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REGULATORY EFFECTS OF ANTIGEN AND ANTIBODY ON THE REAGIN RESPONSE IN RABBITS

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SUMMARY

The influence of antigen dosage on reagin formation to haemocyanin in rabbits was studied. Using large doses of antigen, reagin formation was more readily elicited, the induction period was shorter and the persistence of reagins in serum was longer than when low antigen doses were employed. Reagin synthesis was readily induced after intravenous administration of antigen. Immunization with antigen mixed with varying doses of IgG antibody resulted in a suppressed formation of antibodies reactive in passive haemagglutination and in a total inhibition of reagin formation. The inhibition of reagin synthesis was long-lasting and booster injections of antigen did not result in the appearance of reagins. 'Hyposensitization' with series of injections of antigen or antigen–antibody mixtures resulted in an initial decrease of the reagin titres, but restoration of reagin levels was observed already about 2 weeks following cessation of the treatment. The possible implications of the results for the specific immunologic therapy of allergic disorders are discussed.

INTRODUCTION

The reaginic (homocytotropic) antibodies of the rabbit appear to constitute a unique immunoglobulin class (Lindqvist, 1969; Strannegård & Chan, 1969; Zvaifler & Robinson, 1969; Ishizaka, Ishizaka & Hornbrook, 1970). Although the reaginic antibodies may be heterogeneous (Henson & Cochrane, 1968) the reagins produced in response to stimulation with haemocyanin, which has been used as antigen in the present studies, appear to correspond to human IgE (Strannegård & Belin, 1970). The kinetics of reagin formation in rabbits has been studied recently and several lines of evidence, including the demonstration that passively administered IgG antibodies inhibit primary reagin formation, indicate that reagin formation is subject to antibody-induced regulation (Strannegård & Yurchision, 1969a, b; Strannegård & Belin, 1970). It has also been found that passively administered

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IgM antibodies may, in contrast to IgG antibody, stimulate reagin synthesis (Strannegård & Belin, 1971). It is not known, however, whether or not these regulatory effects of antibody on reagin synthesis operate under physiological conditions. The fact that reagin synthesis appears to be very short-lived in many cases would be compatible with a feedback inhibition. In the present report we have tried to gain some information about this problem by studying the influence of antigen dosage on reagin formation. The inhibitory effect of antibody, which we have demonstrated earlier, has been further studied using immunization with antigen–antibody mixtures.

Since the rabbit may provide a good model for the study of human allergic disease, it was considered of interest to mimic clinical hyposensitization using this model. In the present report the results of such studies are also described.

MATERIALS AND METHODS

Animals. White random-bred rabbits weighing 2–2·5 kg were used for all immunizations. For homologous passive cutaneous anaphylaxis (PCA) tests rabbits weighing 3–3·5 kg were used.

Antigen. Haemocyanin was prepared from *Buccinum undatum* as previously described (Strannegård & Belin, 1970).

The techniques used for homologous PCA, passive haemagglutination (HA), treatment with 2-mercaptoethanol and sucrose density ultracentrifugation have been described previously (Strannegård & Belin, 1970). Preparation of IgG antibodies was performed using DEAE-cellulose chromatography. The IgG antibodies were eluted with a 0·0175 M phosphate buffer, pH 6·3.

Direct skin tests were performed by injection of 0·5, 1, 2 and 4 μg of haemocyanin intradermally in 0·2 ml volumes into animals which had previously been injected intravenously with 2 ml of 1% Evans blue. These amounts of antigen gave negative reactions in normal rabbits whereas higher doses were found to give nonspecific toxic reactions.

RESULTS

Influence of antigen dosage on the reagin response

Five groups of rabbits with four animals in each group were injected in the hind footpads with varying doses of haemocyanin in Freund's adjuvant. The rabbits were bled before the injection, then after 3, 6, 8, 10 and 14 days and thereafter at weekly intervals. All sera were tested for antibodies by means of the HA and homologous PCA tests. The doses of 50 μg, 500 μg and 5 mg induced a reagin response in all of the injected rabbits whereas 5 μg gave rise to reagin formation in three of four animals and 0·5 μg in two of four animals. The kinetics of reagin formation is shown in Fig. 1(a). The induction period for reagin formation was longer with the low antigen dosages and the reagin titres attained were lower than observed after stimulation with high antigen doses. The reagin response elicited by low antigen dosage was usually less well sustained than that caused by high antigen dosage. All antigen doses employed gave rise to marked responses of haemagglutinating antibodies, which were clearly dose dependent (Fig. 1b). Haemagglutinating antibodies were demonstrable 6 days after immunization in all cases. The first antibody to appear was sensitive to treatment with 2-mercaptoethanol. Antibodies resistant to this treatment were demonstrable

