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The influence of IgE-mediated reactions on the expression of delayed hypersensitivity in the rat

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Summary. The effect of IgE-mediated reactions on the expression of delayed hypersensitivity skin reactions was examined in the rat. It was found that the elicitation of an IgE-mediated reaction at the time of skin test could either potentiate or inhibit the development of delayed reactions. At a fixed level of IgE sensitivity, large delayed reactions were potentiated and small delayed reactions suppressed. These interactions were not dependent on the two types of sensitivity being directed against the same antigen but were dependent on the reaction of IgE with its appropriate antigen.

INTRODUCTION

The observed reaction in most immunological responses is the resultant of a number of interactions between B-cell and T-cell compartments. The expression of T-cell functions may be modulated both by other T cells (Zembala & Asherson, 1973) and B cells (Katz, Parker & Turk, 1975). *In vitro* both the induction and the expression of T cell-mediated cytotoxicity may be inhibited by 7S antibody (Sinclair, Lees, Fagan & Birnbaum, 1975). Although the normal functional role of homocytotropic antibodies is unknown there is some evidence to suggest that the protective effects of antibodies of other immunoglobulin classes may be potentiated at sites of IgE-mediated reactions (Steinberg, Ishizaka & Norman, 1974). Were IgE to have a regulatory function, an effect on delayed hypersensitivity might be expected since both IgE antibody production and a phase of delayed-type hypersensitivity may be early and transient features of primary immune responses. Clinical studies have suggested that IgE-mediated reactions may be capable of suppressing the expression of delayed hypersensitivity in man. Delayed-type skin reactions are observed at the site of grass pollen skin tests in some hayfever sufferers in whom the immediate reaction has been suppressed with antihistamine drugs (Brostoff & Roitt, 1969). Here we report observations on the effect of IgE-mediated reactions on the delayed skin reaction to homologous and heterologous antigens in the rat.

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MATERIALS AND METHODS

Animals

Groups of ten inbred PVG/c rats were used between 11 and 14 weeks of age.

Immunization

Tuberculin sensitivity was produced by a single footpad injection of 250 μg freeze-dried, heat-killed human strain *Mycobacterium tuberculosis* contained in 0·05 ml incomplete Freund's adjuvant (I.F., Difco).

Ovalbumin immunization was performed by the injection of a total of 15 μg ovalbumin (OA) in 0·9% saline emulsified with an equal volume of IF or Freund's complete adjuvant (FCA). Animals given OA in FCA received a total of 0·375 μg *Mycobacteria*. Antigen emulsion, 0·05 ml, was injected i.d. into each of three sites: each hind footpad and a site over the sternum.

Nippostrongylus-potentiated anti-OA serum was obtained 24 days after the injection of 1 mg OA i.m. and 2×10^{10} *Bordetella pertussis* i.p. and 14 days after infection with 4000 *Nippostrongylus brasiliensis* larvae. Aliquots of pooled sera were stored at −16°.

Cyclophosphamide pretreatment

In some experiments animals were injected intraperitoneally 3 days before immunization with cyclophosphamide dissolved in sterile distilled water.

Antibody determination

Anti-OA antibodies were determined by either the tanned sheep red blood cell technique or by passive cutaneous anaphylaxis (PCA). The latter was performed using 0·1 ml serum dilution injections, a challenging i.v. dose of 1 mg OA in 0·5 ml 1% Evans blue and a latent period of either 2 or 48 h.

Skin testing

Skin tests were performed by the i.d. injection of 0·05 ml 0·9% saline containing antigen into one flank. Skin test sites were examined 6, 12, 24, 36, 48, and 72 h after challenge for the presence of induration and erythema. Induration was quantitated in each animal by the comparison of the skin-fold thickness measured at each time with an Oditest OOT spring-loaded dial caliper with that observed immediately before challenge.

Histology

Skin specimens were fixed in 10% formol-saline, embedded in paraffin wax, 5-micron sections being stained with haematoxylin and eosin.

Statistical techniques

The significance of differences in the mean increases in skinfold thickness between groups was tested by pooled variance Student's *t*-tests. The statistical significance of interactions between factors influencing increases in skin-fold thickness at the peak of delayed reactions (24 h post challenge) was tested by the factorial analysis of variance.

