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Immunochemical Identification of Mouse IgE

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Summary. Immunochemical identification of a distinct immunoglobulin class associated with mouse reaginic antibody and designated IgE has been performed. An antiserum against mouse reaginic antibody was prepared in rats by immunization with homologous peritoneal mast cells sensitized with mouse reaginic antibody. This antiserum, after adequate absorption, recognized only one component existing in mouse serum or fractions of serum containing reaginic antibody. This component was not any of the already known γ_1, γ_2, IgA or IgM immunoglobulins; its immunoglobulin nature was indicated by its antigen-binding capacity in radioimmunodiffusion analysis. Fractionation experiments (zone electrophoresis, gel filtration on Sephadex G-200) showed that there was a strict association between reaginic anaphylactic activity and IgE immunoglobulin. Molecular weight of IgE was found to be about 200,000. In biological tests, anti-IgE neutralized anaphylactic activity attributed to reaginic antibody, but not that attributed to γ_1-antibody. Anti-IgE degranulates normal and sensitized mouse peritoneal mast cells, and rat peritoneal mast cells after they have been sensitized by mouse reaginic antibody.

INTRODUCTION

Antibody activity has been detected in four major classes of mouse immunoglobulins: γ_1, γ_2, IgA and IgM; γ_2-globulins occur in two subclasses: γ_{2a} and γ_{2b} (Fahey, Wunderlich and Mishell, 1964a, b). Anaphylactic properties have been associated with γ_1 antibody (Nussensweig, Merryman and Benacerraf, 1964; Fahey and Barth, 1965).

More recently, a second type of anaphylactic antibody has been found in the mouse: it has biological and physicochemical properties distinct from those of mouse γ_1 and similar to those of the reaginic antibodies found in man and in various other species (Prouvost-Danon, Silva Lima and Queiroz Javierre, 1966; Mota and Peixoto, 1966; Bloch, 1967; Prouvost-Danon, Peixoto and Queiroz Javierre, 1967, 1968; Prouvost-Danon, 1968; Clausen, Munoz and Bergman, 1969; Mota, Sadun, Bradshaw and Gore, 1969; Revoltella and Ovary, 1969; Vaz and Prouvost-Danon, 1969; Prouvost-Danon and Binaghi, 1970a).

It was recently shown that rat mast cells (Mota, Wong, Sadun and Gore, 1969) and in particular rat peritoneal mast cells (Prouvost-Danon, to be published) could be anaphylactically sensitized by mouse reaginic antibody. This suggested the possibility of preparing an antiserum against mouse reaginic antibody by immunization of rats with sensitized homologous mast cells.

Such an antiserum has been employed for the immunochemical identification of the mouse reaginic antibody. The results indicate that the mouse reaginic antibody does not

belong to any of the previously established classes of mouse immunoglobulins, but is associated with a distinct immunoglobulin class provisionally designated IgE.

MATERIAL AND METHODS

Mouse immunoglobulins and antisera against immunoglobulins

Mouse γ_1, IgA and IgM myeloma immunoglobulins, obtained from myelomas RPC23, MPC1 and MPC103, were kindly supplied by Dr P. del Guercio.

Purified anti-DNP γ_1 antibody was prepared by the method of Farah, Kern and Eisen (1960).

Antisera specific for mouse γ_1, γ_2, IgA and IgM were obtained from Melpar Lab. (U.S.A).

Rabbit anti-Fab was prepared by immunization with mouse immunoglobulin obtained by chromatography on DEAE cellulose at 0·005 M phosphate pH 8.

Reaginic sera

Reaginic antibody was produced in Swiss 55, Copacabana mice. This strain was chosen because of their production of higher concentrations of reaginic antibody (Prouvost-Danon and Binaghi, 1970b). The mice were immunized either by a single injection in the back of 100 μg ovalbumin (Grade V, Sigma, USA) mixed with 20 mg aluminium hydroxide gel (Prouvost-Danon *et al.*, 1966) or by two intraperitoneal injections of 1 μg ovalbumin mixed with 1 mg aluminium hydroxide gel given 4 weeks apart; a method shown to give high concentrations of reaginic antibody (Levine and Vaz, 1970). The animals were bled by puncture of the ophthalmic venous plexus either 8–10 days after immunization in the first procedure or at different times after 8 days of immunization in the second procedure. The sera were separated and stored at $-20°$.

Fractionation of mouse reaginic antisera

(a) The globulin fraction of the antisera was obtained by precipitation at half saturation with ammonium sulphate, followed by chromatography on DEAE cellulose (Whatman DE52). Proteins were eluted step-wise with 0·02 M, 0·05 M and 0·1 M phosphate buffers at pH 8·0 and the fractions obtained with each buffer were pooled separately and concentrated by ultra-filtration. They were designated respectively F1, F2 and F3.

