THE JOURNAL OF IMMUNOLOGY

Vol. 102, No. 4, 1969
Printed in U.S.A.

A COMPARATIVE ANTIGENIC STUDY OF γE-GLOBULIN AND MYELOMA-IgND[1]

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Received for publication November 25, 1968

In the course of previous studies on human reaginic antibodies, Ishizaka, Ishizaka and Hornbrook (1, 2) have reported that reaginic activity to ragweed antigen E is associated with a unique immunoglobulin, which was tenatively designated γE-globulin. The concentration of γE anti-ragweed antibody in atopic patients' sera, as measured by antigen-binding activity, correlated with the ability of the sera to passively sensitize normal human and monkey skin (3, 4). A correlation between reaginic activity and γE-antibody, determined by radioimmunodiffusion, was observed when ragweed sensitive sera, fractionated by diethylaminoethyl (DEAE) cellulose chromatography, were analyzed by gel filtration, sucrose density gradient ultracentrifugation and zone electrophoresis in agarose gel (2). Based on these findings, γE-globulin was isolated from ragweed sensitive serum. Such preparations had high reaginic activity and contained γE-anti-ragweed antibodies but no detectable amounts of IgG, IgA, IgM and IgD antibodies (5). Studies of the antigenic structure of γE-globulin indicated that the protein possesses antigenic determinants of Type K and L light chains as well as characteristic antigenic determinants, which are not shared by any of the immunoglobulins of known classes and subclasses (6). It was also evident that γE-globulin lacks major antigenic determinants in common with human γ, α, μ and δ polypeptide chains.

Quite independently, evidence for the presence in human serum of a previously unrecognized class of immunoglobulins was provided by the discovery of an atypical myeloma protein, provisionally designated myeloma-IgND (7) and its counterpart identified in normal serum, designated IgND (8). The biologic significance of this immunoglobulin class was indicated by the findings of significantly elevated levels of IgND in serum from patients with proven allergy (8–10) and the detection of allergen antibodies of IgND type in atopic allergy in high correlation with clinical states and passive cutaneous anaphylaxis (PCA) reactions in the baboon (11, 12).

In view of these results as well as the apparent similarity in physicochemical properties of myeloma-IgND (7, 11) and γE-globulin (2, 5) a comparative antigenic analysis of the two proteins was initiated. The results of these studies, which were performed in the two laboratories at the same time, will be described in the present report.

MATERIALS AND METHODS

Myeloma proteins. Myeloma protein ND isolated from plasma by precipitation with Na_2SO_4 (18 g/100 ml) was purified by chromatography on DEAE Sephadex A-50, followed by recycling chromatography on Sephadex G-150 as described (13). The final product was contaminated with other immunoglobulins (IgG, IgM and IgD) in amounts corresponding to less than 0.1% of total protein as determined by the single radial immunodiffusion method described by Mancini, Carbonara and Heremans (14).

IgG of four subclasses IgG_1, IgG_2, IgG_3 and IgG_4, IgA myeloma proteins of different subclasses (Fu, Ma and He) (15, 16), IgD myeloma and IgM macroglobulin are the same preparations described in (6).

γE-globulin. A serum sample from a ragweed sensitive patient (A) and a reagin-rich fraction (Fr. A_{abs} in (5)) prepared from the serum were employed in the present experiments. The γE-globulin-containing fractions were obtained by a

[1] Part of this work was supported by Research Grants AI-04985 from the United States Public Health Service and GB-4646 from the National Science Foundation. Requests for reprints should be directed to K. Ishizaka, Children's Asthma Research Institute and Hospital, Denver, Colorado.

combination of salt precipitation, DEAE-cellulose column chromatography, recycling chromatography on Sephadex G-200, and DEAE Sephadex column chromatography. IgG and IgA remaining in one of the fractions (Fr. A) were absorbed by purified rabbit antibodies specific for these immunoglobulins. In order to avoid contamination of the sample with rabbit IgG, the rabbit antibodies had been precipitated with goat antibody specific for the F_c portion of rabbit IgG and the specific precipitates were suspended in Fr. A for the absorption. As described in (5), no human serum protein except γE-globulin was detected in the preparation by immunoelectrophoresis. Another preparation composed of γE-globulin and IgG was obtained from a reaginic serum (Pr) which contained no detectable amount of IgA globulin. The purification of the preparation was described in (6).

