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Antigen Combining Activity Associated with Immunoglobulin D

Abstract. *The titers of antibodies to the benzylpenicilloyl antigenic determinant are increased with specific antiserum to immunoglobulin D, as shown by the enhancement method. This increase in titer is blocked by purified immunoglobulin D protein but not by immunoglobulins of the other four classes.*

Immunoglobulin D (γD) was discovered by Rowe and Fahey during studies of myeloma proteins (*1*). They demonstrated that γD was present in normal human serum and was structurally similar to the other classes of immunoglobulins but possessed unique antigenic determinants associated with the heavy chain. Also γD-containing cells were present in human spleen sections and resembled plasma cells (*2*). However, in spite of these similarities to the other immunoglobulins, antibody activity in γD has been difficult to demonstrate.

We have analyzed human serums containing antibody reactive with the benzylpenicilloyl antigenic determinant. Fresh type O, Rh-positive erythrocytes were sensitized with penicillin G, as described by Levine and his associates (*3*). Serums from subjects allergic to penicillin G were diluted to determine the lowest concentration necessary for agglutination of sensitized erythrocytes. The cells were washed, and the titer was again determined in the presence of buffer or specific goat antiserum to γD. An increase in titer in the presence of this antiserum is referred to as "enhancement," and presumably reflects the presence of γD antigenic determinants on the erythrocytes. This antiserum was produced in a goat by immunization with purified γD from myeloma serum, the protein being injected in complete Freund's adjuvant. The antiserum was rendered specific for γD by absorption with γG, with lambda-type Bence Jones protein, and with normal human serum deficient in γD protein. The absorbed antiserum formed a single band with γD myeloma serum in Ouchterlony double-diffusion experiments and contained 160 μg of antibody nitrogen per milliliter, as judged by quantitative precipitin analysis with purified γD protein. In addition, the absorbed antiserum to γD did not form precipitin bands in Ouchterlony double-diffusion with immunoglobulins of the other four classes. Serums from allergic subjects which contained detectable γD and which agglutinated penicillin-sensitized erythrocytes in high titer were screened to determine whether their titers were enhanced by antiserum to γD. Three such serums containing from 3.4 to 6.5 mg of γD per 100 ml were found and studied further with antiserum to γD and various inhibitors. The direct agglutination reactions were specific for the benzylpenicilloyl antigenic determinant in that they were inhibited by the hapten, α-D-benzylpenicilloyl-ε-aminocaproate, $1 \times 10^{-2}M$, but not by the unrelated hapten, dinitrophenyllysine, $2 \times 10^{-3}M$ (Table 1). The marked enhancement of the titer, 32-fold, in the presence of antiserum to γD is blocked by purified γD but not by immunoglobulins of the four other classes. Similar results were obtained with serums from two other subjects whose titers were enhanced 8-fold and 16-fold. The enhancement of titer by antiserum to γD was demonstrated several times with each serum.

These results indicate that serums from some subjects allergic to penicillin G contain γD-globulins specific for the benzylpenicilloyl antigenic determinant. Although we have not studied serums before and after exposure to antigen to demonstrate a rise in the concentration of reactive γD proteins, these results do suggest that γD has antibody activity. Ritchie (*4*) has reported antibody activity against cell nuclei which is associated with γD; Heiner and his associates (*4*) have shown that antibody activity to diphtheria toxoid and bovine γ-globulin is associated with γD. Levine and Redmond (*5*) also found γD on erythrocytes from patients with hemolytic anemia caused by penicillin G. However, blocking of these reactions by purified γD protein or absorption of activity with antiserum to γD was not demonstrated.

Table 1. Enhancement of titer by antiserum to γD. Normal erythrocytes and erythrocytes (RBC) sensitized with penicillin G were incubated with dilutions of serum in buffer [dextran, fetal calf serum, tris-buffered saline (*3*)] and washed thrice with 3.0 ml of 0.15*M* NaCl, 0.02*M* tris(hydroxymethyl)aminomethane, *p*H 8.2. Cells were resuspended in 0.2 ml of buffer, anti-γD, or anti-γD and inhibitor and incubated for 45 minutes at 24°C; the titer was then determined. Reactions were graded as described by Levine and his associates (*3*). Anti-γD was used at 1.3 μg of antibody nitrogen per tube, while the inhibitors were used in amounts from 24 to 29 μg of nitrogen per tube. Anti-γD and the various inhibitors were absorbed with normal and sensitized erythrocytes to remove naturally occurring agglutinins. γA, γM, and γD were purified myeloma proteins free of other immunoglobulins by immunodiffusion analysis while Cohn fraction II was used as γG. As a source of γE, a serum was used which contained γE (2 g/100 ml) but was deficient in γD protein (*6*). As additional controls, sensitized and normal erythrocytes were incubated with all the inhibitors and their reactions were tested directly and after addition of antiserum to γD. Moreover, normal goat serum did not produce enhancement of the titer. Finally, pooled normal human serum containing 5 mg of γD per 100 ml and treated with an insoluble benzylpenicilloyl immunoabsorbent to remove penicilloyl antibodies did not directly agglutinate sensitized or normal erythrocytes, and after being washed the cells were not agglutinated by specific antiserum to γD.

RBC incubated with	$10^3 \times$ Reciprocal dilution of patient's serum								
	Normal RBC	Sensitized RBC							
	4	4	16	32	64	128	256	512	1024
Buffer	0	1+	Tr	0	0	0	0	0	0
Anti-γD	0	—	1+	1+	1+	1+	1+	Tr	0
Anti-γD + γD	0	—	Tr	0	0	0	0	0	0
Anti-γD + γG	0	—	1+	1+	1+	1+	1+	Tr	0
Anti-γD + γM	0	—	1+	Tr	1+	1+	1+	Tr	0
Anti-γD + γA	0	—	—	—	1+	1+	1+	Tr	0
Anti-γD + γE	0	—	1+	1+	1+	1+	1+	Tr	0

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