RESULTS

Characterization of delayed hypersensitivity

The production of delayed hypersensitivity skin reactions to OA in rats was examined in animals skin-tested with 25 μg OA 14 days after immunization with 15 μg OA in either IF or FCA. Three days before immunization animals were untreated or treated with 100 mg/kg or 200 mg/kg cyclophosphamide, injected i.p. In non-cyclophosphamide treated animals haemagglutinating antibodies could be detected against OA in high titre by the 14th day. Arthus-type reactions were seen in animals immunized with OA in IF and FCA. These reactions were only detectable 4 h after challenge when marked and were most frequently noticable at 6 or 12 h after challenge being macroscopically most obvious at 12 h. In IF-immunized animals these lesions which on the 1st day were pale and markedly indurated were still plainly visible at 24 h when they were erythematous; erythema being still visible in some at 36 h. Twelve hours after challenge such lesions showed oedema and marked perivascular polymorphonuclear infiltration on microscopic examination. Non-cyclophosphamide treated OA in FCA-immunized animals had less marked 'Arthus' reactions, induration which was maintained for longer and more marked erythema which was frequently observed later than 48 h after challenge. Pretreatment of OA in IF-immunized animals with 100 mg/kg or 200 mg/Kg cyclophosphamide reduced the haemagglutinating antibody response and the size of the 'Arthus' reaction without potentiating the delayed component of the OA skin reaction. Anti-OA antibody was not detectable by either the tanned SRBC or PCA techniques in sera obtained on the 14th day after immunization with OA and FCA from 100 mg/kg or 200 mg/kg cyclophosphamide pretreated animals. OA skin test in such animals produced reactions macroscopically similar to tuberculin reactions in the absence of 'Arthus' reactions. These delayed reactions were also histologically similar to tuberculin reactions with a predominantly mononuclear cell

infiltrate from 24 to 48 h after challenge. Similar reactions could also be elicited within 24 h of cell transfer in the recipients of lymph node or spleen cells obtained 11 days after immunization with OA in FCA, from 100 mg/kg cyclophosphamide pretreated donors.

Characterization of IgE-mediated reactivity

In the classical PCA reaction in the rat in which antigen and dye are injected i.v. positive reactions are usually adjudged those in which staining of greater than 5 mm diameter is observed. By this criterion the titre of our anti-OA Nippostrongylus potentiated serum pool (anti-OA IgE) was 1 in 800 using a latent period of 48 h. However definitely blued lesions of less than 5 mm diameter were observed at sites sensitized with serum dilutions up to 1 in 1000. When antigen was injected i.v. in the absence of Evan's blue, induration was plainly visible within 20 min at sites injected 48 h before with 1 in 500 anti-OA IgE serum and by measurement of skinfold thickness, induration could be detected at this time at sites sensitized with 1 in 1000 serum dilutions. Significantly greater changes in skinfold thickness could also be detected at sites sensitized with anti-OA IgE 48 h before local challenge with 100 μg OA than at similarly challenged sites injected with normal rat serum (NRS). In all such reactions induration was maximal between 15 and 25 min after antigen challenge, skin-fold thickness increases decreasing in an apparently exponential manner after the peak and progressively greater doses of sensitizing serum produced greater peak induration. After small reactions skin-fold thickness had returned to pretest values within 1 h of challenge but in larger reactions the length of time that induration was detectable was proportional to the peak of the response at 15–20 min post challenge, with in very large reactions, significant induration detectable for more than 24 h. The capacity of anti-OA IgE serum to sensitize skin for immediate and late changes in skinfold thickness on antigen challenge 48 h later was progressively diminished by repeated freezing and thawing and the ability to sensitize for 2 h and 48 h PCA reactions was completely abolished by heating at 56° for 30 min.