(b) Reaginic fractions of mouse antiserum were further fractionated by zone electrophoresis. Eight wells were cut in a 6 × 18-cm slide. Eighty microlitres of the sample was applied to the eight wells and, following electrophoresis, 4-mm segments were cut along seven wells and each was eluted with saline by freezing and thawing. The eluates were tested for reaginic activity by PCA. The remaining well was employed for an immunoelectrophoresis with the appropriate antiserum.

(c) Gel filtration was performed on Sephadex G-200 with 0·15 M phosphate buffered saline pH 8·0 in a 2·5 by 88-cm column (volume 430 ml) at a flow rate of 7–9 ml/hour. In order to calculate molecular weight, calibration of the column was previously made with a mixture of human IgG, ovalbumin (EA), bovine serum albumin (BSA) and dextran blue (DB).

Absorption of mouse reaginic antibody on to rat peritoneal mast cells

Peritoneal mast cells from four rats were collected in Bloom saline medium (Bloom,

Fredholm and Haegermark, 1967) containing 2 per cent rat serum. They were washed three times by centrifugation and resuspended in 3·6 ml medium to which 0·4 ml of a reaginic fraction of mouse antiserum was added. The suspension was incubated for 2½ hours at 37°, washed five times by centrifugation and resuspended in half the initial volume. Sensitization of the mast cells was demonstrated by the degranulation of an aliquot of the suspension after 10 minutes incubation at 37° with ovalbumin.

Immunization of rats

Two millilitres of the above sensitized suspension of rat peritoneal mast cells was emulsified with an equal volume of Freund's complete adjuvant and injected in the footpads of four rats (0·8 ml per rat). Two boosters were made in the same way, at intervals of 2 weeks and 4 weeks, and the antisera were harvested after 7 and 10 days.

Absorption of antisera

The rat antisera were absorbed with mouse γ_1 and IgA globulins, with normal mouse serum (NS) or with F1, F2 or F3, by incubation with adequate volumes for 1 hour at 37°, 24 hours at 4° followed by centrifugation.

Mouse reaginic sera were also treated in the same way with adequate volumes of antisera specific for the mouse immunoglobulins.

In all cases, the completeness of absorption was verified by Ouchterlony analysis.

Immunodiffusion

Ouchterlony analyses were performed in 1·5 per cent agar in buffered saline 0·15 M. Immunoelectrophoresis was performed according to Scheidegger (1955) in 1·25 per cent agar in veronal buffer 0·025 M pH 8·2. Radioimmunodiffusion was carried out by the method of Yagi, Maier and Pressman (1962). Ovalbumin was labelled with I^{125} by the method described by McConahey and Dixon (1966) using chloramine T. After precipitin bands developed, the slides were washed thoroughly for at least 2 days, the labelled antigen was added in the antibody trough or well. After 24 hours, the plates were washed for 2 or 3 days, dried and stained. Radioautographs were obtained by placing X-ray film (Kodirex, Kodak) in contact with the plates for 1–3 weeks.

Passive cutaneous anaphylaxis

Passive cutaneous anaphylaxis (PCA) was performed both in the dorsal skin and in the ear of normal Swiss mice with 20 µl and 10 µl respectively of mouse reaginic sera or fractions of sera as previously described (Prouvost-Danon and Binaghi, 1970b). The incubation time was 48–72 hours for reaginic activity and 2 hours for γ_1 activity. Titres were expressed as the reciprocal of the highest dilution giving a blue spot 3–4 mm in diameter.

Sensitization and degranulation of peritoneal mast cells

In vitro experiments were performed as previously described (Prouvost-Danon and Binaghi, 1970a) using peritoneal mast cells collected from Swiss mice or Wistar rats. Barron-modified Ringer medium (Barron, Meyer and Miller, 1948) and salt medium described by Bloom *et al.* (1967) and containing 0·1 per cent human serum albumin were used respectively for mouse and rat peritoneal mast cells. The cells were collected from the peritoneal cavity, washed three times by centrifugation and resuspended in 0·2–0·4 ml.

Sensitization of rat mast cells was performed by incubation at 37° with dilutions of mouse reaginic fraction, then thoroughly washing by five centrifugations and resuspensions in the same medium. The normal or sensitized cells were incubated at 37° for 15 minutes, the specific antigen (10 μg/ml ovalbumin) or antiserum was added in a volume of 10–20 μl and incubation was continued at 37° for a further 5 or 10 minutes. The degranulated and intact mast cells were counted after staining with toluidine blue.

RESULTS

IMMUNOCHEMICAL RECOGNITION OF MOUSE REAGINIC ANTIBODY

Fractions F1 and F2 obtained by DEAE-cellulose chromatography from mouse reaginic sera were shown by Ouchterlony analysis with specific antisera to contain γ_1 and γ_2 immunoglobulins, and F_3 to contain γ_1, γ_2, IgA and IgM immunoglobulins. Albumins and non-immunoglobulin proteins were also contained in the fractions, as observed on immunoelectrophoresis. PCA analysis showed reaginic activity in F2 (recovery about 100 per cent) but not in F1 or F3.