Antisera specific for each immunoglobulin. The preparation of antiserum specific for myeloma protein ND was described in (7). Rabbits and sheep were immunized with the F_c fragment of myeloma IgND included in complete Freund's adjuvant, and the antisera were absorbed with normal human serum and F_{ab} fragment of the myeloma IgND. The supernatant anti-IgND was specific for the F_c portion of the myeloma protein. Rabbit antisera specific for each immunoglobulin, i.e., IgG, IgA, IgM, IgD and γE-globulin, were the same samples described in previous reports (2, 5). In immunoelectrophoresis against serum A, the anti-γE-globulin serum gave a precipitin arc in the α globulin region in addition to the γE-globulin arc but did not show any precipitin arcs of IgG, IgA, IgM or IgD. A monospecific anti-γE-globulin serum was prepared in guinea pigs by immunization with a purified reagin-rich fraction (Fr. B_{abs}) described previously (17). The preparation was included in complete Freund's adjuvant and injected into the footpads. After 6 weeks the guinea pigs were bled and the antiserum was absorbed with normal IgG and myeloma IgD. The antiserum gave a single precipitin arc of identical specificity with serum A and a reagin-rich fraction from serum Pr. No visible reaction was obtained with any of the myeloma IgG, IgA, IgD or IgM (Fig. 1).

Radioimmunodiffusion. A purified ragweed allergen (Fr. IVc; antigen E prepared according to King *et al.* (18) for the Committee on Standardization of Allergens) was labeled with ^{131}I using chloramine T (19). Immunodiffusion was carried out by the method of Ouchterlony (20) using 1% agarose. After precipitin bands were formed, the plates were washed and radioactive antigen was applied to the antibody wells. Radioautographs were taken by applying x-ray film to the plate (Kodak Industrial x-ray film Type KK).

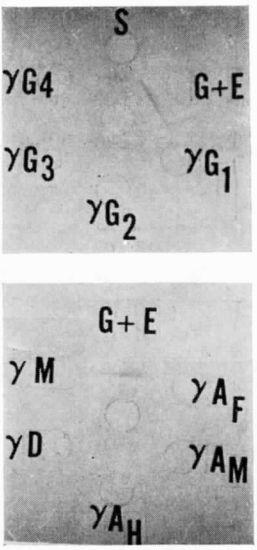

Figure 1. Ouchterlony analysis of anti-γE-globulin guinea pig serum. The antiserum was placed in center wells. Peripheral wells were filled with serum A (S), a reagin-rich fraction from serum Pr containing IgG and IgE (G + E), IgG myeloma proteins of four subclasses, IgA myeloma proteins of Fu type (γA_F), Ma type (γA_M), He type (γA_H), IgD and IgM proteins.

Skin reactions. Prausnitz-Küstner (P-K) reactions were carried out in normal individuals, who gave negative skin reactions to ragweed allergen, and in *Macaca irus* (4, 21). One-twentieth milliliter of serum dilutions containing reagin was injected intracutaneously and the skin sites were challenged with 0.3 μg of antigen E in 0.02 ml of saline. One per cent of Evans blue was injected intravenously into *Macaca irus* immediately before the injection of antigen. Diameters of the skin reactions were measured 15 to 20 min after the challenge.

RESULTS

Radioimmunodiffusion experiments were carried out using radioactive antigen E. The center