Anti-OA IgE and the delayed skin-test to OA

In subsequent experiments skin sites were passively sensitized with ten times the minimum sensitizing dose of anti-OA IgE (0·1 ml 1 in 100 serum). In this experiment skin sites were prepared with either anti-OA or NRS 72 h before antigen challenge. A second serum injection was also given at a contralateral site so that equal numbers of animals received either two injections of NRS, anti-OA IgE at the challenge site and NRS at the contralateral site (local IgE) or NRS at the challenge site and anti-OA IgE at the contralateral site (distant IgE). Each serum treatment was applied to unimmunized animals and animals immunized with OA in FCA following 100 mg/Kg cyclophosphamide pre-treatment. Fourteen days after active immunization skin tests were performed using 100 μg OA. The design of the experiment is illustrated in Fig. 1. Controls to investigate the possible effects of cyclophosphamide and immunization with CFA without ovalbumin on the local skin reaction to OA at sites sensitized with anti-OA IgE were carried out separately. Groups of ten rats were used. Significant effects were not found on skin reactivity.

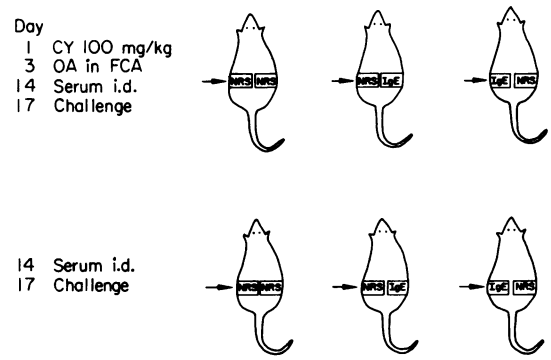

Figure 1. To illustrate the design of the experiment to investigate the interaction of IgE and cell-mediated reactivity to ovalbumin.

Injection of anti-OA IgE at a site distant to that subsequently challenged with OA had no significant effect on the macroscopic appearances of the delayed skin reaction or on the changes in skin-fold thickness observed. Local anti-OA sensitization appeared to have a biphasic influence on the macroscopic appearances of the delayed OA skin reaction. Within the first 24 h lesions were more marked but then faded more rapidly. Twenty-four hours after challenge 4/10 locally anti-OA IgE sensitized actively immunized animals had erythema of greater than 20 mm diameter as opposed to 1/10 of each of the other two actively immunized groups. The means of increases

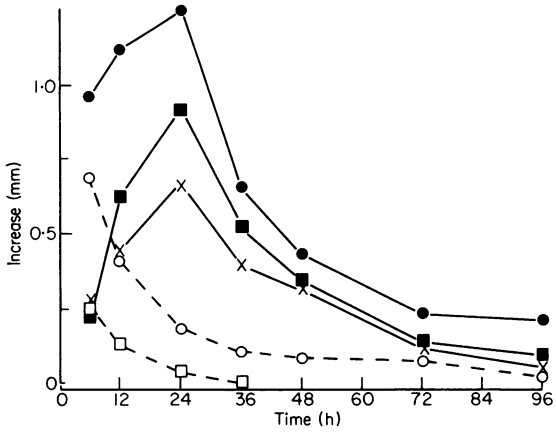

Figure 2. Changes in skin thickness following 100 μg OA challenge. Mean changes for groups of ten animals. Challenge sites pre-treated with: (●, ○) local anti-OA IgE; (■, □) distant anti-OA IgE; (×) normal rat serum; (——) active OA immunized; (– – –) no active immunization.

in skin-fold thickness of the former group were significantly greater than those of non-IgE sensitized animals at 72 h ($P < 0{\cdot}05$) as well as at 24 h after challenge ($P < 0{\cdot}01$) (Fig. 2).

Comparing locally IgE sensitized and non-IgE injected animals, the effect of the interaction between anti-OA IgE injection and active immunization represents an increase of 55% in the induration expected were the two influences to summate ($P < 0{\cdot}05$). However, the interaction calculated between locally and distantly anti-OA IgE injected groups does not reach statistical significance. To circumvent possible central influences of antibody injection on reactivity to the same antigen experiments were then performed to examine the influence of an OA/anti-OA reaction on the expression of tuberculin sensitivity.