To obtain a rat antiserum against mouse reaginic antibody, rat peritoneal mast cells were sensitized with 0·4 ml F2 as described in Materials and Methods (PCA titre of F2: 128; O.D. at 280 mμ: 14·8). Addition of ovalbumin (10 μg/ml) to an aliquot of the sensitized cells produced 46 per cent of degranulation of the mast cells, while controls were not degranulated. Four rats were immunized with the sensitized rat peritoneal mast cells. The antiserum obtained from one of the rats after two boosters was designated A-286.

Mouse reaginic sera (RS) and fractions (F2) with high PCA titres (500–2000 for the sera, 2000 for F2) were analysed by immuno- and radioimmunodiffusion in Ouchterlony and immunoelectrophoresis tests against the rat antiserum A-286.

In Ouchterlony analysis, A-286 revealed a strong precipitin band against normal serum (NS) or F3, and an additional lighter band against RS.

Absorption of A-286 by γ_1, IgA globulins or F_1 had no effect upon the precipitin bands. After absorption by NS or by F3, A-286 revealed uniquely the lighter precipitin band against RS, and nothing against NS, F1, F3, γ_1 or IgA (Fig. 1) nor against IgM. After further absorption by F2, A-286 did not react any more against RS. For ease of presentation, the rat antiserum A-286 absorbed by F3 and recognizing a band only in reaginic serum and not in normal serum, will be provisionally designated anti-IgE (a-IgE). Anti-IgE also yielded a precipitin band against F2. The specificity of the precipitin band formed between anti-IgE and RS or F2 was compared with bands formed by anti-γ_2, anti-IgA and anti-IgM using Ouchterlony analysis. The results show that the specificity of the band yielded by anti-IgE was entirely different from those yielded by anti-γ_2, anti-IgA and anti-IgM. An immunoelectrophoretic analysis (Fig. 2) showed that the specificity of the IgE band was also different from that of the band yielded by anti-γ_1. It may be concluded that the precipitin band formed between reaginic antibody and anti-IgE was not due to any of the known immunoglobulins.

The antibody nature of the material precipitated from RS or F2 by anti-IgE was tested by radioimmunodiffusion. Radioautography demonstrated antigen binding in the precipitin band formed between anti-IgE and all the mouse reaginic sera tested (PCA titres 500–2000) as well as F2(PCA titre:2000). No antigen binding was demonstrable by normal serum nor by a precipitin band yielded by anti-γ_1 against myeloma γ_1 globulin.

Radioimmunoelectrophoresis was also performed. A-286 yielded in the region of IgG

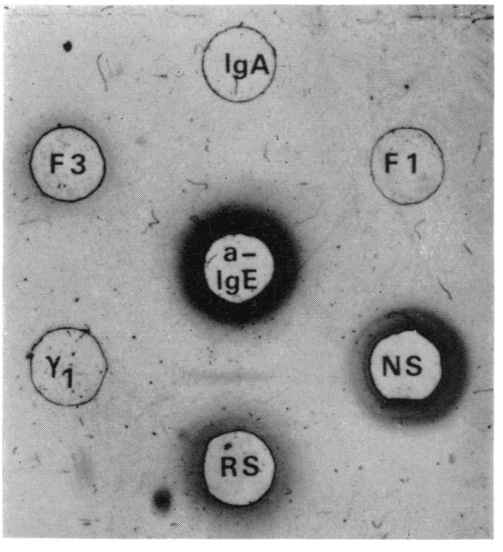

FIG. 1. Immunodiffusion analysis of anti-IgE.