after 8 days in the rabbits given high antigen dosage but not until after 10–14 days in the rabbits given the lowest antigen dosage. By sucrose density gradient ultracentrifugation it was shown that the antibodies, which were resistant to treatment with 2-mercaptoethanol, sedimented as 7S and those sensitive to this treatment, as 19S antibodies. They are therefore in the following referred to as IgG and IgM antibodies, respectively.

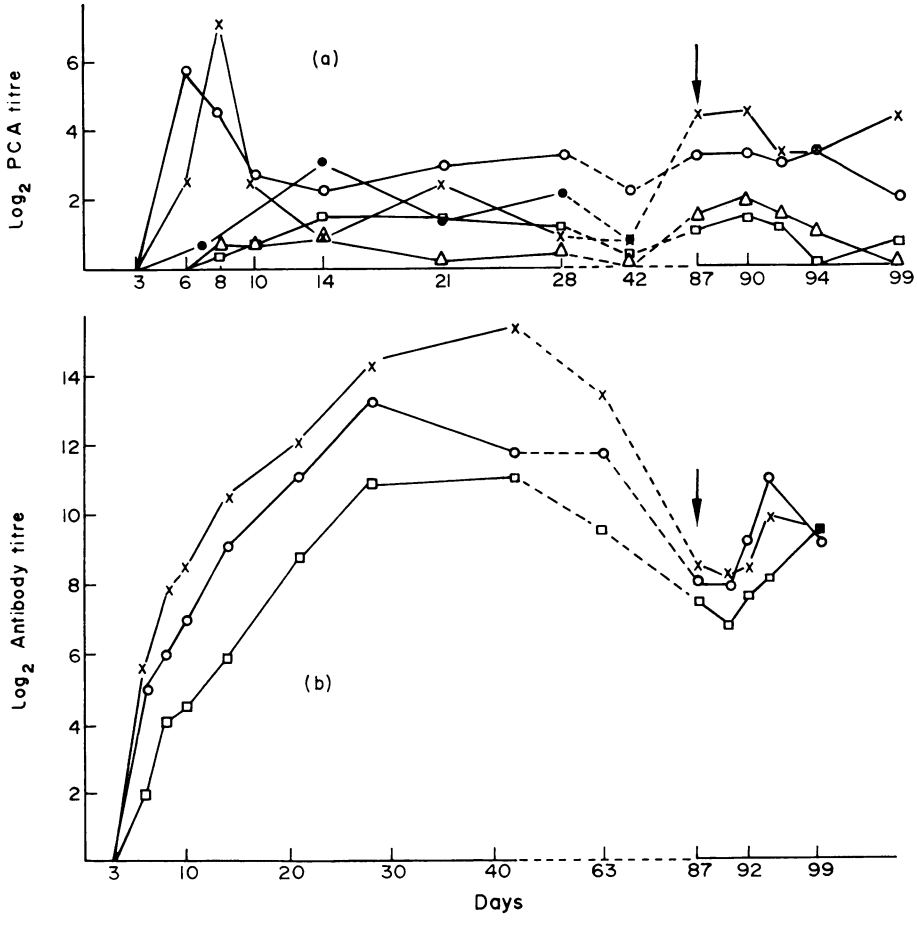

FIG. 1. Influence of antigen dosage on the formation of reaginic (a) and haemagglutinating (b) antibodies. Each point represents geometric mean antibody titre of sera from four animals injected with the following amounts of haemocyanin: ×, 5 mg; ○, 500 μg; ●, 50 μg; △, 5 μg; □, 0·5 μg. The arrow indicates time for restimulation of each animal with 1 mg haemocyanin in Freund's complete adjuvant.

A subcutaneous booster injection of 1 mg haemocyanin 12 weeks after the primary injection resulted in a secondary response of haemagglutinating antibodies. Two out of eight rabbits which had been given 0·5 or 5 μg haemocyanin as a primary stimulus developed a secondary reagin response but none of the rabbits which had been immunized with 0·5 or 5 mg haemocyanin showed a similar secondary response. The rabbits which had not

responded to the primary antigen stimulation did not respond after the secondary stimulation. In several cases there was a significant fall of reagin titres after the secondary stimulation.

