circles which were placed on pieces of moist sponge in petri dishes. The dishes were placed in a humid incubator at 27°C for 4-5 days during which time the eggs hatch. The larvae were then harvested by adding water at 37°C to the petri dishes. The water was filtered under suction on to course filter paper on which the larvae are retained. This filter paper was then inverted on an Endecott sieve (mesh 400) in a Baerman apparatus filled with water at 37°C. The larvae which swim through the sieve were run off after sedimentation. The larvae in aliquots of suspension were counted and the suspension was adjusted to contain 4000 larvae/ml. Rats were infected by injecting 4000 larvae subcutaneously in the flank region.

c) Con A - 400 µg in 2.5 M saline was injected intra-peritoneally.

Statistical analysis

Antibody levels from more than 2 groups were compared by analysis of variance. Students T test was used to

compare antibody levels of one group with another. All tests were carried out at a significance level of $p < 0.05$.

All other materials and methods have already been described previously in this thesis.

Results

Effect of adjuvant used in initial immunisation on enhanced IgE responses brought about by a booster dose of antigen or *N. brasiliensis* infection

Three groups of 10 rats were primed with 10 µg EA together with one of the adjuvants *B. pertussis* (preparation D in Materials and Methods), $Al(OH)_3$ or CFA. One month later, each of the three groups was sub-divided into two, whereupon 5 rats were given a booster of 1 µg EA and the other 5 were infected with *N. brasiliensis*.

Table 7 shows the EA PCA titres 12 days after initial immunisation and 4 and 12 days after antigen challenge or *N. brasiliensis* infection respectively.

The difference between the primary IgE response of rats immunised with *B. pertussis*, $Al(OH)_3$ or CFA, was not statistically significant, i.e. analysis of variance indicated that the three groups could be regarded as having come from identical populations. The enhanced responses however segregated into two distinct patterns, namely high level booster response associated with poor potentiated

TABLE 7. Effect of EA challenge or N. brasiliensis infection on primary EA IgE response induced by immunisation with EA and one of the adjuvants B. pertussis, $Al(OH)_3$ or CFA.

Immunisation	Geometric Mean EA PCA titre (range)		
	12 days after immunisation	4 days after antigen challenge	12 days after N. brasiliensis infection
10 µg EA + B. pertussis	60 (8-128)	1176 (512-16,000)	512*
10 µg EA in $Al(OH)_3$	150 (64-512)	338 (128- 1,024)	6203 (512-32,000)
10 µg EA in CFA	56 (4-256)	76 (16- 128)	4083 (512- 8,192)

Each group of 10 animals immunised ther. divided one month later into two groups of 5 for challenge (1 µg EA) or infection with 4000 N. brasiliensis larvae.

*All animals in this group had the same titre.

response in the B. pertussis group, and conversely poor to absent booster response associated with high level potentiated response in the $A\ell(OH)_3$ and CFA groups. For both types of enhanced response, analysis of variance of the three groups showed that they originated from separate populations.

In order to test for variation of enhancing effects following priming with antigen and different preparations of B. pertussis, I carried out the experiment outlined in Table 8. Here, 5 groups of 10 rats were primed with 10 µg EA together with one of the five B. pertussis suspensions described in the Materials and Methods. Again, one month after immunisation each group was sub-divided into two, and the rats of each sub-group were either given a further injection of 1 µg EA or were infected with N. brasiliensis. The results (Table 8) show that the primary IgE response of the rats primed with alum absorbed B. pertussis suspensions (A and B) were somewhat higher than those of the groups primed with B. pertussis in simple suspension, and this difference in level was statistically significant. The experiment also showed that priming with alum absorbed B. pertussis led eventually to the best potentiated response but that no elevation occurred following a booster dose of antigen.

TABLE 8. Primary and enhanced IgE responses to EA in rats immunised with *B. pertussis* from different sources (with and without alum).

Immunisation	Geometric Mean EA PCA titre (range)		
	12 days after immunisation	4 days after antigen challenge	12 days after *N. brasiliensis* infection
10 µg EA + *B. pertussis* A	147 (128-152)	147 (32- 512)	1349 (512-2048)
10 µg EA + *B. pertussis* B	111 (32-512)	128 (32- 512)	1551 (512-4096)
10 µg EA + *B. pertussis* C	42 (8-128)	337 (64-1024)	256 (2- 512)
10 µg EA + *B. pertussis* D	42 (1-256)	587 (128-4096)	388 (64-1024)
10 µg EA + *B. pertussis* E	37 (8-128)	512*	152 (64- 512)

Each group of 10 animals immunised then divided one month later into two groups of 5 for challenge (1 µg EA) or infection with 4000 *N. brasiliensis* larvae.

*All animals in this group had the same titre.

The results of both above experiments indicated that the inclusion of $A\ell(OH)_3$ in the priming event prepared the animals well for potentiation of the response by subsequent N. brasiliensis infection, but inhibited subsequent antigen stimulated booster responses. Since this effect apparently occurred whether $A\ell(OH)_3$ was absorbed to EA or to B. pertussis we considered the possibility that the $A\ell(OH)_3$ per se might influence the response. However, in experiments in which $A\ell(OH)_3$ was administered by itself either one week before or simultaneously with but not absorbed to EA and B. pertussis it was not found to influence either the potentiated or booster response.

Since the occurrence of booster IgE antibody responses following immunisation of Hooded Lister rats with EA and B. pertussis is largely inhibited if the priming dose of antigen is greater than 10 μg (Jarrett and Stewart, 1974), we considered the possibility that the inhibition of booster responses following immunisation with EA-$A\ell(OH)_3$ might be the result of more effective utilisation of the EA dose so that 'large dose suppression' might be induced by priming with a comparatively smaller amount of antigen. Consequently, I immunised groups of rats with 20, 2, 0.2 and 0.02 μg EA in $A\ell(OH)_3$ and challenged with 10 ng EA one month later. A primary response occurred in the first two groups (20 and 2 μg).

This response was not increased but rather diminished following challenge. The smaller doses of EA did not result either in primary responses or booster responses following antigen challenge.

Con A as an adjuvant and an enhancing agent for the IgE response to EA and KLH

To determine if Con A could function as an adjuvant for the induction of an IgE response to EA or as an agent for the subsequent enhancement of the IgE response, two groups of rats were immunised with 100 μg EA and B. pertussis (E) or 100 μg EA and Con A respectively as described in the Materials and Methods. One month later the rats of both groups were injected with 400 μg Con A and were bled 4 and 12 days later.