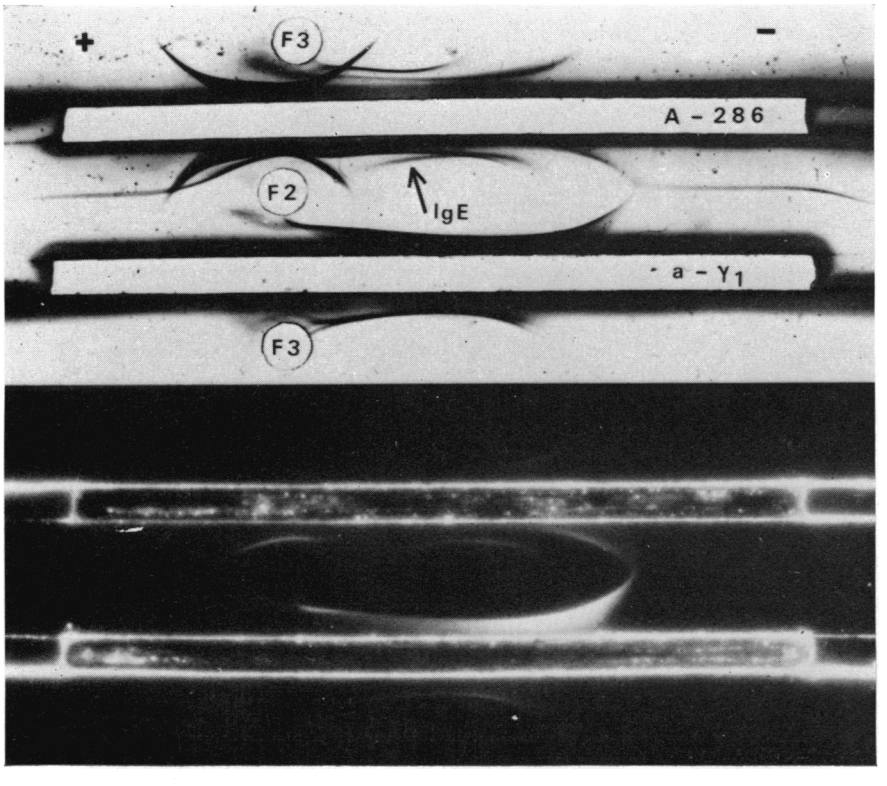

FIG. 2. Radioimmunoelectrophoretic analysis of the reagin rich fraction F2. Both stained slide (upper) and corresponding radioautograph (lower) are shown.

globulins a precipitin band against F2, and not against F3, distinct from the precipitin band obtained with anti-γ_1 (Fig. 2a) or with anti-γ_2. Radioautography demonstrated antigen binding by this distinct precipitin band, which was designated IgE (Fig. 2b). It can be seen in Fig. 2 that A-286 recognized other materials, some of non-antibody nature, as well as IgE. When anti-IgE was used against RS in radioimmunoelectrophoresis, only the IgE precipitin band was yielded. No precipitin band was yielded by anti-IgE against NS.

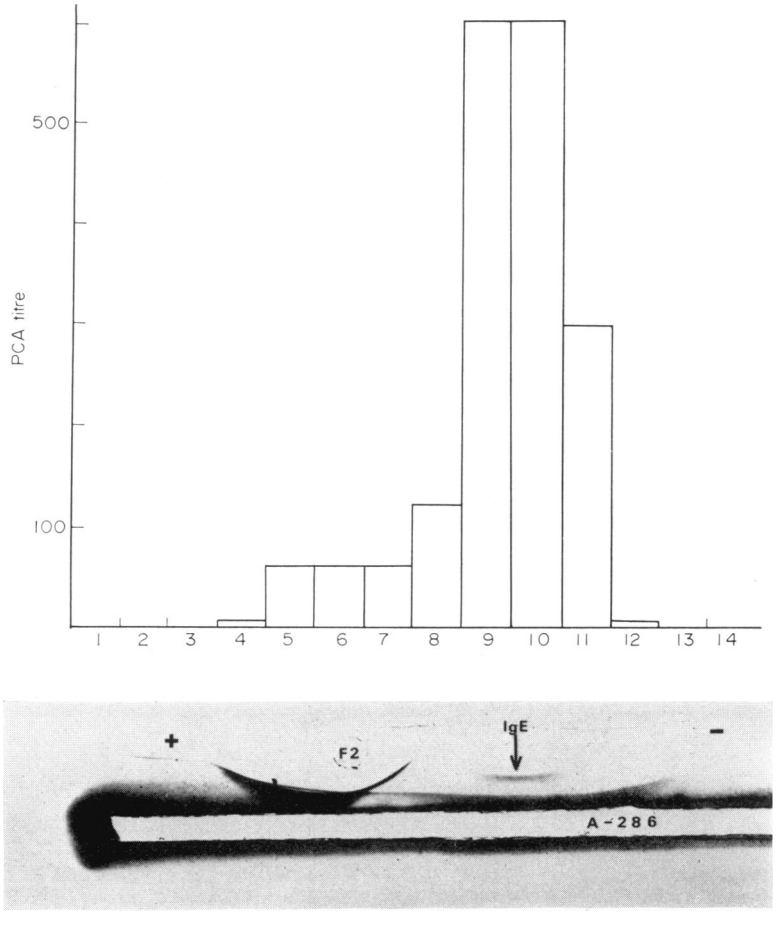

FIG. 3. Zone electrophoresis of the reagin rich fraction F2: distribution of reaginic activity (PCA titre) and of IgE precipitin band.

CORRELATION BETWEEN DISTRIBUTION OF IgE AND THAT OF REAGINIC ACTIVITY ON ZONE ELECTROPHORESIS

Electrophoresis was carried out with 80 μl of F2 (PCA titre:2000) as described in methods. Immunoelectrophoresis was performed with A-286. The results (Fig. 3) show that PCA activity was eluted from the segments corresponding exactly with the IgE band (PCA titre:600, in relation to the initial volume of 80 μl, in segments 9 and 10).