Reagin formation after intravenous administration of antigen

One intravenous injection of 5 mg of haemocyanin resulted in an evident reagin response and a moderate response of agglutinating antibodies in all of the injected rabbits (Fig. 2). The reagin and HA antibody response was more slowly mounted than observed after injection of the same amount of antigen in adjuvant. A secondary injection subcutaneously of 0·5 mg haemocyanin in Freund's adjuvant 3 weeks after the primary injection resulted in a boosted

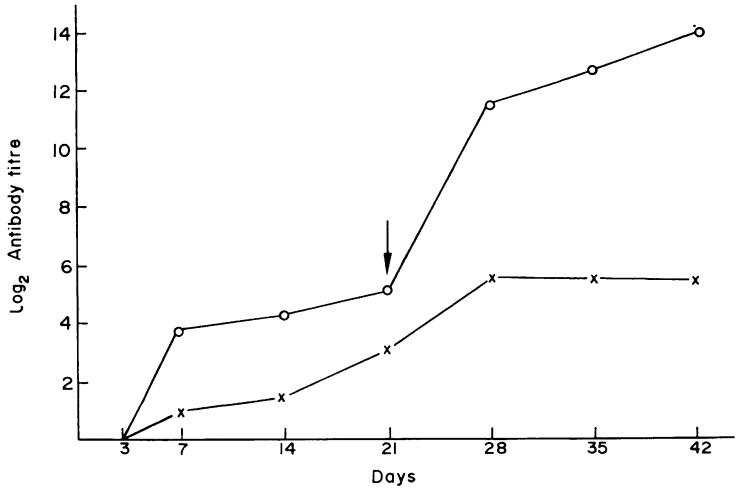

FIG. 2. Formation of reaginic and haemagglutinating antibodies after intravenous administration of antigen. Each point represents geometric mean antibody titre of sera obtained from four rabbits immunized with 5 mg haemocyanin intravenously. Arrow indicates restimulation with 0·5 mg haemocyanin in Freund's complete adjuvant. ○, HA antibodies; ×, reagins.

response of reaginic as well as agglutinating antibodies. When 5 mg haemocyanin was given intravenously, 1 day prior to stimulation with 0·5 mg haemocyanin subcutaneously in Freund's adjuvant, a reagin response followed that was about equal in magnitude to that elicited by antigen in adjuvant only.

Effect of injection of antigen–antibody mixtures

A pool of IgG antihaemocyanin antibodies was made from rabbit hyperimmune sera submitted to DEAE chromatography. The HA titre of the pooled IgG antibody was 2^{13}. Three groups of rabbits with four animals in each group were injected with mixtures of 50 μg haemocyanin and 0·5 ml of the IgG fraction diluted 1/100, 1/10 or undiluted, respectively. All three mixtures contained antibody in excess as shown by HA-tests performed on supernatants of similar, centrifuged, mixtures. After allowing the antigen–antibody mixtures to stand at room temperature for 30 min they were emulsified in Freund's complete adjuvant and injected in the hind footpads of the rabbits. All twelve rabbits responded by

the formation of agglutinating antibodies but none of them produced humoral reagins. The formation of agglutinating antibodies was suppressed according to dose of IgG antibody in the injection mixtures (Fig. 3). 6 weeks after the primary injection of antigen plus antibody a secondary injection of 0·5 mg haemocyanin in Freund's complete adjuvant was given. None of the twelve rabbits responded with reagin formation upon the booster injection although they showed a boosted response of agglutinating antibodies, and none gave a positive skin test upon injection of subtoxic doses of haemocyanin intradermally.