The results (Table 9) showed that Con A had an adjuvant effect for the induction of an IgE response to EA in rats. The response induced with Con A as adjuvant was significantly greater than that of the rats immunised with EA and B. pertussis. Con A injected alone one month after priming also had an enhancing effect on the already existing EA IgE response induced with B. pertussis or with Con A as adjuvant. The Con A enhanced response occurred by day 4 after Con A injection, and had declined again to low levels by day 12. In this respect the Con A response resembles the booster response elicited by challenge with antigen and differs from the

TABLE 9. Con A in the induction and enhancement of IgE antibody response to EA.

Immunisation	Geometric Mean EA PCA titre (range)		
	12 days after immunisation	4 days after Con A	12 days after Con A
100 μg EA + B. pertussis	28 (2- 64)	601 (256-2048)	84 (32-128)
100 μg EA + Con A	111 (32-256)	859 (512-2048)	26 (4-128)

enhanced response stimulated by N. brasiliensis infection which reaches a peak 12-14 days after infection. I also measured the levels of total serum IgE before and after the enhancing injection of Con A and found that total IgE was not significantly increased by administration of this agent. This again is in contrast to enhancement brought about by N. brasiliensis which is accompanied by a great increase in total IgE (Jarrett and Bazin, 1974).

In order to test if the adjuvant effect of Con A was restricted to the EA IgE response, I repeated the above experiment using instead keyhole limpet haemocyanin as antigen. I found that Con A did not act as an adjuvant for the induction of a response to KLH, nor did it enhance the IgE response to KLH induced by immunisation with this antigen and B. pertussis.

Finally, I show here the results of an experiment to compare the Con A and N. brasiliensis enhanced IgE responses following immunisation with the various adjuvants used in this study. Four groups of 10 rats were immunised with 10 μg EA given with B. pertussis, Con A, $A\ell(OH)_3$ or CFA respectively. One month later each group of rats was divided into two and the 5 rats of each subgroup were either given Con A or were infected with N. brasiliensis. Table 10 shows that the Con A enhancing effect occurred only in the animals initially immunised with B. pertussis or Con A as adjuvants

TABLE 10. Comparison of the enhancing effects of N. brasiliensis and Con A on the IgE responses induced by immunisation with EA and the adjuvants shown below.

Immunisation	Geometric Mean EA PCA titre (range)		
	12 days after immunisation	4 days after Con A	12 days after N. brasiliensis
10 μg EA 10^{10} B. pertussis (E)	12 (2-128)	361 (32-2048)	16 (0- 256)
10 μg EA + Con A	64 (16-128)	445 (256-2048)	31 (0- 8)
10 μg EA + $A\ell(OH)_3$	101 (32-128)	14 (2- 256)	1349 (512-8192)
10 μg EA + CFA	56 (4-128)	8*	890 (128-8192)

Each group of 10 animals immunised then divided one month later into two groups of 5 for Con A infection (400 μg) or N. brasiliensis infection.

*All animals in this group had the same titre.

and not at all in those immunised with $A\ell(OH)_3$ or CFA. Conversely, a potentiated response only occurred in the $A\ell(OH)_3$ and CFA immunised animals and not in these immunised with B. pertussis or Con A.

DISCUSSION

The production of IgE antibody involves the proliferation and maturation of IgE B cells under the influence of helper and suppressor T cells in turn influenced by the products of other types (Tada, 1976; Ishizaka, 1976; Bourne et al., 1974). Clearly, the system offers numerous points of access to intervention by adjuvants.

Adjuvants are usually used in conjunction with antigen in order to facilitate the induction of an immune response and IgE responses in particular cannot usually be induced unless adjuvant is used. The adjuvants which have been most commonly used for this purpose are $A\ell(OH)_3$, B. pertussis and CFA (Mota, 1964; Revoltella and Ovary, 1969; Levine and Vaz, 1970; Strannegard and Chan, 1969; Kishimoto and Ishizaka, 1973; and reviewed by Tada 1975).

Adjuvants used to induce specific responses may function by increasing and prolonging the effect of antigen dose, by enhancing the interaction of B cells with antigen, by expanding helper T cell populations or abrogating the effect of suppressor cells and by stimulating the processing of antigen by macrophages which may indirectly lead to the above effects (Freund, 1956; Dresser and Phillips, 1973; Unanue et al., 1969; Hamaoka and Katz, 1973; Allison and Davies, 1971; Tada et al., 1972;

WHO Technical Report 595, 1976). These effects may occur singly or in combination depending on a complexity of variables including the individual antigen and the manner of its use, the dose and form of antigen, and the nature of the test system and species.

Infection of the rat with live helminth parasites has a rather different and restricted stimulating effect: it is selective for IgE and is capable of non-specifically enhancing only already existing IgE antibody responses. Thus, parasites cannot assist in the priming of an IgE response to heterologous antigen administered at or around the time of infection. In order for a response to be potentiated it must first be induced by immunisation with the antigen and one of the conventional adjuvants (Orr and Blair, 1969; Jarrett et al., 1972). Evidently the potentiating effect of parasites is exerted directly or indirectly on primed IgE B cells.

Some time ago however we began to realise that the mere induction of a primary IgE response was not a sufficient prerequisite for its subsequent potentiation. This is amply borne out in the results of these experiments which show that while the IgE responses to EA induced with B. pertussis, $Al(OH)_3$ or CFA are of a similar level, potentiated responses following infection occur more consistently and to a higher level in animals immunised with the aid of the latter two adjuvants.

In a similar way the state of readiness of the rats to produce an antigen driven booster IgE response cannot be prejudged from the level of the primary response. It has previously been shown that although primary IgE responses of Hooded Lister rats immunised in the dose range of 1 μg - 1 mg EA (with B. pertussis) cannot be distinguished, booster responses can subsequently be elicited only in animals initially immunised with the lower doses of antigen (Jarrett and Stewart, 1974). As can be seen from the experiments reported here the booster response is quite variable, not only between individual animals, but also between experiments. We have been unable to relate this variability to different batches of B. pertussis (with the exceptions discussed below) or antigen and can at present only suppose that it results from variable environmental or physiological host factors which influence the balance in expression between helper and suppressor mechanisms.

Clearly, factors other than the dose of antigen used in initial immunisation can affect this balance. Here it is shown that booster IgE responses cannot be evoked in rats primed even with a small amount of antigen if the adjuvant used in the priming event is $Al(OH)_3$ or CFA. These adjuvants thus have an effect, not evident in terms of the amount of antibody produced in the primary response,

which prepares the immune system more effectively than B. pertussis for potentiation of the response by parasites and for suppression of the booster response following reactivation by antigen. Perhaps the most straightforward explanation for this combination of effects is that $Al(OH)_3$ and CFA by comparison with B. pertussis lead to further rounds of proliferation of both memory B cells and memory T cells. In this way a second dose of antigen could reactivate an enlarged suppressor T cell population to a position of predominance in the booster response. Parasitic infection would lead to an enhanced potentiated response through the availability of a larger EA specific B cell pool on which to act through antigen non-specific mechanisms.