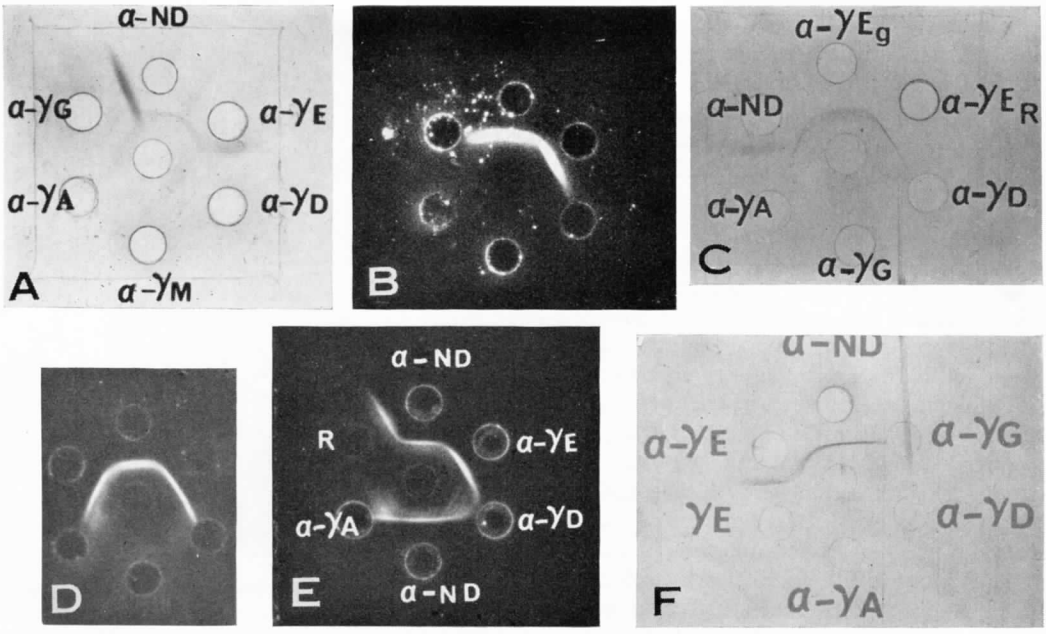

Figure 2. Radioimmunodiffusion of a reaginic fraction. Both the stained slide (*A*, *C*) and corresponding radioautograph (*B*, *D*) are shown. Center wells were filled with a reagin-rich fraction (Fr. A_{abs}) and peripheral wells were filled with antisera specific for one of the immunoglobulins and with anti-IgND (a-ND). Both rabbit and guinea anti-γE (a-γE_R , a-γE_g) were used in plate (*C*). A center well of plate (*E*) was filled with serum A and peripheral wells were filled with antisera specific for each immunoglobulin class and Fr. A_{abs} (R). *F*, Immunodiffusion analysis of myeloma ND and γE. The center well of the plate was filled with myeloma-IgND. The peripheral wells were filled with antisera specific for γG, γD, γA, γE, anti-ND and with Fr .A_{abs} (γE). Myeloma ND and γE-globulin gave a precipitin band of identical specificity with anti-γE and anti-ND.

wells of Ouchterlony plates were filled with Fr. A_{abs} from serum A against antisera specific for each immunoglobulin and anti-IgND was placed in peripheral wells. As shown in Figures 2*A* and 2*C*, both rabbit and guinea pig anti-γE-globulin sera as well as anti-IgND serum gave a single precipitin band of identical specificity. The antisera specific for the other immunoglobulins failed to give a visible precipitin reaction. Radioautographs of the plates showed that the precipitin arcs formed with anti-γE-globulin and anti-IgND combined radioactive antigen (Fig. 2*B*, *D*). No radioactive arc was formed by the other antisera and the radioactive precipitin arcs formed by anti-γE and anti-IgND went into the adjacent wells which contained the antisera specific for IgA, IgG or IgD. These findings indicate that anti-γE and anti-IgND precipitated the same protein which is different from the four known immunoglobulins, and that the protein is a carrier of antibody activity.

The identity of the anti-γE and anti-IgND in their antigenic specificity was confirmed using serum A (Fig. 2*E*). In an Ouchterlony plate, the serum was placed in a center well and antisera specific for one of the IgA, IgD or γE-globulins and anti-IgND were placed in peripheral wells. One peripheral well adjacent to anti-IgND was filled with Fr. A_{abs}. The anti-IgND and anti-γE sera gave a precipitin band of identical specificity with serum A and Fr. A_{abs}. The anti-IgA and anti-IgD sera gave precipitin bands with serum A but the IgA and IgD precipitin bands crossed the band formed by anti-IgND. The radioautograph of the plate clearly shows that the γE-globulin band contains radioactive antigen and that the specificity of the precipitin band was different from that of either IgA or IgD band. It is evident that anti-IgND, which is specific for the F_c portion of myeloma-IgND, precipitated γE-globulin with which anti-ragweed antibodies were associated.