OA/anti-OA IgE reactions and the tuberculin skin reaction

In further experiments the influence of IgE-mediated reactions on tuberculin skin reactions was examined in animals sensitized with anti-OA IgE at the site of a subsequent PPD challenge and injected i.v. with OA immediately following PPD challenge. A similar level of anti-OA IgE sensitivity to that employed in the previous experiment was used, the magnitude of the tuberculin reaction being varied by using two test doses of tuberculin. In each experiment either anti-OA IgE or NRS was injected i.d. into both *Myco-*

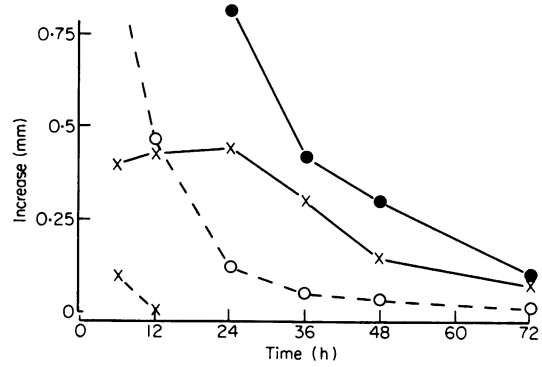

Figure 3. Changes in skin thickness following 100 u PPD challenge and i.v. OA. Mean changes for groups of ten animals. Challenge sites pre-treated with: (●, ○) anti-OA IgE; (×) normal rat serum; (——) active OA immunized; (– – –) no active immunization.

bacteria in oil immunized or unimmunized animals 48 or 72 h before skin test. Neither anti-OA IgE sensitization in the absence of OA challenge nor intravenous OA administration in the absence of anti-OA IgE sensitization were observed to have a significant effect on the tuberculin skin reaction. The skin reaction to 100 u PPD elicited 14 days after immunization was potentiated at sites of OA/anti-OA IgE reactions, erythema being of slightly greater diameter and persisting for longer. The mean skin-fold thickness increases of animals experiencing both an anti-OA IgE-mediated reaction and a positive tuberculin reaction at the same site were significantly greater than those of animals experiencing a tuberculin reaction alone at 24 ($P < 0{\cdot}001$) and 48 h ($P < 0{\cdot}02$)

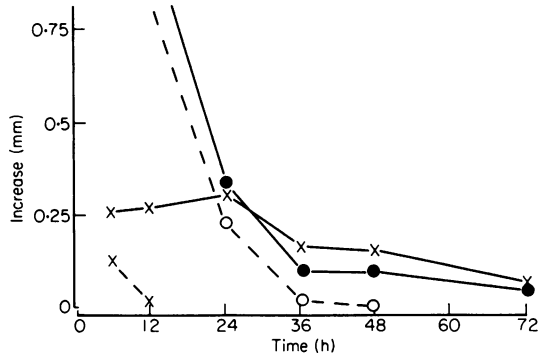

Figure 4. Changes in skin thickness following 10u PPD challenge and i.v. OA. Mean changes for groups of ten animals. Challenge sites pre-treated with: (●, ○) anti-OA IgE; (×) normal rat serum; (——) active OA immunized; (– – –) no active immunization.

after challenge (Fig. 3). At 24 h the effect of the interaction between the two reactions represents an increase of 45% in the increase in skin-fold thickness expected if the two reactions were to summate ($P < 0.01$). When an IgE-mediated reaction of similar size was superimposed on reactions to 10 u PPD the opposite effect was observed (Fig. 4). Twenty-four hours after challenge with 10 u PPD erythema was only seen in 1/10 actively immunized animals which had also experienced an OA/anti-OA IgE reaction as opposed to 7/10 NRS treated actively immunized animals. Although the mean increase in skinfold thickness of the former group is not significantly smaller than that of the latter 24 h after challenge, the influence of the interaction between the two reactions represents a decrease in induration of 39% ($P < 0.01$ by factorial analysis).