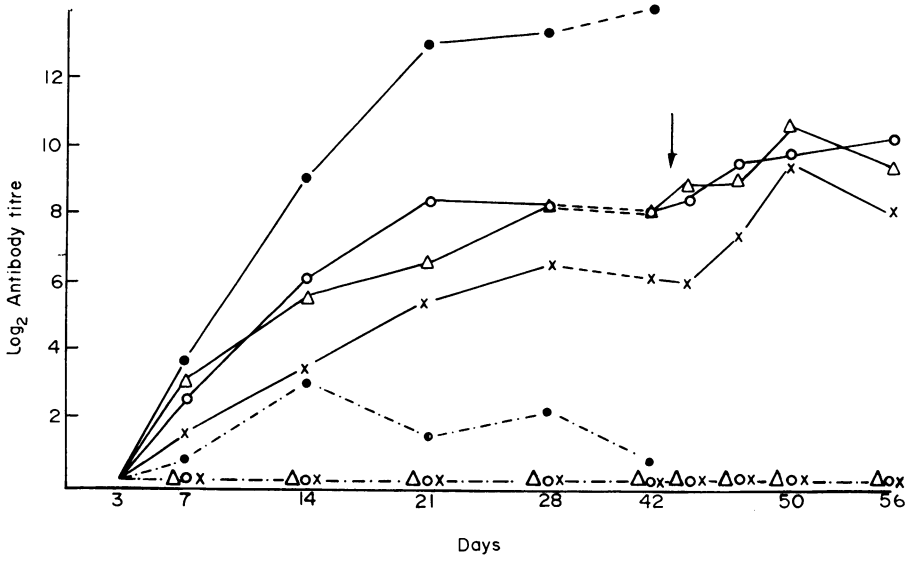

FIG. 3. Antibody response after injection of antigen–antibody mixtures. The figure illustrates the haemagglutinating antibody response after injection of 50 μg haemocyanin mixed with an IgG antibody preparation, diluted as indicated. No reagin response was obtained in any of the rabbits immunized with antigen plus antibody. Titres of agglutinating and reaginic antibodies (●) of sera from control animals injected with 50 μg haemocyanin only are also indicated. Arrow represents restimulation with 0·5 mg haemocyanin. ×, Antibody 1:1; △, antibody 1:10, ○, antibody 1:100; ●, no antibody; –·–, reagins.

'Hyposensitization' experiments

Seven rabbits which had been stimulated with haemocyanin twice and had persistent levels of serum reagins were each given a series of subcutaneous injections of haemocyanin three times daily according to the following scheme: day 0: 0·1 μg, 1 μg and 10 μg; day 1: 50 μg, 100 μg and 200 μg; day 2: 400 μg, 800 μg and 1·6 mg. Six control rabbits which had previously been given similar antigen injections as the test rabbits were not given the series of subcutaneous injections. The reaginic antibody titres of the sera of the test and control rabbits following hyposensitization are given in Fig. 4. As seen in the figure the hyposensitization treatment decreased the reagin titres notably. This result could be repeated with the rabbits from the initial control group which were hyposensitized after another 2 months. The depression of the reagin levels was most evident at an initial stage following hyposensitization, but it was not permanent, as shown by a rise in reagin titre 2 weeks later.

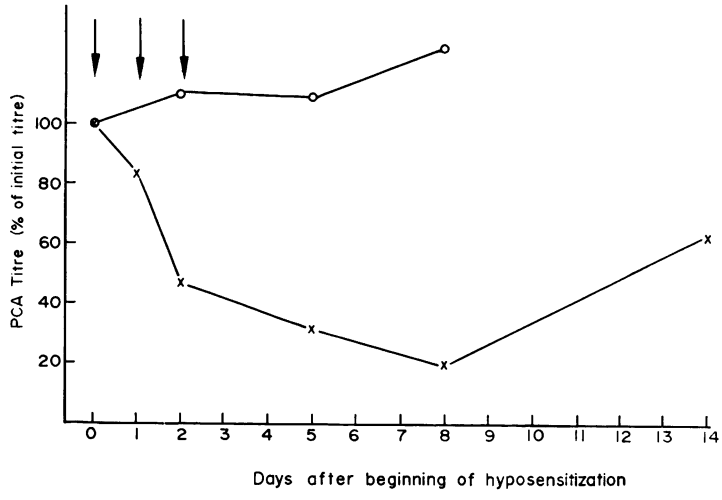

FIG. 4. Effect of 'hyposensitization' with a series of increasing doses of antigen. The figure gives geometric mean homologous PCA titres, expressed as percentage of initial titre, of sera from five rabbits undergoing hyposensitization day 0, 1 and 2 (arrows) and from four untreated control rabbits. The mean titre of sera from the untreated rabbits, obtained 63 days after the beginning of hyposensitization, was 77% of the initial titre. ○, Untreated; ×, hyposensitized.