The problem of the declining efficacy of B. pertussis in producing potentiable IgE responses has not really been solved. It has however been found that the absorption of B. pertussis to $Al(OH)_3$ increases its efficacy in this respect, and also that initial immunisation with B. pertussis in this form, by comparison with a simple suspension leads to the complete suppression of booster responses. The observation that $Al(OH)_3$ had a similar effect whether absorbed to EA or to B. pertussis suggested it might per se influence the immune responses. Walls (1977) has shown that the injection of $Al(OH)_3$ causes an eosinophil response in mice

in the absence of specific antigen stimulation and that this response is T cell dependent. However, in an experiment here in which $A\ell(OH)_3$ was injected intramuscularly in rats immunised simultaneously with EA and B. pertussis intraperitoneally it did not have any detectable influence on primary or subsequent enhanced IgE responses.

B. pertussis has two adjuvant principles and each acts on several cell types involved in the immune response. The heat labile lymphocytosis promoting/histamine sensitising factors (Morse and Morse, 1976; Lehrer et al., 1975) is a potent adjuvant for haemagglutinating and reaginic antibody in rats and mice (Clausen et al., 1969; Tada et al., 1972; Lehrer et al., 1976). The bacterial lipopolysacharide (LPS) has also been shown to be an adjuvant for the induction of an IgE response in mice (Newburger et al., 1974; Danneman and Michael, 1976) and is thought to be the main active component for IgE adjuvant activity in guinea pigs (Mota et al., 1974). LPS is a B cell mitogen in vitro (Janossy and Greaves, 1971; Anderson et al., 1972) but its adjuvant activity in vivo is not solely dependent on this property (Newburger et al., 1974; Ness et al., 1976).

It is possible that the relative proportions of these two adjuvants in a given B. pertussis preparation could influence the degree of stimulation of one cell type relative to another, and that the proportions could be influenced by perhaps quite minor changes in culture and processing techniques. It would be interesting to investigate this possibility in the model described here.

Con A, a selective T cell mitogen (Anderson et al., 1972) can either stimulate or inhibit immune responses probably through the activation of helper or suppressor T cells respectively (Dutton, 1972; Rich and Pierce, 1973; Markham et al., 1977; Nirmal et al., 1972; Egan et al., 1974; Romball and Weigle, 1975). Gollapudi and Kind (1975) showed that Con A could act as an adjuvant for the induction of an IgE response to EA in mice and further that pre-immunisation with Con A alone could prime mice to EA so that higher levels of IgE to EA were produced on subsequent immunisation with EA alone (Gollapudi and Kind, 1977). The explanations put forward for this second phenomenon include the possibility that EA and Con A cross-react at the T cell level and indeed the Con A enhancing effect was not found to apply to the antigens human or bovine serum albumin or normal rabbit serum. Here I have shown that Con A can function as an adjuvant for the induction of an IgE response to EA in the rat and also that a subsequent

injection of Con A alone will enhance the response to EA.

By contrast with the enhanced response produced by parasites the Con A response has the characteristics of a booster response. Both antigen and Con A enhanced responses occur 4 days after injection of antigen or Con A respectively. Both responses occur only in animals initially immunised with EA together with B. pertussis or Con A and are inhibited in rats immunised with EA in $A\ell(OH)_3$ or CFA. The potentiated response, on the other hand, reaches a peak 12-14 days after N. brasiliensis infection, and occurs most strikingly in rats initially immunised with EA in $A\ell(OH)_3$ or CFA. The potentiated response is associated with a great increase in total serum IgE whereas this increases by at most 2-3 μg/ml during the booster and Con A responses.

These findings are compatible with the idea of cross-reaction of Con A and EA at the T cell level, more so since we have found that Con A functions neither as adjuvant nor enhancing agent for the IgE response to KLH. The evidence that mouse T and B cells recognise different determinants on the EA molecule (Takatsu and Ishizaka, 1975) is interesting in this context.

Another possibility for a Con A enhancing effect on IgE production is through the non-specific activation of T lymphocytes which however would be expected to influence the KLH response. Alternatively, by its activity as an IgE-directed ligand (Magro and Bennich, 1977) there is a possibility that Con A might cause direct activation of IgE B cells. This however would be expected to influence the level of total circulating IgE.

The experiments reported here were done initially to overcome a practical problem and were not therefore designed to answer questions about the mechanism of adjuvant action. They do however help to explain discrepancies between laboratories in occurrence of the potentiated IgE response following N. brasiliensis infection, and also they exemplify the importance of initial adjuvant in the elicitation of subsequent IgE booster responses. These experiments illustrate very clearly the stimulation of different combinations of cell types, or difference in degree of stimulation of one cell type relative to others by the different adjuvants used and the system clearly offers unusual scope for further investigation of the mechanism of adjuvant action.

3. IgE PRODUCTION IN DIFFERENT RAT STRAINS

Introduction

From the literature on the IgE response in rats, it would seem that Hooded Lister rats by comparison with other rat strains are exceptionally good IgE producers. However, in most of the previous studies, rats were immunised with a large amount of antigen which, as we now know, is inhibitory to the production of booster responses even in Hooded Lister rats. Levine and Vaz (1970) showed that mouse strains could be segregated into poor or good IgE antibody producers according to their response to immunisation with a small dose of antigen. Similar although rather more limited studies in rats have been reported by Murphey et al., (1974). From their studies, it emerged that Brown Norway rats responded to immunisation with 1 μg EA in $A\ell(OH)_3$ with a prolonged IgE response similar to that which is seen in Hooded Listers, other rat strains (ACl and F344) did not respond. Booster responses did not occur to any extent in any of the strains which these authors studied. However, $A\ell(OH)_3$ was used as the adjuvant in their immunisations and as was shown in the previous section this adjuvant, while being effective in the induction of rat IgE antibody, causes effective suppression of booster responses.

Smith and Petillo (1976) have reported that the potentiated IgE response to egg-albumin following infection of rats with N. brasiliensis is more marked in some rat strains than others. Again, Brown Norway rats were shown to be good responders.

In order to confirm, and enlarge on, these findings a number of different strains have been examined here for their ability to produce booster and potentiated responses to EA following immunisation with a high or low dose of the antigen and B. pertussis adjuvant.

Materials and Methods

Rats

CFHB and CFY rats were purchased from Anglia Laboratory Animals Ltd., Hooded Lister (HL) rats from Animal Suppliers (London) Ltd., and Sprague Dawley (SD) rats from Olac Northern Ltd. The Albino Swiss (AS), Brown Norway (BN) and hybrid AS/BN rats were donated by the Animal House of the Western Infirmary, Glasgow. All other materials and methods have been previously described.