GEL FILTRATION

Further purification of reaginic antibody was undertaken by gel filtration through Sephadex G-200 1·2 ml of F2 (PCA titre:8·000; O.D. at 280 mμ:65) containing dextran blue (DB) was applied to the column. The distribution of IgE in the eluate was tested by Ouchterlony analysis with anti-IgE and that of reaginic activity by PCA. Fig. 4 shows the results: distribution of IgE paralleled exactly that of reaginic activity. Both were found in the ascending portion of the IgG peak. The elution volume of the peak of reaginic activity (PCA) corresponded to a molecular weight of 200,000. γ_1 and γ_2, as tested by Ouchterlony analysis with anti-γ_1 and anti-γ_2, were recovered in the IgG peak, corresponding to a molecular weight of 155,000.

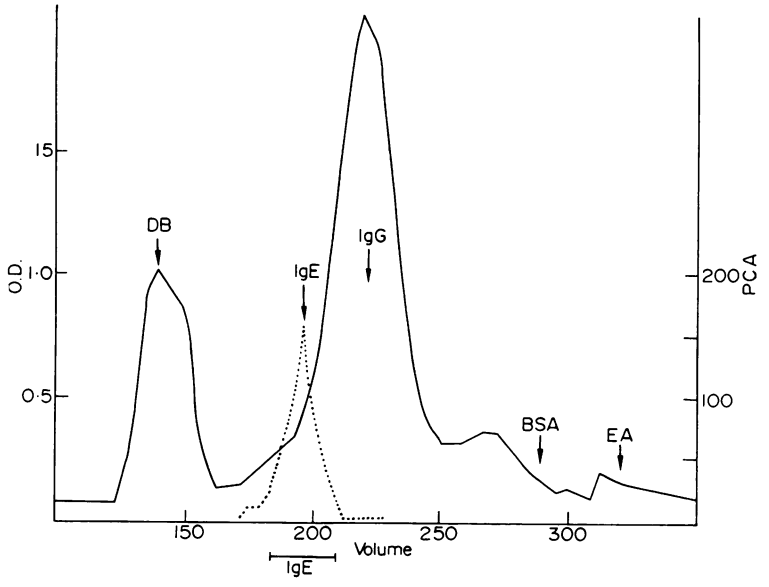

FIG. 4. Gel filtration of the reagin rich fraction F2 through a Sephadex G-200 column: distribution of reaginic activity (PCA titre: broken line) and of IgE as detected by anti-IgE. Continuous line represents absorption at 280 mμ. Elution volumes of human IgG, BSA, EA and dextran blue (DB), are indicated by arrows.

NEUTRALIZATION OF PCA ACTIVITY OF REAGINIC SERUM BY ANTI-IgE

Anti-IgE (A-286 absorbed with either F3 or NS) completely neutralized the PCA activity of mouse reaginic serum (initial PCA titre:1000). In contrast, treatment of the reaginic serum by anti-γ_2, anti-γ_1, anti-IgA or anti-IgM did not reduce at all the PCA activity. Treatment of the reaginic serum with anti-Fab, which produced precipitate, reduced the PCA titre four-fold. The results are shown in Table 1.

Experiments were also performed to differentiate the 2 hours PCA activity due to γ_1-antibody from the 72 hours PCA activity due to reaginic activity: the 2 hours PCA activity of a specifically purified preparation of anti-DNP γ_1-antibody (titre:160) was not at all affected after absorption by anti-IgE, while it was greatly reduced after absorption by anti-γ_1 (titre <30).

TABLE 1

NEUTRALIZATION OF PCA ACTIVITY OF MOUSE REAGINIC SERUM (RS) BY SPECIFIC ANTI-IMMUNOGLOBULIN SERA

Treatment		PCA titre†
Antiserum	Volume*	
Saline	0·5	1000
Normal rat serum	0·5	1000
Anti-IgE	0·5	< 12
Anti-γ1	20	1000
Anti-γ2	10	1000
Anti-IgA	5	1000
Anti-IgM	5	1000
Anti-Fab	20	250

* Volume per 1 volume RS.
† Dilution in relation to initial volume of RS.

TABLE 2

DEGRANULATION OF MOUSE PERITONEAL MAST CELLS BY ANTI-IgE

Mast cells	Antiserum	
	Nature	Dilution inducing 50 per cent degranulation
Normal	Anti-IgE	1:1,500
Sensitized	Anti-IgE	1:15,000
Sensitized	a-IgE absorbed by F2	1:300
Normal	Anti-γ1	1:10

TABLE 3

ACTION OF ANTI-IgE ON RAT PERITONEAL MAST CELLS, NORMAL OR SENSITIZED BY MOUSE REAGINIC ANTIBODY

Mast cells	Anti-IgE	Degranulation (per cent)
Normal	No	0
Normal	1:80	0
Sensitized*	No	0
Sensitized	1:80	33
Sensitized	1:200	24
Sensitized	1:1000	23

* Incubated with mouse F2 (PCA titre 2000) diluted to 1:20 for 2 hours at 37° followed by washing. Antigen (EA 10 μg/ml) induced respectively 0 and 73 per cent degranulation of normal and sensitized cells.