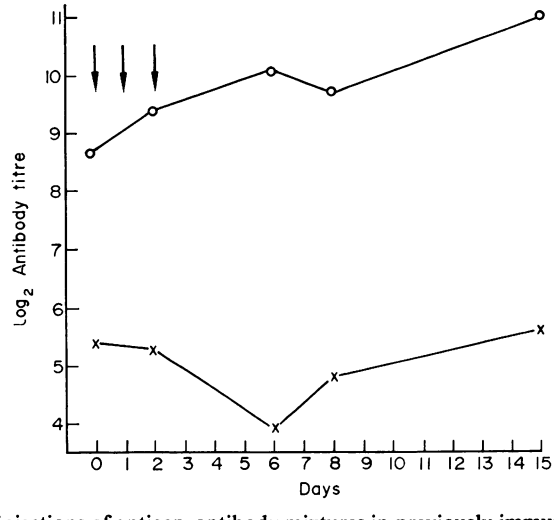

FIG. 5. Effect of injections of antigen–antibody mixtures in previously immunized rabbits. The geometric mean titres of sera from seven rabbits injected day 0, 1 and 2 (arrows) with antigen–antibody mixtures, as given in the text, are illustrated. ○, HA antibodies; ×, reaginic antibodies.

The hyposensitization resulted in a secondary type response of haemagglutinating antibodies, and no significant decrease of the HA titres comparable to that noted for the reagin titres was observed.

In other experiments seven rabbits which had high reagin titres resulting from intravenous and subcutaneous immunizations were injected five times during 3 days with mixtures of

IgG antibody and haemocyanin. Each mixture consisted of 0·5 ml of a 1/10 dilution of the IgG-fraction described above and varying doses of haemocyanin, the first injection mixture containing 5 μg and the following 50 μg, 0·5 mg, 1 mg and 1 mg, respectively. As shown in Fig. 5, this combined treatment resulted in a decrease of the serum reagin titres which, however, were restored about 2 weeks following the cessation of treatment. Similarly as was observed for hyposensitization with antigen only, the HA titres were raised upon treatment without any preceding initial decrease of the titres.

DISCUSSION

The present report confirms earlier findings (Strannegård & Belin, 1970) of an antibody-induced suppression of the reagin response in rabbits. It is not clear whether this suppression operates during the normal antibody response but judging from the results of Graf & Uhr (1969) concerning antibody synthesis *in vivo*, in animals continuously deprived of the produced antibody, such a mechanism seems probable. Antibody-induced suppression of reagin synthesis can evidently be easily obtained during the induction of reagin synthesis but so far there is no strong evidence for a suppressive effect later on during the reagin response. Haemocyanin is known to elicit a long-lived 19S antibody response (Dixon, Jacot-Guillarmod & McConahey, 1967; Strannegård & Belin, 1970) in contrast to many other antigens. In analogy with this the reagin response was also found to be long lived, especially after stimulation with high antigen doses. It is therefore possible that feedback inhibition *in vivo* of the synthesis of various classes of immunoglobulins is not a prominent feature in the haemocyanin system. In several other systems reagin synthesis has been shown to be more shortlived.

The dose–response relationship obtained with varying doses of haemocyanin shows that, within the dose range studied, the reagin response is similarly dependent on the antigen dose as is the synthesis of IgM and IgG antibodies. The results indicated that a higher dose of the antigen was needed for the elicitation of a reagin response than to elicit IgG and IgM antibody formation. This finding would be compatible with an occurrence of fewer potential reagin-producing cells than of IgG- or IgM-producing cells. In fact IgE occurs in extremely low concentrations in human serum (Johansson & Bennich, 1967) and with the aid of fluorescent antibody tracing it has been shown that IgE-producing cells are very scarce in most body tissues (Tada & Ishizaka, 1970). Other investigators have proposed that low antigen doses preferentially give rise to reagin formation (Revoltella & Ovary, 1969). These results may be explained if it is assumed that the postulated antibody-induced suppression of reagin synthesis is a more prominent feature in their systems than in the haemocyanin system used by us.

The failure to obtain reagin production upon challenge of animals which had previously shown an IgM and IgG response but no reagin response, suggests that the antibody-induced suppression may be a determining factor for the keeping of a non-reagin-responding state. The results suggest that such an unresponsiveness may be achieved either by suppressing the primary reagin response with passive antibody or by stimulating the animals with very low doses of antigen.