Results

Experiment 1

This experiment involved groups of 10 rats of the strains SD, CFHB, CFY and HL. Each group was divided into two sub-groups of 5. Rats of sub-group A were

immunised with 1 mg EA and of sub-group B with 1 μg EA in each case injected in saline solution intraperitoneally with 10^{10} B. pertussis as adjuvant. Twenty four days later, all rats were challenged with 1 μg EA given intraperitoneally without adjuvant and 34 days after this (i.e. 58 days after immunisation) by which time the booster response is known to have declined (Jarrett and Stewart, 1974), all the rats were infected with 4000 N. brasiliensis larvae. The rats were bled 12 days after immunisation, 4 days after antigen challenge and 12 days after N. brasiliensis infection.

The resultant IgE antibody levels to EA are shown in Table 11 and Fig. 4. In the Sprague Dawley group, a few animals only responded to immunisation with either 1 mg or 1 μg EA. Nevertheless, booster responses occurred in 4/5 animals of both groups A and B following the booster dose of antigen. However, subsequent parasitic infection was without effect at all, and in fact 12 days after infection, EA IgE antibodies were indetectable in the serum.

Both CFHB and CFY strains performed rather better. Primary responses occurred in both strains following immunisation with 1 mg of EA. 1 μg EA induced a response

TABLE 11. Primary, booster and potentiated IgE responses to EA in different rat strains.

		Rat Strain											
		Sprague Dawley			CFHB			CFY			Hooded Lister		
		EA PCA Titres											
		D12 after sens^{n}.	D4 after chall.	D12 after inf^{n}.	D12 after sens^{n}.	D4 after chall.	D12 after inf^{n}.	D12 after sens^{n}.	D4 after chall.	D12 after inf^{n}.	D12 after sens^{n}.	D4 after chall.	D12 after inf^{n}.
A Immunised with 1 mg EA	1	2	32	0	2	8	0	4	16	256	128	128	512
	2	4	128	0	4	32	8	64	128	512	8	16	512
	3	0	128	0	4	32	512	8	32	0	128	1024	Died
	4	0	16	0	2	4	0	2	16	-	16	32	64
	5	0	0	0	0	8	0	8	128	256	128	128	512
B Immunised with 1 µg EA	1	0	4	0	1	128	256	0	16	0	8	512	256
	2	0	4	0	1	128	0	0	32	0	4	128	512
	3	0	128	0	0	128	0	0	16	256	8	2048	512
	4	2	8	0	4	128	0	0	32	64	64	2048	-
	5	0	0	0	0	1024	0	0	16	0	16	512	512

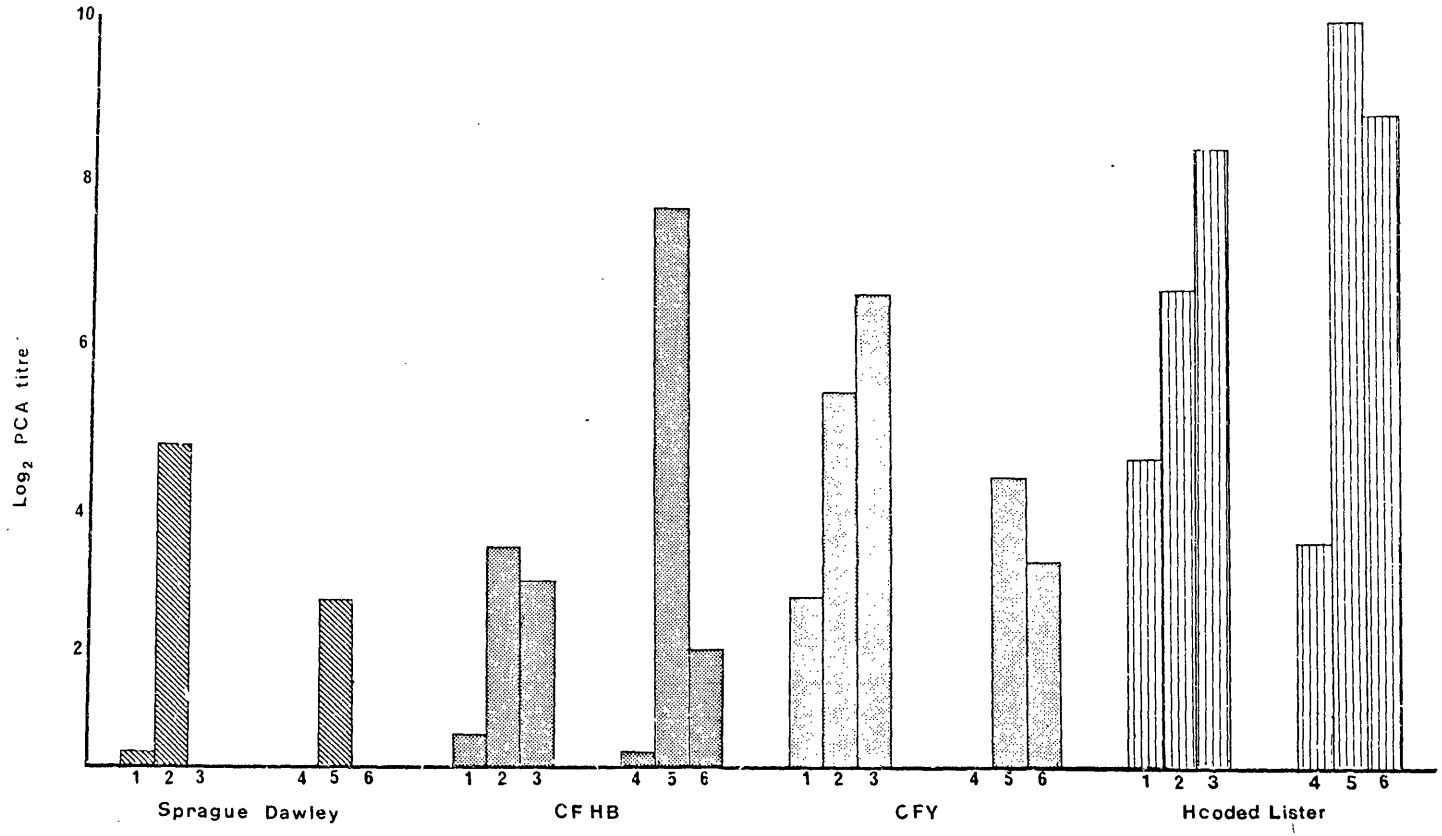


Fig. 4 IgE responses of various rat strains following immunisation, antigen challenge and N. brasiliensis infection. Rats were immunised with either 1 mg EA (1) or 1 μg EA (4) with B. pertussis. The rats were subsequently challenged with 1 μg EA (2 and 5) 24 days later, and were infected with N. brasiliensis 34 days after challenge (3 and 6). The values above are the mean IgE antibody levels 12 days after immunisation, 4 days after challenge and 12 days after infection.

in 3/5 CFHB rats, but was sub-threshold for rats of the CFY strain. Booster responses however occurred even in previously negative animals, and in the CFHB strain rats at least were consistently higher level in group B initially immunised with 1 μg EA. In both strains the potentiated response varied considerably between individual animals, occurring rather more frequently in the CFY rats.

