Lambda-Gamma Determinant of Human IgG

Antigenic determinants on protein molecules have been shown to be dependent upon the amino acid sequence and three dimensional folding of the molecule. Alteration of the primary structure or of the configuration of the molecule results in altered specificity.

During the past few years many workers have described antigenic determinants both for the classes and the subclasses of the immunoglobulins. These determinants have been ascribed to particular areas of the polypeptide chains, as for example, in the localization of the Gm and the Inv sites on the heavy chain and light chain, respectively. It has been shown in more recent studies that for their expression, some antigenic determinants depend upon the close proximity of the heavy chain with the light chain in the tertiary and quarternary structure of the molecule[1-5]. During attempts to raise an anti human lambda chain antiserum in rabbits, by immunizing with an IgG λ myeloma, a $\lambda_2\gamma_2$ antigenic determinant was observed which relied upon the association of the four chains for its expression.

Materials and methods. Preparation of the antisera. Antisera were raised in rabbits by hind foot pad injection of approximately 1 mg of an IgG λ myeloma protein emulsified with Freunds complete adjuvant. Simultaneously 10 mg of an IgG $\varkappa$ myeloma protein of the same subclass was administered i.v. in an attempt to suppress antibody formation to other than λ chain determinants, after the method of HENNEY and ISHIZAKA[6].

Fourteen days later a second injection of the IgG λ myeloma protein in adjuvant was given i.m. into the shoulders with a second i.v. injection of the IgG $\varkappa$ myeloma protein. After a further period of 14 days the animals were bled and the serum tested.

One antiserum (AS13) was obtained using γ G3 myeloma proteins and another (AS12) by using γ G1 proteins. Both antisera showed weak reactions against Fc, and were rendered specific by absorption with a small amount of an IgG2 $\varkappa$ myeloma protein. AS12 also contained antibodies to siderophilin which were removed by absorption with a purified preparation of siderophilin kindly supplied by Mr. R. DREW, Department of Experimental Pathology, Medical School, Birmingham.

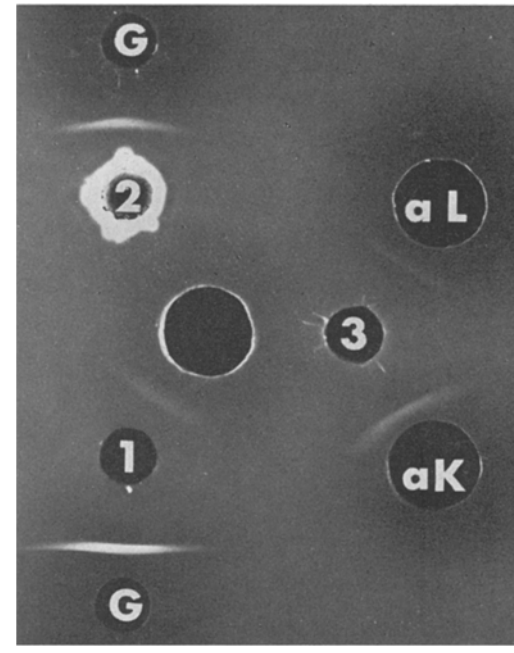

Fig. 2. Ouchterlony analysis of whole human IgG (1) and its separated heavy (2) and light (3) polypeptide chains against AS13 in the large central well. Commercial anti kappa (aK) and anti lambda (aL) light chain antisera and anti whole human IgG (G) are also shown.

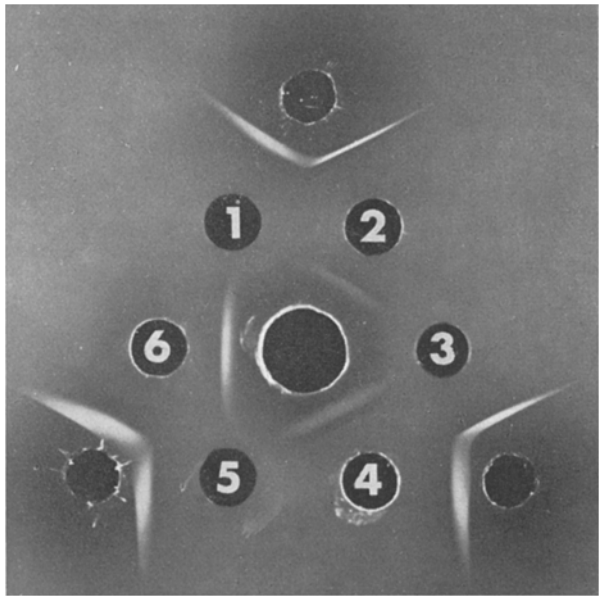

Fig. 1. Comparative Ouchterlony analysis of 3 $\varkappa$ IgG myeloma proteins (IgG1, IgG2 and IgG3 in wells 1, 3 and 5, respectively) with 3 λ IgG myeloma proteins (IgG1, IgG1 and IgG3 in wells 2, 4 and 6, respectively). AS13 was placed in the centre well, and anti whole human IgG in the outermost wells.

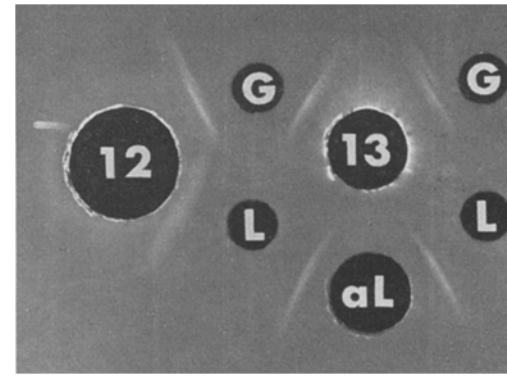

Fig. 3. Double diffusion of whole human IgG (G) and isolated light chains (L) against prepared antisera AS12 (12) and AS13 (13). The former gives a spur of whole IgG over light chains. A commercial preparation of anti λ light chain antiserum (aL) is also shown.