It is of interest to note that reagin formation may take place in the absence of adjuvant added to the antigen. In studies using bovine serum albumin as antigen it was not possible to elicit reagin formation with intravenously administered antigen (Strannegård & Yurchi-

sion, 1969a). In the present system, however, this was readily achieved and it was not possible to produce a state analogous to partial tolerance or immune deviation in the way described for delayed hypersensitivity (Axelrad, 1968). Many orally introduced antigens readily pass the mucosa to get into the circulation (Bernstein & Ovary, 1968). Since intravenous administration of antigen evidently may result in reagin production, orally or intranasally introduced antigens should therefore be able to give rise to circulating reagins. In fact both the oral and nasal routes have been successfully used in the rabbit for the elicitation of a reagin response (Strannegård & Yurchision, 1969b; Strannegård & Belin, to be published).

Our earlier studies have indicated that one mechanism for the effect of hyposensitization therapy may be suppression of reagin response by IgG antibody. The present results suggest still another mechanism, namely consumption of reagins by the injected antigen. Such a consumption may give a beneficial effect on an allergic state, provided the antigen reacts primarily with circulating, and to a lesser extent with skin-fixed reagins. A decrease of reagin titres has been obtained in hyposensitization of patients with penicillin allergy (Levine *et al.*, 1967; Fellner *et al.*, 1970). The recent experiments in humans using the radioallergosorbent test, by Berg & Johansson (1970), however, indicate that the reagin titres are not significantly affected during the first few days of hyposensitization treatment, but rise to high levels thereafter in many cases. The difference between these results and the present ones probably reflects differences in type of antigen and in the antigen dosages employed.

The present results suggest that a high dose of antigen would be desirable in hyposensitization, since it will induce the formation of large quantities of IgG antibodies which are potential suppressors of reagin synthesis and also will result in consumption of certain amounts of reagins. On the other hand, such large antigen doses will react with skin-fixed reagins and thereby cause immediate symptoms. The latter difficulty can be circumvented to some extent by giving gradually increasing doses of antigen as is done in clinical hyposensitization. Another possibility for giving high doses of antigen without provoking allergic reactions would be to inject antigen–antibody mixtures, thereby preventing the appearance of large quantities of free antigen in the circulation and at the same time administering passive, potentially suppressive, antibody. Injection of antigen–antibody mixtures containing small amounts of antibody will result in an initially suppressed response of IgG and IgM antibody but the serum antibody titres may eventually reach levels which are higher than in animals given antigen only (Dixon *et al.*, 1967). This phenomenon might be advantageous if antigen–antibody mixtures were to be used in hyposensitization.

It is evident that antigen–antibody mixtures may stimulate IgG and IgM antibody formation in the absence of a reagin response. If potentially reagin forming cells are not triggered after this stimulation, there are no primed cells to be stimulated upon a following antigen challenge, and the previously formed IgG antibodies will then presumably act as effective inhibitors of reagin synthesis. A long-lasting unresponsiveness as regards reagin formation will result.

Thus, in the primary response, reagin synthesis can be conveniently depressed, using antigen–antibody mixtures. In the clinical situation, however, where hyposensitization is considered, i.e. when reagin synthesis is already induced, permanent depression of reagin formation is probably much more difficult to achieve. In the present experiments attempts to use antigen–antibody mixtures for hyposensitization experiments resulted in a depression of the serum reagin levels although they were restored after cessation of the treatment. If it is indeed possible to give higher antigen doses using antigen–antibody mixtures rather

than antigen alone the use of antigen–antibody mixtures should be tried for the treatment of allergic disorders.

The present results and those of others concerning the possible mechanisms of hyposensitization would suggest several different modes of action. Firstly, blocking antibody resulting from injection therapy may combine with unintentionally introduced antigen preventing the latter from reacting with reagins. Secondly, blocking antibody may suppress the formation of reagins. Thirdly, injected antigen may consume circulating reagins, leading to temporarily reduced serum reagin levels. The first two of these mechanisms would be presumed to be most important clinically, although the second of these probably operates after prolonged treatment only. It is of great interest to note that hyposensitization may result in partial tolerance to grass pollen allergen in patients with hay fever, and that this partial tolerance is much better correlated to clinical improvement than is the production of IgG (blocking) antibodies (Brostoff & Roitt, 1970). Also, hyposensitization treatment appears to result in changes of cellular response mechanisms leading to decreased antigen-induced release of histamin from leucocytes of treated individuals (Levy & Lichtenstein, 1970).