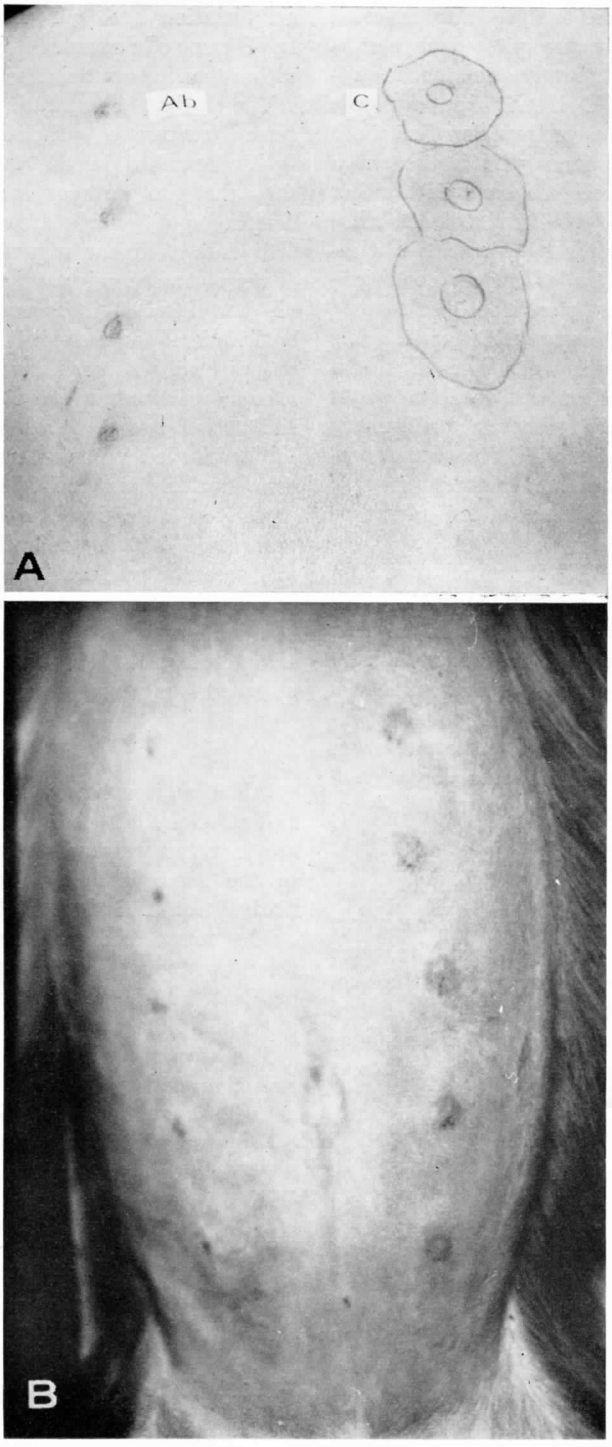

Figure 3. Absorption of reaginic activity with antiserum specific for myeloma IgND. P-K reaction in humans (*A*) and in *Macaca irus* (*B*). Skin sites at left received serial twofold dilutions of supernatant after absorption of a reaginic serum with the antiserum and the sites at right received the same dilutions of the original serum. All sites were challenged with 0.3 μg of purified ragweed allergen.

The immunodiffusion analysis also revealed that anti-γE antibody precipitated myeloma-IgND (Fig. 2*F*). Comparative immunoelectrophoretic analysis of γE-globulin and myeloma IgND in 1.5% agarose gel revealed that both myeloma ND protein and γE fraction from serum A gave a γ_1 precipitin band with either anti-IgND or anti-γE. No significant difference was observed between the two proteins in electrophoretic mobility. Under certain conditions double precipitin arcs were formed between anti-IgND and myeloma IgND or reaginic sera. This was not seen when anti-γE serum was used. The reason for this reaction is not quite clear at present, although preliminary experiments by Bennich and Johansson (unpublished data) indicate that hidden antigenic as well as immunogenic determinants might be involved.

In a previous work (1, 2) it was reported that anti-γE-globulin serum precipitated the reaginic factor of atopic patients' sera which induced Prausnitz-Küstner reactions in humans (1, 2) and passive cutaneous anaphylaxis in monkeys (4). If anti-IgND reacts with γE-globulin containing skin-sensitizing antibody, the antiserum should precipitate the reaginic factor in patients' sera. The anti-IgND serum containing 4 μg AbN/ml was incubated with an equal volume of serum A at 0°C for 24 hr and appropriate dilutions of the supernatants were injected intracutaneously into normal individuals and *Macaca irus* (Fig. 3*a*, *b*). Control sites received the same dilutions of unabsorbed serum. When the skin sites were challenged with antigen E, the control sites showed erythema-wheal reactions in humans and blueing reactions in *Macaca irus*, whereas the sites receiving the supernatants did not. It is clear that anti-IgND serum precipitated the reaginic antibodies in the patient's serum.