Hooded Lister rats included as a control group, performed more or less as expected, with a primary response to each of the doses of antigen, booster responses much higher in group B and potentiation occurring in both groups regardless of size of initial immunising dose.

Experiment 2

This experiment involved groups of AS, BN and AS/BN cross rats. The rats were immunised with 10 μg EA and 10^{10} *B. pertussis* and were challenged 23 days later with 1 μg EA. Thirteen days after this, the animals were infected with 4000 *N. brasiliensis* larvae. Table 12 and Fig. 5 show the EA IgE antibody levels 12 days after immunisation, 4 days after antigen challenge and 12 days after infection. Clearly, the BN rats responded to all three stimuli with a greater EA IgE antibody response than the other groups. AS rats responded poorly to both primary and booster immunisations and did not produce a

TABLE 12. Primary booster and potentiated IgE responses to EA in AS, BN and AS/BN hybrid rats.

Rat Strain								
AS			AS/BN			BN		
EA PCA Titres								
D12 after sensitisation	D4 after challenge	D12 after infection	D12 after sensitisation	D4 after challenge	D12 after infection	D12 after sensitisation	D4 after challenge	D12 after infection
0	8	0	32	128	2	32	512	4096
2	32	0	4	128	0	128	512	1024
0	0	2	4	256	0	64	2048	512
4	32	0	4	512	0	32	512	4096
0	128	died	4	128	0	128	512	4096
2	128	0	32	128	0	128	8192	1024
			16	128	0			

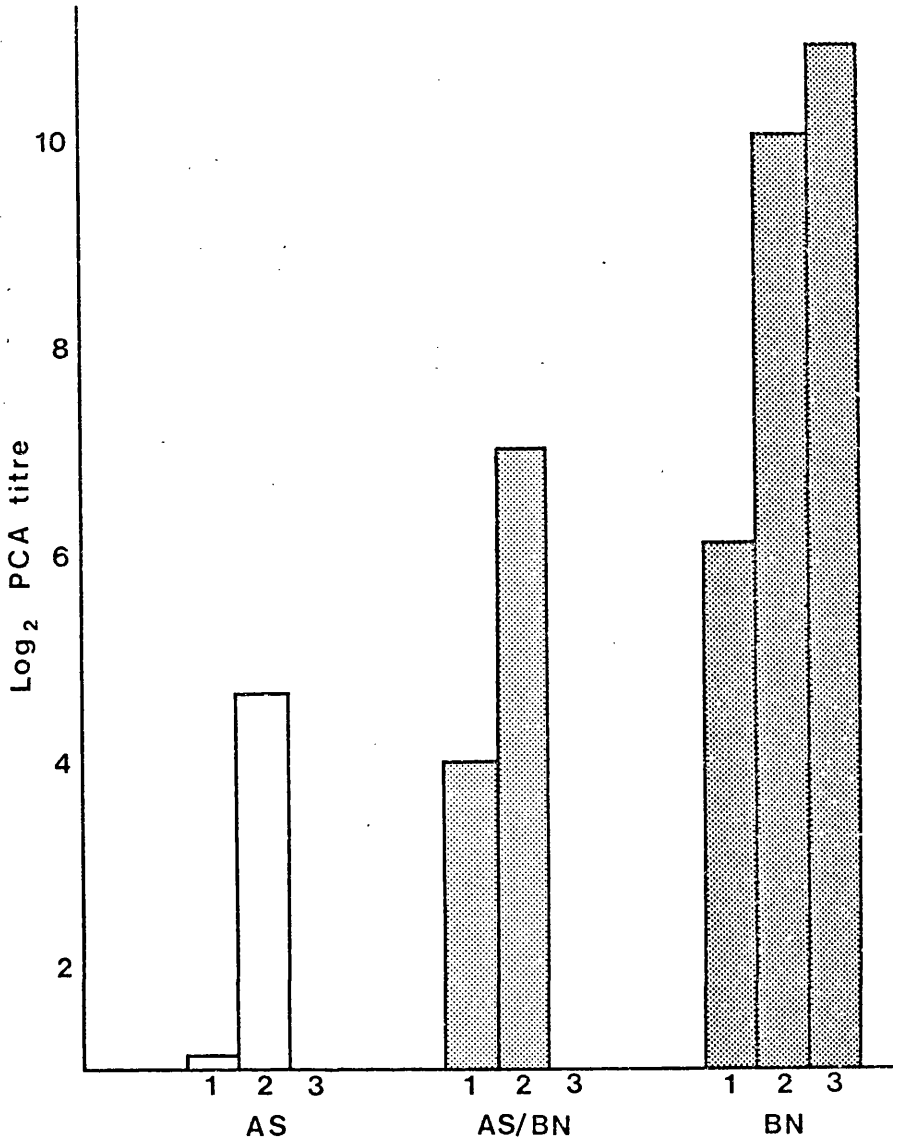


Fig. 5 The IgE response of Albino Swiss (AS), Brown Norway (BN) and AS/BN cross rats to immunisation with egg-albumin and subsequent egg-albumin challenge and N. brasiliensis infection.

potentiated response following N. brasiliensis infection. The responses of the AS/BN rats were interesting in that IgE production was intermediate during both primary and booster responses, but after N. brasiliensis infection absence of response was dominant.

Total serum IgE following N. brasiliensis infection

Total serum IgE levels were measured after N. brasiliensis infection on pooled serum samples from Experiment 1, and individual sera from Experiment 2. The results are shown in Table 13 from which it can be seen that a great measure of variation exists among the different strains in the extent to which parasitic infection causes an increase in total IgE levels. Also, it can be seen that the total serum IgE level in AS/BN rats is intermediate between AS having very low response and BN which have a very high response.

Discussion

These experiments have demonstrated strain differences for reaginic antibody production in the rat. The effects of a large immunising dose and a small immunising dose given with B. pertussis and their effect on subsequent challenge and infection have been examined.

TABLE 13. Total serum IgE in different rat strains 12 days after N. brasiliensis infection.