DEGRANULATION OF PERITONEAL MAST CELLS BY ANTI-IgE

The ability of anti-IgE to induce the degranulation of mouse peritoneal mast cells was tested at 37°. Table 2 shows the results. Anti-IgE induced the degranulation of normal mouse peritoneal mast cells at a dilution of 1:1,500 and that of sensitized peritoneal mast cells at a dilution of 1:15,000 (the sensitized peritoneal mast cells were collected from mice immunized 10 months before against ovalbumin, and were able to degranulate upon the addition of ovalbumin at 37°). The ability of anti-IgE to induce the degranulation of mouse peritoneal mast cells was reduced to 2 per cent after absorption with F2 from reaginic serum. In contrast, anti-γ_1 induced degranulation of normal mouse peritoneal mast cells only at a dilution of 1:10, which represents about 1 per cent of the activity of anti-IgE.

Table 3 shows that anti-IgE had no activity upon normal rat peritoneal mast cells. However, after *in vitro* passive sensitization of rat peritoneal mast cells by mouse reaginic antibody, degranulation was obtained either by the addition of antigen (ovalbumin) or of anti-IgE.

DISCUSSION

The immunochemical, physicochemical and biological data presented in this paper indicate that mouse reaginic antibody is associated with an immunoglobulin class different from the already known γ_1, γ_2, IgA and IgM. In line with the equivalent findings in man (Ishizaka, Ishizaka and Hornbrook, 1966a, b), rabbit (Ishizaka, Ishizaka and Hornbrook, 1970), rat (Stechschulte, Orange and Austen, 1970) and cow (Hammer, Kickhofen and Schmid, 1971), we have provisionally designated this class of immunoglobulin IgE.

It was recently shown that mouse reaginic antibody is able to anaphylactically sensitize rat peritoneal mast cells, probably by its fixation on the membrane of the mast cells (Prouvost-Danon, to be published). This property of mouse reaginic antibody was utilized for production of an antiserum against mouse reaginic antibody by immunizing rats with homologous peritoneal mast cells sensitized with mouse reaginic antibody.

The antiserum produced (A-286) was able to recognize on immunodiffusion analysis a component which, according to all the evidence, is an immunoglobulin possessing reaginic anaphylactic activity. This component was present in mouse antiserum obtained in conditions of immunization producing high titres of reaginic antibody, or in fractions of these antisera rich in reaginic antibody. On the contrary, no reactions were observed with normal mouse serum or with non-reaginic fractions.

It must be noted that A-286 reacted with some other serum components. A-286 could, in fact, be rendered specific for the reaginic antibody by absorption with normal serum or with non-reaginic fractions containing the other immunoglobulin classes. After absorption, the antiserum obtained recognized only one component. This component was not any of the known mouse immunoglobulins γ_1, γ_2, IgA or IgM, as demonstrated by Ouchterlony and immunoelectrophoresis analysis using appropriate purified myeloma proteins or specific antisera. The antibody nature of this component was ascertained by radioimmunodiffusion tests with labelled antigen, indicating that it is an immunoglobulin. In view of these results, we have designated this new class of immunoglobulin IgE, and the specific antiserum anti-IgE.

A strict association was found between reaginic antibody and IgE immunoglobulin in fractionation experiments.

By zone electrophoresis of mouse reaginic serum, the reaginic activity as tested by PCA was recovered in exactly the same fractions that gave a precipitin band with anti-IgE. After fractionation by gel filtration on Sephadex G-200, a perfect correlation was found between the reaginic PCA activity of the fractions and a positive reaction with anti-IgE. This experiment indicated that the molecular weight of mouse IgE was about 200,000 (while that of γ_1 is about 155,000).

The specificity of anti-IgE antiserum was also shown by biological tests. In a series of experiments it was shown that the anaphylactic activity due to reaginic antibody could be removed by treatment with anti-IgE. On the contrary, antisera specific for γ_1, γ_2, IgA or IgM were completely inactive. It is important to note that anti-IgE eliminated the skin sensitization to PCA persistent after 72 hours, due to reaginic antibody, but did not affect that obtained after 2 hours due to γ_1 antibody; in contrast, anti-γ_1 antiserum eliminated the 2 hours activity, but not the 72 hours activity. These results confirm by an immunochemical procedure the distinction between anaphylactic activities of γ_1 and reaginic antibodies.