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Preparation of immunoglobulin and isolation of fragments. Human IgG was prepared by column chromatography with diethylaminoethyl cellulose (Whatman DE 52) equilibrated in 0.01 *M* phosphate buffer at pH 8.0. Heavy and light polypeptide chains were obtained from 300 mg IgG by reaction with 10 mg dithiothreitol (Clelands reagent) buffered at pH 8.0 with 0.2 *M* *tris*-HCl. After 30 min the preparation was alkylated using a slight excess of iodoacetamide dissolved in the same buffer. Separation of the heavy and light chains was carried out an G100 Sephadex in 1 *M* acetic acid by the method according to FLEISCHMAN[7].

Papain digestion of IgG was carried out using a method essentially similar to that of PORTER[8]. Pepsin digest fragments (Fab)[2] from normal human IgG was kindly supplied by Dr. R. JEFFERIS, Department of Experimental Pathology, Medical School, Birmingham.

Immunoelectrophoresis was carried out in 1.5 g/100 ml Difco Noble agar in 0.045 *M* barbitone buffer pH 8.6 after the method of SCHEIDEGGER[9]. Double diffusion analysis was performed in 1.2 g/100 ml agarose gel in phosphate buffered saline pH 7.2.

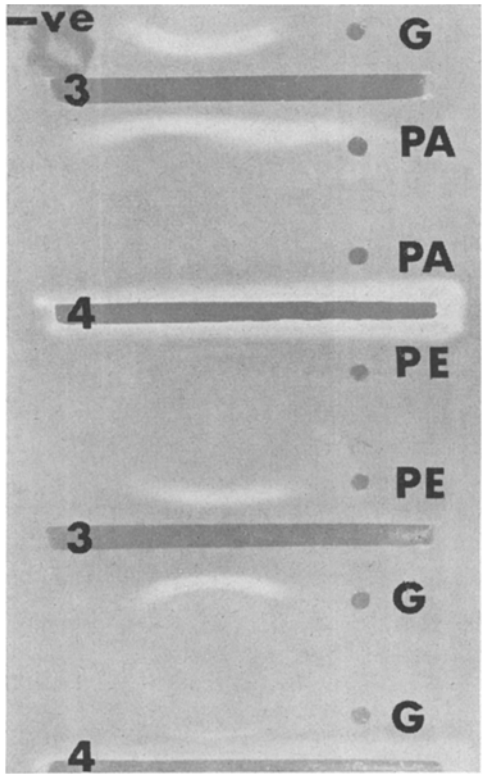

Fig. 4. Immunoelectrophoretic analysis of whole human IgG (G), papain digested IgG (PA), and pepsin digested IgG (PE) against anti whole human IgG (3) and AS13 (4). The latter reacted weakly with the pepsin digest but not at all with the papain digested IgG.

Anti λ-chain and anti $\varkappa$-chain antisera were also obtained from Hoechst Pharmaceuticals Ltd. The former reacted with free λ-chains, but not with IgGλ myeloma proteins or with normal human IgG. Anti whole human IgG antiserum was raised in a rabbit by the i.m. injections of whole IgG prepared as described.

Results. Both antisera AS12 and AS13 reacted with isolated λ myeloma proteins but not with $\varkappa$ myeloma proteins of the four IgG subclasses 3 pairs of which, against AS13, are shown in Figure 1.

Electrophoretic analysis showed the reaction to occur in the position of the myeloma protein. Both antisera reacted with normal Human IgG. One of the antisera, AS13, however did not give a precipitation line with free light chains either as Bence Jones protein or after dissociation from whole IgG (Figure 2); nor did it react with free heavy chains. The other antiserum AS12 gave a weak precipitin line with free light chains, but the line against whole IgG spurred over it (Figure 3). It was confirmed that this antiserum reacted weakly with 3 λ, but not with 3 $\varkappa$ Bence Jones proteins.

On digestion of whole IgG with papain the reaction with AS13 was lost, although the antiserum still reacted against the pepsin digest (Figure 4). This antiserum also did not react with isolated human colostrol IgA, prepared as described previously[10], nor with a partially purified preparation of serum IgM, free from other immunoglobulins.

Discussion. There have been a number of reports of configurational antigenic determinants of IgM and IgA with $\varkappa$ light chains. An antigenic determinant requiring interaction of light and heavy chains was described by POLMAR and STEINBERG[11] in which light chains were necessary for the expression of the allotypic antigen Gm 3 on the heavy chain of the IgG molecule. Removal of the light chains resulted in loss of antigenic activity.

The results presented in this paper demonstrate the interaction of IgG heavy chains and λ light chains in the production of a specific antigenic determinant. This determinant is present in the intact molecule and pepsin fragment (Fab)[2] but is absent from the separated light chains and heavy chains, type $\varkappa$ IgG myeloma proteins and the papain fragments Fab and Fc.

Thus the specificity requires the integrity of the 4 polypeptide chains, and is not just a $\lambda\gamma$ determinant; or alternatively, the contribution of the γ chain to a $\lambda\gamma$ determinant may be in the hinge region, in the portion of the molecule susceptible to papain but not pepsin digestion.

Zusammenfassung. Die Antigendeterminanten der IgG wurden mit Hilfe des Myelomproteins charakterisiert.

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