Strain		µg/ml Total IgE
SD	A	20.5
	B	20.1
CFHB	A	21.4
	B	20.4
CFY	A	100
	B	118
HL	A	160
	B	121
AS		2.3 ± 0.45
AS/BN		20.1 ± 3.74
BN		171 ± 23.2

*Mean ± Standard Error.

Sprague Dawley rats responded poorly to 1 mg and 1 μg of EA but did produce low level secondary responses. In CFHB and Hooded Lister rats, the secondary responses were higher after initial immunisation with 1 μg EA. CFY produced similar but low level secondary responses after both initial immunising doses even although a primary response was not detected after 1 μg.

As was discussed in the Introduction to this section, it is now known that suppressor and helper T cells play an important role in the IgE responsiveness of mice and rats. There are antigen-specific helper and suppressor T cells which respectively enhance and suppress IgE response to specific antigen (Tada and Okumura, 1971; Ishizaka, 1976; Tada, 1976). Recently, a second population of antigen non-specific suppressor T cells has been described, whose action is apparently to prevent the induction of all IgE antibody responses (Watanabe et al., 1976; Chiorazzi et al., 1977).

Hooded Lister and Brown Norway rats by comparison with the other 'poor IgE producing' strains used here are either deficient in this latter population of cells or in a mechanism influencing their expression. The antigen-specific suppressive mechanism is however activated following immunisation with a large dose of antigen and

is thought to cause the inhibitory effect on booster IgE responses. It is likely that the other rat strains examined here possess more active versions of both suppressive mechanisms.

It is interesting in this context, that the lymphocytes of Brown Norway rats have been found to respond less vigorously than those of Lewis rats to the T cell mitogens phytohaemagglutinin and concanavalin A (Newlin and Gasser, 1973; Williams et al., 1973). Thus, the incorporation of tritiated thymidene was much lower in Brown Norway lymph node cells following stimulation of cell cultures with the above mitogens. These results reflected a difference between the two strains either in relative numbers of reactive T cells or in their ability to divide as a result of influences from other cell types. It is interesting also that elevation of total serum IgE occurred after infection in the poor responder strains of rat, even where the potentiated response was absent. An interesting feature of the AS/BN hybrid rat, is that the IgE levels after N. brasiliensis infection, are intermediate between those of the parent strains, neither of the characteristics of the parent strains, i.e. high level/BN nor low level/AS, being obviously dominant.

It seems probable that the total serum IgE produced in infection is related directly to the basal serum IgE level which also varies considerably among different rat strains (Bennich et al., 1976). Similar strain dependent differences in rat total IgE before and after Schistosoma mansoni infection have been demonstrated by Rousseaux-Prevost et al., (1977).

It is clear that the variation in IgE response seen in the different rat strains following conventional immunisation or helminth infection reflects quantitative or qualitative differences in the components of the IgE regulatory network. The preliminary experiments reported here will be extended in an attempt to obtain a clearer understanding of the immunoregulatory effect.

REFERENCES

Allison, A.C. and Davies, A.J.S. (1971). Nature, 233, 330.

Anderson, J., Moller, G. and Sjoberg, O. (1972). Cellular Immunol., 4, 381.

Augustin, R. (1967). "Handbook of Experimental Immunology", Ed. D.M. Weir. Blackwell, Oxford, p. 42.

Avrameas, S. and Ternynck, T. (1969). Immunochemistry, 6, 5 .

Barth, E.E.E., Jarrett, W.F.H. and Urquhart, G.M. (1966). Immunology, 10, 459.

Bazaral, M., Orgel, Alice and Hamburger, R.N. (1973). Clin. Exp. Immunol., 14, 117.

Bazin, H., Beckers, A., Deckers, C. and Moriame, M. (1973). J. Natn. Cancer Inst., 51, 1351.

Bennich, H. and Dorrington, K. (1973). "Biological Role of the Immunoglobulin E System", Eds. K. Ishizaka and D.H. Dayton. Nat. Inst. of Chld. Hlth. and Human Dev., Bethseda, Maryland, p.19.

Bennich, H. and Johansson, S.G.O. (1968). "Gammaglobulins", Ed. K. Killander. Wiley Interscience, New York, p.199.

Bennich, H. and Johansson, S.G.O. (1971). Adv. Immunol., 13, 1.

Bennich, H., Ishizaka, K., Johansson, S.G.O., Rowe, D.S., Stanworth, D.R. and Terry, W.H. (1968). Bull. Wld. Hlth. Org., 38, 51.

Bennich, H., Johansson, S.G.O., Von-Bahr-Lindstrom, H. and Karlsson, T. (1976). "Molecular and Biological Aspects of the Acute Allergic Reaction." Eds. S.G.O. Johansson, K. Strandberg and B. Uvnas. Plenum, New York, p.175.

Berg, T. and Johansson, S.G.O. (1969a). Int. Arch. Allergy and Applied Immunol., 36,219.

Berg, T. and Johansson, S.G.O. (1969b). Acts. Paediat. Scand., 58, 513.

Berglund, G., Finnstrom, O., Johansson, S.G.O. and Moller, K.L. (1968). Acta. Paediat. Scand., 57, 89.

Bernstein, I.D. and Ovary, Z. (1968). Int. Arch. Allergy, 33, 521.

Bienstock, J. (1974). "Progress in Immunology II", Vol. 4. Eds. L. Brent and J. Holborrow. p.197.

Binaghi, R.A. and Benacerraf, B. (1964). J. Immunol., 92, 920.

Binaghi, R.A., Benacerraf, B., Bloch, K. and Kourilsky, F. (1964). J. Immunol., 92, 927.

Blackeley, C.H. (1893). Experimental Researches on the causes and nature of Catarrhus aestivus (hay fever or hay asthma). Dawsons of Pall Mall, London.

Bourne, H.R., Lichenstein, L.M., Melman, K.L., Henney, C.S., Weinstein, Y. and Shearer, G.M. (1974). Science, 184, 19.

Brocklehurst, W.E. (1973). "Handbook of Experimental Immunology". Ed. D.M. Weir. Blackwell, Oxford, p.21.

Ceska, M. and Lundkvist, U. (1972). Immunochemistry, 9, 1021.

Chiorazzi, N., Fox, D.A. and Katz, D.H. (1977). J. Immunol., 118, 48.

Clausen, C.R., Munoz, J. and Bergmann, R. (1969). J. Immunol., 103, 768.

Coca, A.F. and Grove, E.F. (1925). J. Immunol., 10, 445.

Conrad, D.H., Bazin, H., Sehon, A.H. and Froese, A. (1975). J. Immunol., 114, 1688.

Coombs, R.A., Howard, A.N. and Mynors, L.S. (1953). Brit. J. Exp. Path., 34, 525.

Coombs, R.A., Hunter, A., Jonas, W.E., Bennich, H., Johansson, S.G.O. and Panzani, R. (1968). Lancet i, 115.