The reaginic activity could also be reduced by an anti-Fab antiserum. In this case, the activity could not be completely eliminated but only reduced four-fold. The reasons for this are not clear. A similar observation has been made in the rat (Jones and Edwards, 1970).

Degranulation of and histamine release from mouse peritoneal mast cells by anti-mouse immunoglobulin antiserum has been previously observed, and it was suggested that the mouse immunoglobulin involved is cell-bound IgE reaginic antibody (Prouvost-Danon, Peixoto and Queiroz Javierre, 1970). In the present experiments, normal or sensitized mouse peritoneal mast cells were degranulated after treatment with anti-IgE. The effect of anti-IgE was much more marked when sensitized mast cells were employed, probably because the concentration of IgE on the membrane was higher, as a consequence of the increase of IgE following immunization. Anti-γ_1 had very little effect. These results may be taken as an evidence that IgE, but not γ_1, is firmly fixed on mast cells, as already demonstrated in a different way (Prouvost-Danon, 1968; Prouvost-Danon and Binaghi, 1970a; Prouvost-Danon *et al.*, 1967). Similar results have been obtained with human leucocytes and anti-IgE, and in this case also the effect of anti-IgE is much more marked when the cells come from an atopic individual than when normal cells are employed (Ishizaka, Ishizaka, Johansson and Bennich, 1969; Ishizaka, Tomioka and Ishizaka, 1971).

Furthermore, anti-mouse IgE was able to induce degranulation of rat peritoneal mast cells after they have been sensitized with mouse reaginic antibody, but not without such treatment. This result also confirms that heterologous sensitization is possible between rat and mouse reaginic antibodies.

The mouse was the first animal in which two different types of anaphylactic antibodies, γ_1 and reaginic, were recognized by their biological and physicochemical properties. The findings of the present work indicate that these antibodies belong to two different classes of immunoglobulins. The same situation probably also exists in guinea-pig (Catty, 1969). It would be interesting to know whether this is a general case in animal species.

REFERENCES

BARRON, E. S., MEYER, J. and MILLER, Z. B. (1948). 'The metabolism of skin. Effect of vesicant agents.' *J. invest. Derm.*, **11**, 97.

BLOCH, K. J. (1967). 'The anaphylactic antibodies of mammals including man.' In Kallos and Waksman, *Progr. Allergy*, **10**, 84. (Karger, Basel).

BLOOM, G. D., FREDHOLM, B. and HAEGERMARK, O. (1967). 'Studies on the time course of histamine release and morphological changes induced by histamine liberators in rat peritoneal mast cells.' *Acta physiol. scand.*, **71**, 270.

CATTY, D. (1969). *The Immunology of Nematode Infections. Trichinosis in Guinea-Pig as a Model.* Karger, Basel.

CLAUSEN, C. R., MUNOZ, J. and BERGMAN, R. K. (1969). 'Reaginic-type of antibody in mice stimulated by extracts of Bordetella pertussis.' *J. Immunol.*, **103**, 668.

FAHEY, J. L. and BARTH, W. F. (1965). 'Heterologous and homologous skin-sensitizing activity of mouse 7Sγ-$_1$ and 7Sγ-$_2$ globulins.' *Nature (Lond.)*, **206**, 730.

FAHEY, J. L., WUNDERLICH, J. and MISHELL, R. (1964a). 'The immunoglobulins of mice. I. Four major classes of immunoglobulins: 7Sγ-$_2$, 7Sγ-$_1$, γ-1A (β-2A) and 18Sγ-1M globulins.' *J. exp. Med.*, **120**, 223.

FAHEY, J. L., WUNDERLICH, J. and MISHELL, R. (1964b). 'The immunoglobulins of mice. II. Two subclasses of mouse 7Sγ-$_2$ globulins: γ-2a and γ-2b globulins.' *J. exp. Med.*, **120**, 243.

FARAH, F. S., KERN, M. and EISEN, H. N. (1960). 'The preparation and some properties of purified antibody specific for 2–4 dinitrophenyl groups.' *J. exp. Med.*, **112**, 1195.

HAMMER, P. K., KICKHOFEN, B. and SCHMID, T. (1971). 'Detection of homocytotropic antibody associated with a unique immunoglobulin class in the bovine species.' *Eur. J. Immunol.*, **1**, 249.

ISHIZAKA, K., ISHIZAKA, T. and HORNBROOK, M. M. (1966a). 'Physicochemical properties of reaginic antibody. IV. Presence of a unique immunoglobulin as a carrier of reaginic activity.' *J. Immunol.*, **97**, 75.

ISHIZAKA, K., ISHIZAKA, T. and HORNBROOK, M. M. (1966b). 'Physicochemical properties of reaginic antibody. V. Correlation of reaginic antibody with γE-globulin antibody.' *J. Immunol.*, **97**, 840.