DISCUSSION

The physicochemical properties of γE-globulin and myeloma-IgND are similar to each other. The electrophoretic mobility of both proteins in agarose gel was γ_1 (2, 7). The sedimentation coefficient of γE-globulin with antibody activity to ragweed antigen E was estimated to be about 8 S by sucrose density gradient ultracentrifugation (2). This value is in close agreement with 8.2 S ($s^0_{20,w}$) for purified myeloma-IgND as determined by analytical ultracentrifugation (13). On gel filtration both γE-globulin and myeloma-IgND came off Sephadex G-200 or G-150 columns earlier than IgG but similar to IgA (5, 7).

The results of comparative studies of the antigenic structure of γE-globulin and myeloma-IgND described in the present paper confirm that the two proteins share major antigenic determinants, which are characteristic of a fifth class of human immunoglobulins. Presence of the common major antigenic determinants in γE and IgND is supported by the fact that both anti-γE and anti-IgND precipitated human reaginic antibodies and induced reversed type allergic reactions in normal human skin (17). In addition, both non-antibody γE and myeloma IgND blocked passive sensitization of human skin with reaginic antibodies, whereas myeloma proteins of other immunoglobulin classes failed to do so (6, 22). On the basis of these findings it was recently proposed that this new class of immunoglobulins be designated IgE (or γE) (22) in accordance with "Nomenclature for Human Immunoglobulins" (23) and that myeloma IgND be called E myeloma protein.

SUMMARY

IgND, represented by a myeloma protein ND and γE-globulin, detected in and isolated from atopic patients' sera with reaginic activity, belong to the same immunoglobulin class, which is distinct from IgG, IgA, IgM and IgD.

The antigenic determinants characteristic of the fifth immunoglobulin class, which should be designated IgE, are localized in the F_c portion of the molecule.

The association of reaginic activity with IgE was confirmed by the fact that anti-γE globulin as well as an antiserum specific for the F_c portion of myeloma-IgND precipitated the reaginic factor in atopic patients' sera.

REFERENCES

1. Ishizaka, K., Ishizaka, T. and Hornbrook, M. M., J. Immun., *97:* 75, 1966.
2. Ishizaka, K., Ishizaka, T. and Hornbrook, M. M., J. Immun., *97:* 840, 1966.
3. Ishizaka, K., Ishizaka, T. and Hornbrook, M. M., J. Immun., *98:* 490, 1967.
4. Ishizaka, K., Ishizaka, T. and Arbesman, C. E., J. Allerg., *39:* 254, 1967.
5. Ishizaka, K. and Ishizaka, T., J. Immun., *99:* 1187, 1967.

6. Ishizaka, K., Ishizaka, T. and Terry, W. D., J. Immum., *99:* 849, 1967.
7. Johansson, S. G. O. and Bennich, H., Immunology, *13:* 381, 1967.
8. Johansson, S. G. O., Bennich, H. and Wide, L., Immunology, *14:* 265, 1968.
9. Johansson, S. G. O. and Bennich, H., 3rd Nobel Symposium, p. 199, Almqvist & Wiksell, Stockholm, 1967.
10. Johansson, S. G. O., Lancet, *2:* 951, 1967.
11. Wide, L., Bennich, H. and Johansson, S. G. O., Lancet, *2:* 1105, 1967.
12. Coombs, R. R. A., Hunter, A., Jonas, W. E., Bennich, H., Johansson, S. G. O. and Panzani, R., Lancet, *1:* 1115, 1968.
13. Bennich, H., Biochemical J., In press.
14. Mancini, G., Carbonara, A. O. and Heremans, J. F., Immunochemistry, *2:* 235, 1965.
15. Vaerman, J. P. and Heremans, J. F., Science, *153:* 647, 1966.
16. Terry, W. D. and Robert, M. S., Science, *153:* 1007, 1966.
17. Ishizaka, K. and Ishizaka, T., J. Immun., *100:* 554, 1968.
18. King, T. P., Norman, P. S. and Connell, J. T., Biochemistry, *3:* 458, 1964.
19. Yagi, Y., Maier, P., Pressman, D., Arbesman, C. E. and Reisman, R. E., J. Immun., *91:* 83, 1963.
20. Ouchterlony, O., Progr. Allerg., *5:* 1, 1958.
21. Layton, L. L., Yamanaka, E. and Renko, C. W., J. Allerg., *33:* 271, 1962.
22. Stanworth, D. R., Humphrey, J. H., Bennich, H. and Johansson, S. G. O., Lancet, *2:* 330, 1967.
23. World Health Organization, Bull. W. H. O., *38:* 151, 1968.