Crabbe, P.A., Nash, D.R., Bazin, H., Eysson, H. and Heremans, J.F. (1969). J. Exp. Med., 130, 723.

Dale, H.H. (1913). J. Pharmacol., 4, 167.

Danneman, Pamela J. and Micheal, J.G. (1976). Cellular Immunol., 22, 128.

Dresser, D.W. and Phillips, J.M.(1973). "Immunopotentiation, Ciba Foundation Symposium No. 18". Elsevier, Amsterdam, p.3.

Dutton, R.W. (1972). J. Exp. Med., 136, 1445.

Egan, H.S., Reeder, W.J. and Ekstedt, R.D. (1974). J. Immunol., 112, 63.

Franklin, E.C. (1964). Prog. Allergy, 8, 58.

Freund, J. (1956). Adv. Tuberc. Res., 7, 130.

Gleich, G.J., Averbeck, A.K. and Swedlund, H.A. (1971). J. Lab. Clin. Med., 77, 690.

Gleich, G.J. and Jacob, G.L. (1975). Science, 190, 1106.

Gollapudi, U.S.S. and Kind, L.S. (1975). Int. Arch. Allergy, 48, 94.

Gollapudi, U.S.S. and Kind, L.S. (1977). Int. Arch. Allergy, 53, 569.

Goodfriend, L., Kovacs, B.A. and Rose, B. (1966). J. Allergy, 37, 122.

Greenwood, F.C., Hunter, W.M. and Glover, J.S. (1963). Biochem. J., 89, 114.

Hamaoka, T. and Katz, D.H. (1973). J. Immunol., 111, 1554.

Herbert, W.J. (1973). "Handbook of Experimental Immunology". Ed. D.M. Weir. Blackwell, Oxford, Appendix 2.

Heremans, J.F. (1969). Nat. Inst. Chld. Hlth. and Human Dev., Bethseda, Maryland, p.309.

Heremans, J.F. and Vaerman, I.P. (1962). Nature, 193, 1091.

Hoffman, D.R., Yamamoto, F.Y., Geller, B. and Haddad, Z. (1975). J. Allergy Clin. Immunol., 55, 256.

Hogarth-Scott, R.S., Johansson, S.G.O. and Bennich, H. (1969). Clin. Exp. Immunol., 5, 617.

Huldt, G., Johansson, S.G.O. and Lantto, S. (1973). Arch. Environ. Hlth., 26, 36.

Ishizaka, K. (1976). "Molecular and Biological Aspects of the Acute Allergic Reaction". Eds. S.G.O. Johansson, K. Strandberg and B. Uvnas. Plenum, New York, p.59.

Ishizaka, K. and Ishizaka, T. (1968c). J. Immunol., 101, 68.

Ishizaka, K. and Ishizaka, T. (1971). "Biochemistry of the Acute Allergic Reaction". Eds. K.F. Austin and E.L. Becker. Blackwell, Oxford, p.13.

Ishizaka, K., Ishizaka, T. and Hornbrock, M.M. (1966b). J. Immunol., 97, 75.

Ishizaka, K., Ishizaka, T. and Hornbrook, M.M. (1966c). J. Immunol., 97, 840.

Ishizaka, K., Ishizaka, T. and Lee, E.H. (1966a). J. Immunol., 37, 336.

Ishizaka, K., Ishizaka, T. and Menzel, A.E.O. (1967). J. Immunol., 99, 610.

Ishizaka, K., Ishizaka, T. and Terry, W. (1967c). J. Immunol., 99, 849.

Ishizaka, K. and Okudaira, H. (1972). J. Immunol., 109, 84.

Ishizaka, T., Soto, C. and Ishizaka, K. (1972b). J. Immunol., 109, 1290.

Ishizaka, T., Soto, C. and Ishizaka, K. (1973). J. Immunol., 111, No. 2, 500.

Ishizaka, K., Tomioka, H. and Ishizaka, T. (1970). J. Immunol., 105, No. 6, 1459.

Janossy, G. and Greaves, M.F. (1971). Clin. Exp. Immunol., 9, 483.

Jarrett, E.E.E. (1976). "Molecular and Biological Aspects of the Acute Allergic Reaction". Eds. S.G.O. Johansson, K. Strandberg and B. Uvnas. Plenum, New York, p.105.

Jarrett, E.E.E. and Bazin, H. (1974). Nature, 251, 613.

Jarrett, E.E.E., Henderson, D. Riley, P. and White, R.G. (1972). Int. Arch. Allergy, 42, 475.

Jarrett, E.E.E., Orr, T.S.C. and Riley, P. (1971). Clin. Exp. Immunol., 9, 585.

Jarrett, E.E.E. and Stewart, D. (1972). Immunology, 23, 749.

Jarrett, E.E.E. and Stewart, D. (1974). Immunology, 27, 365.

Jarrett, E.E.E., Jarrett, W.F.H. and Urquhart, G.M. (1968a). Parasitology, 58, 625.

Johansson, S.G.O. (1967). Lancet ii, 951.

Johansson, S.G.O. (1968a). Int. Arch. Allergy, 34, 1.

Johansson, S.G.O. and Bennich, H. (1967). Immunology, 13, 381.

Johansson, S.G.O., Bennich, H. and Wide, L. (1968). Immunology, 14, 265.

Johansson, S.G.O., Mellbin, T. and Vahlquist, B. (1968c). Lancet i, 1118.

Kishimoto, T. and Ishizaka, K. (1973). J. Immunol., 111, 1194.

Korenblatt, P.E., Rothberg, R.M., Minden, P. and Farr, R.S. (1968). J. Allergy, 41, 226.

Korotzer, J.L., Haddad, Z.H. and Lopapa , A.F. (1971). Immunology, 20, 245.

Lehrer, S.B., Vaughan, J.H. and Tan, E.N. (1975). J. Immunol., 114, 34.

Lehrer, S.B., Vaughan, J.H. and Tan, E.N. (1976). J. Immunol., 116, 178.

Levine, B. and Vaz, N.M. (1970). Int. Arch. Allergy, 39, 156.

Lindstrom von-Bahr, H. and Bennich, H. (1974). FEBS letters, 40, 57.

Magro, A.M. and Bennich, H. (1977). Immunology, 33, 51.

Mancini, G., Carbonara, A.O. and Hereman, J.F. (1965). Immunochemistry, 2, 235.

Mann, D., Granger, H. and Fahey, J.L.(1969). J. Immunol., 102, 618.

Markham, P., Reed, N.D., Stashak, P.W., Prestcott, B., Amsbaugh, D.F. and Baher, P.J. (1977). J. Immunol., 119, 1163.