DISCUSSION

Treatment with large doses of cyclophosphamide specifically depletes B-cell areas of peripheral lymphoid tissue (Turk & Poulter, 1972). In guinea-pigs, treatment with 300 mg/kg cyclophosphamide potentiates the cell-mediated response to subsequently presented contact sensitizing agents and antigens injected in IF, but does not potentiate the delayed component of reactions to antigens injected in FCA (Turk, Parker & Poulter, 1972). In mice, the potentiating effects of cyclophosphamide treatment and BCG infection on delayed reactivity to SRBC are summative (Mackaness, Lagrange & Ishibashi, 1974). In our experiments, delayed-type reactions were not seen in cyclophosphamide pretreated animals immunized with OA in IF but were observed in the absence of detectable circulating antibody in rats immunized with OA in FCA after treatment with 100 mg/kg or 200 mg/kg cyclophosphamide. We have found 300 mg/kg to be greater than an LD 75 dose in PVG/c rats. Thus the difference between our results and those of Turk *et al.* may be due to species differences or be due to the cyclophosphamide dosages employed. In our experiments greater delayed reactions were seen in rats pretreated with 100 mg/kg cyclophosphamide than in those given 200 mg/kg. We consider that OA in FCA immunized, cyclophosphamide pretreated rats in these experiments demonstrated a state of pure delayed hypersensitivity against OA.

Nippostrongylus brasiliensis infection potentiates the IgE antibody response to other antigens without influencing the haemagglutinating antibody response (Orr, Riley & Doe, 1971). The use of such sera in a long latency passive system provides a means by which pure IgE-mediated skin reactivity may be examined. In our experiments significantly raised skinfold thickness could be observed for longer than 12 h post challenge at passively sensitized sites. In such animals skinfold thickness decreased between 6 and 12 h after OA challenge whereas in 'Arthus' reactions induration increased over this period. Induration observed within 30 min of an OA challenge at passively sensitized sites was greatly in excess of that induced by the local injection of 0·05 ml saline; the technique of measuring skin thickness used in these experiments is capable of detecting statistically significant changes for up to 6 h after the injection of this volume of saline.

The results of the experiments on the interactions of IgE-mediated and delayed hypersensitivity reactions demonstrate that the expression of delayed hypersensitivity may be either potentiated or inhibited by the occurrence of an IgE-mediated reaction at the same time. These interactions are not dependent upon the two responses being directed against the same antigen but are dependent on the triggering of IgE by specific antigen. The nature of the observed interaction appears to be related to the relative magnitudes of the two types of reaction. At a fixed level of IgE-mediated reactivity large delayed reactions may be potentiated whilst small delayed reactions are suppressed.

Local changes in vascular permeability consequent upon mast-cell degranulation may have a large number of effects. It has been suggested that histamine may increase the spread of antigen from the site of injection so that a less persistent reaction could occur over a greater area (Pepys, 1955). The entry of the effector cells of delayed hypersensitivity into skin sites may be dependent upon the release of mast cell products (Gershon, Askenase & Gershon, 1975). A local increase in cell traffic through an area secondary to IgE-mediated increases in blood flow and vascular permeability would be expected to both accelerate and potentiate delayed responses. The effect of histamine on blood vessels is mediated by receptors of H_1 type (Ash & Schild, 1966), whereas lymphocytes possess histamine receptors of H_2 type (Plaut, Lichenstein, Gillespie & Henney, 1973). The observation that T cell-mediated cytotoxicity may be inhibited by histamine (Plaut *et al.*, 1973) suggests that the

effector cells of delayed hypersensitivity may be regulated directly by mast cell products. *In vivo* suppression of delayed hypersensitivity in the guinea-pig may be reversed by the H_2 antagonists burimamide and metiamide (Rocklin, 1976). Early in allogeneic responses T cell-mediated cytotoxicity is only poorly inhibited by histamine, whereas later inhibition becomes marked (Plaut *et al.*, 1973). Thus it would be expected that early in an immune response the stimulatory effects of IgE-mediated reactions due to increases in cell traffic would predominate and that inhibition of delayed reactions would become more significant as the response progressed. We should therefore like to suggest that IgE may play a regulatory role in immune responses, the nature of which alters dynamically with the time course of the response. Early, before circulating antibody is available in appreciable quantity, protection by IgE-mediated potentiation of cell-mediated immunity could occur, to be followed when circulating antibody of other classes is first available, by preferential localization of such antibody. Later when IgE antibody is present in high titre, inappropriate degrees of host-tissue damage by delayed hypersensitivity reactions could be prevented by IgE-mediated inhibition.

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