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REFERENCES

AXELRAD, M.A. (1968) Suppression of delayed hypersensitivity by antigen and antibody. Is a common precursor cell responsible for both delayed hypersensitivity and antibody formation? *Immunology*, **15**, 159.

BERG, T. & JOHANSSON, S.G.O. (1970) IgE specific reagins during hyposensitization. *Acta paediat. scand.* **59**, Suppl. 206. p. 85.

BERNSTEIN, I.D. & OVARY, Z. (1968) Absorption of antigens from the gastro-intestinal tracts. *Int. Arch. Allergy*, **33**, 521.

BROSTOFF, J. & ROITT, I.M. (1970) Induction of tolerance to grass pollen in patients with summer hay fever. *Excerpta med., Int. Congr. Ser.* **211**, 136.

DIXON, F.J., JACOT-GUILLARMOD, H. & MCCONAHEY, P. (1967) The effect of passively administered antibody on antibody synthesis. *J. exp. Med.* **125**, 1119.

FELLNER, M.J., VAN HECKE, E., ROZAN, M. & BAER, R.L. (1970) Mechanisms of clinical desensitization in urticarial hypersensitivity to penicillin. *J. Allergy*, **45**, 55.

GRAF, M.N. & UHR, J.W. (1969) Regulation of antibody formation by serum antibody. I. Removal of specific antibody by means of immunoadsorption. *J. exp. Med.* **130**, 1175.

HENSON, P.M. & COCHRANE, C.G. (1969) Immunological induction of increased vascular permeability. I. A rabbit passive cutaneous anaphylactic reaction requiring complement, platelets and neutrophils. *J. exp. Med.* **129**, 153.

ISHIZAKA, K., ISHIZAKA, T. & HORNBROOK, M.M. (1970) A unique rabbit immunoglobulin having homocytotropic activity. *Immunochemistry*, **7**, 515.

JOHANSSON, S.G.O. & BENNICH, H. (1967) Studies on a new class of human immunoglobulins. I. Immunological properties. *Nobel Symposium* 3, *Gamma Globulins, Structure and Control of Biosynthesis*, p. 193. Almqvist & Wiksell, Stockholm.

LEVINE, B. B., REDMOND, A. P., VOSS, H. E. & ZOLOV, D.M. (1967) Prediction of penicillin allergy by immunological tests. *Ann. N.Y. Acad. Sci.* **145**, 298.

LEVY, D. & LICHTENSTEIN, L.M. (1970) Mechanisms of immunotherapy in pollen allergy. *Excerpta med. Int. Congr. Ser.* **211**, 136.

LINDQVIST, K.J. (1968) A unique class of rabbit immunoglobulins eliciting passive cutaneous anaphylaxis in homologous skin. *Immunochemistry*, **5**, 525.

REVOLTELLA, R. & OVARY, Z. (1969) Preferential production of rabbit reaginic antibodies. *Int. Arch. Allergy*, **36**, 282.

STRANNEGÅRD, Ö. & CHAN, P.C.Y. (1969) Association of rabbit reaginic antibody with an immunoglobulin distinct from IgG, IgA and IgM. *J. Allergy*, **43**, 224.

STRANNEGÅRD, Ö. & BELIN, L. (1970) Suppression of reagin synthesis in rabbits by passively administered antibody. *Immunology*, **18**, 775.

STRANNEGÅRD, Ö. & BELIN, L. (1971) Enhancement of reagin formation in rabbits by passively administered 19S antibody. *Immunology*, **20**, 427.

STRANNEGÅRD, Ö. & YURCHISION, A. (1969a) Formation of rabbit reaginic antibodies to protein and hapten–protein conjugates. *Immunology*, **16**, 387.

STRANNEGÅRD, Ö. & YURCHISION, A. (1969b) Formation of agglutinating and reaginic antibodies in rabbits following oral administration of soluble and particulate antigens. *Int. Arch. Allergy*, **35**, 579.

TADA, T. & ISHIZAKA, K. (1970) Distribution of γE forming cells in lymphoid tissues of the human and monkey. *J. Immunol.* **104**, 377.

ZVAIFLER, N.J. & ROBINSON, J. (1969) Rabbit homocytotropic antibody. A unique rabbit immunoglobulin analogous to human IgE. *J. exp. Med.* **130**, 907.