McDougall, W.A. (1974). B.Sc., Honours Thesis, Faculty of Science, University of Glasgow.

McLaughlin, P., Stanworth, D.R., Kennedy, J.F. and Cho Tun, H. (1971). Nature (New Biol.), 232, 245.

Miller, H.R.P. and Jarrett, W.F.H. (1971). Immunology, 20, 277.

Morgan, C.R. and Lazerow, A. (1963). Diabetes, 12, 115.

Morse, S.I. and Morse, J.H. (1976). J. Exp. Med., 143, 1483.

Mota, I. (1964). Immunology, 7, 681.

Mota, I. (1967). Immunology, 12, 343.

Mota, I., Perini, A. and Truidale, V.S. (1974). Int. Arch. Allergy, 47, 425.

Murphey, S.H., Brown, S., Miklos, N. and Fireman, P. (1974). Immunology, 27, 245.

Murray, M. (1972). "Immunity to Animal Parasites". Ed. E.J.L. Soulsby. Academic Press, p.155.

Ness, D.B., Smith, S., Talcott, J.A. and Gruner, F.C. (1976). Eur. J. Immunol., 6, 650.

Newburger, P.E., Hamaoka, T. and Katz, D.H. (1974). J. Exp. Med., 113, 824.

Newlin, C. and Gasser, D. (1973). J. Immunol., 110, No. 3, 622.

Nirmul, G., Severin, C. and Taub, R.N. (1972). Transplantation, 14, 91.

Ogawa, M., Berger, P.A., McIntyre, O.R., Glendinning, W.E. and Ishizaka, K. (1971). Arch. Dermatol., 103, 575.

Ogawa, M., Kochwa, S., Smith, C., Ishizaka, K. and McIntyre, O.R. (1969). New Eng. J. Med., 281, 1217.

Ogilvie, B. (1967). Immunology, 12, 113.

Okumura, K. and Tada, T. (1974). J. Immunol., 112, 783.

Orr, T.S.C. and Blair, A.M.J. (1969). Life Sciences, 8, II, 1077.

Oudin, J. (1946). Complete Rend. Ac. Sci., 222, 115.

Ovary, Z. (1952). Int. Arch. Allergy and Applied Immunol., 3, 293.

Ovary, Z. (1964). "Immunological Methods Cloms Symposium". Ed. J.F. Ackroyd. Blackwell, Oxford, p.259.

Ovary, Z. (1965). Fed. Proc., 24, 94.

Parkins, R.A., Dimitriadou, A. and Booth, C.C. (1960). Clin. Sci., 19, 595.

Perelmutter, L., Liakopoulou, A. and Phills, J.A. (1973). "Mechanisms in Allergy". Eds. L. Goodfriend, A. Sehon and R.P. Orange. Marcel Decker Inc., New York, p.235.

Polmar, S.H., Waldmann, T.A. and Terry, W.D. (1973). J. Immunol., 110, 1253.

Prausnitz, C. and Kustner, H. (1921). Zbl. Bact. I. Abt. Orig., 86, 160.

Revoltella, R. and Ovary, Z. (1969). Immunology, 17, 45.

Rich, R.R. and Pierce, C.W. (1973). J. Exp. Med., 137, 649.

Romball, C.G. and Weigle, W.O. (1975). J. Immunol., 115, 556.

Rosenberg, E.B., Whalen, G.E., Bennich, H. and Johansson, S.G.O. (1971). New Eng. J. Med., 283, 1148.

Rousseaux-Prevost, R., Bazin, H. and Capron, A. (1977). Immunology, 33, 501.

Rowe, D.S. (1969). Bull. Wld. Hlth. Org., 40, 613.

Rowe, D.S., Tackett, L., Bennich, H., Ishizaka, K., Johansson, S.G.O. and Anderson, S.G. (1970). Bull. Wld. Hlth. Org., 43, 609.

Salmon, S.E., Mackey, G. and Fudenberg, H.H. (1969). J. Immunol., 103, 129.

Sanyal, R.J. and West, G.B. (1958). J. Physiol. (London), 142, 571.

Smith, S.R. and Pertillo, J. (1976). Int. Arch. Allergy, 52, 21.

Stanworth, D.R. (1973). "The Molecular Basis of the Allergic Response."

Stanworth, D.R., Humphrey, J.H., Bennich, H. and Johansson, S.G.O. (1968). Lancet ii, 17.

Stanworth, D.R. and Kuhns, W.J. (1965). Immunology, 8, 323.

Stechsculte, D.J., Orange, R.P. and Austen, K.F. (1970). J. Immunol., 105, 1083.

Stewart, D.F. (1953). Aust. J. Agric. Res., 4, 100.

Strannegard, O. and Belin, L. (1970). Immunology, 18, 775.

Strannegard, O. and Chan, P.C.Y. (1969). J. Allergy, 43, 224.

Tada, T. (1975). Prog. in Allergy, 19, 122.

Tada, T. (1976). "Molecular and Biological Aspects of the Acute Allergic Reaction". Eds. S.G.O. Johansson, K. Strandberg and B. Uvnas. Plenum, New York, p.79.

Tada, T. and Ishizaka, K. (1970). J. Immunol., 104, 377.

Tada, T. and Okumura, K. (1971). J. Immunol., 106, 1002.

Tada, T. and Okumura, K. (1971). J. Immunol., 107, 1137.

Tada, T., Okumura, K. and Iwara, S. (1972). Int. Arch. Allergy, 43, 207.

Takatsu, K. and Ishizaka, K. (1975). Cellular Immunol., 20, 276.

Taylor, B., Norman, A.F., Orgel, H.A., Stokes, C.R., Turner, M.W. and Soothill, J.F. (1973). Lancet ii, 111.

Waldmann, T.A. (1969). New Eng. J. Med., 281, 1170.

Walls, R.S. (1977). Proc. Soc. Exp. Biol.,Med., 156, 431.

Watanabe, N., Kojima, S. and Ovary, Z. (1976). J. Exp. Med., 143, 833.

W.H.O. Technical Report 595 (1976). Immunological Adjuvants. World Hlth Org., Geneva.

Wide, L., Bennich, H. and Johansson, S.G.O. (1967). Lancet ii, 1105.

Wide, L. and Porath, J. (1966). Biochem, Biophys, Acta., 130, 257.

Williams, R.M., Moore, M.J. and Benacerraf, B. (1973). J. Immunol., 111, No. 5, 1571.

Unanue, E.R., Askonas, B.A. and Allison, A.C. (1969). J. Immunol., 103, 71.

Urquhart, G.M., Mulligan, W., Eadie, R.M. and Jennings, F.W. (1965). Exp. Parasitol., 17, 210.

Vaz, N.M. and Levine,B.(1970). Sciences, 168, 852.