ISHIZAKA, K., ISHIZAKA, T. and HORNBROOK, M. M (1970). 'A unique rabbit immunoglobulin having homocytotropic antibody activity.' *Immunochemistry*, **7**, 515.

ISHIZAKA, T., ISHIZAKA, K., JOHANSSON, S. G. and BENNICH, H. (1969). 'Histamine release from human leukocytes by anti-γE antibodies.' *J. Immunol.*, **102**, 884.

ISHIZAKA, T., TOMIOKA, H. and ISHIZAKA, K. (1971). 'Degranulation of human basophil leukocytes by anti-γE antibody.' *J. Immunol.*, **106**, 705.

JONES, V. E. and EDWARDS, A. J. (1971). 'Preparation of an antiserum specific for rat reagin (rat γE?).' *Immunology*, **21**, 383.

LEVINE, B. B. and VAZ, N. M. (1970). 'Effect of combinations of inbred strain, antigen, and antigen dose on immune responsiveness and reagin production in the mouse. A potential mouse model for immune aspects of human atopic allergy.' *Int. Arch. Allergy*, **39**, 156.

MCCONAHEY, P. J. and DIXON, F. J. (1966). 'A method of trace iodination of proteins for immunological studies.' *Int. Arch. Allergy*, **29**, 185.

MOTA, I. and PEIXOTO, J. M. (1966) 'A skin sensitizing and thermolabile antibody in the mouse.' *Life Science*, **5**, 1723.

MOTA, I., SADUN, E. H., BRADSHAW, R. M. and GORE, R. W. (1969). 'The immunological response of mice infected with *Trichinella spiralis*. Biological and physicochemical distinction of two homocytotropic antibodies.' *Immunology*, **16**, 71.

MOTA, I., WONG, D., SADUN, E. H. and GORE, R. W. (1969). 'Mouse homocytotropic antibodies.' In *Cell and Humoral Mechanisms in Anaphylaxis and Allergy*, p. 23. (Karger, Basel).

NUSSENSWEIG, R. S., MERRYMAN, C. and BENACERRAF, B. (1964). 'Electrophoretic separation and properties of mouse anti-hapten antibodies in passive cutaneous anaphylaxis and passive hemolysis.' *J. exp. Med.*, **120**, 315.

PROUVOST-DAMON, A. (1968). 'Mechanisms of antibody mediated histamine release from mouse mast cells.' In *CIOMS Symposium on the Biochemistry of the Acute Allergic Reactions*, (Ed. by K. Frank Austen and Elmer L. Becker), p. 175. Blackwell Scientific Publications, Oxford.

PROUVOST-DANON, A. and BINAGHI, R. (1970a). '*In vitro* sensitization of mouse peritoneal mast cells with reaginic antibody.' *Nature (Lond.)*, **228**, 66.

PROUVOST-DANON, A. and BINAGHI, R. (1970b). 'Reaginic antibody in adult and young mice. Production and biological properties.' *Int. Arch. Allergy*, **38**, 648.

PROUVOST-DANON, A., PEIXOTO, J. M. and QUEIROZ JAVIERRE, M. (1967). 'Reagin-like antibody mediated passive anaphylactic reaction in mouse peritoneal mast cells *in vitro*.' *Life Science*, **6**, 1793.

PROUVOST-DANON, A., PEIXOTO, J. M. and QUEIROZ JAVIERRE, M. (1968). 'Antigen-induced histamine release from peritoneal mast cells of mice producing reagin-like antibody.' *Immunology*, **15**, 271.

PROUVOST-DANON, A., PEIXOTO, J. M. and QUEIROZ JAVIERRE, M. (1970). 'Immune injury of mouse peritoneal mast cells by antibodies to mouse serum and mouse γ-globulin.' *Immunology*, **18**, 749.

PROUVOST-DANON, A., SILVA LIMA, M., and QUEIROZ JAVIERRE M. (1966). 'Active anaphylactic reaction in mouse peritoneal mast cells *in vitro*.' *Life Science*, **5**, 289.

REVOLTELLA, R. and OVARY, Z. (1969). 'Reaginic antibody production in different mouse strains.' *Immunology*, **17**, 45.

SCHEIDEGGER, J. J. (1955). 'Une microméthode de l'immunoélectrophorèse.' *Int. Arch. Allergy*, **7**, 103.

STECHSCHULTE, D. J., ORANGE, R. P. and AUSTEN, K. F. (1970). 'Immunochemical and biological properties of rat IgE. Immunochemical identification of rat IgE.' *J. Immunol.*, **105**, 1082.

VAZ, N. M. and PROUVOST-DANON, A. (1969). 'Behaviour of mouse mast cells during anaphylaxis *in vitro*.' *Progr. Allergy*, **13**.

YAGI, Y., MAIER, P. and PRESSMAN, D. J. (1962). 'Immunoelectrophoretic identification of guinea-pig anti-insulin antibody.' *J. Immunol.*, **89**, 736.