COMMON AND SPECIFIC DETERMINANTS ON THE Fd FRAGMENTS OF GUINEA-PIG IMMUNOGLOBULINS

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1. Introduction

Elucidation of the 4-chain structure of antibody molecules [1] provided a means for recognising that individual classes of immunoglobulins (Ig) have common light chains, but structurally distinct heavy chains [2]. Studies on guinea-pig immunoglobulins revealed that the *C*-terminal halves of heavy chains which are known as Fc fragments are structurally distinct when derived from the different Ig classes known as γ1G and γ2G [3]. On the other hand, the light chains and *N*-terminal halves of heavy chains (known as Fd), carry common antigenic determinants [4, 5].

The present study shows that the common determinants present on Fd of γ1G and γ2G can also be demonstrated on a third class of guinea-pig Ig, namely, γM; in addition, it is shown that Fd contains determinants which are specific for different classes of guinea-pig immunoglobulin.

2. Materials and methods

Isolation of Igs. Serum was obtained from guinea-pigs previously immunised with bovine γ-globulin in Freund's complete or incomplete adjuvant, in order to increase the Ig levels. Preparation of γ1G and γ2G was facilitated by finding that the former is more readily precipitated by $(NH_4)_2SO_4$. γ1G was prepared by precipitation of guinea-pig serum in 32% saturated $(NH_4)_2SO_4$ solution followed by chromatography on DEAE cellulose using tris buffers. The precipitate formed between 32% to 40% saturated $(NH_4)_2SO_4$ was used for isolation of γ2G by DEAE chromatography and gel filtration on Sephadex G200. IgM was isolated from euglobulin solutions by ultracentrifugation and gel filtration. All Ig preparations were pure as judged by immunoelectrophoresis using a polyvalent rabbit antiserum to guinea-pig serum [6].

Ig heavy and light chains were isolated from partially or completely reduced and alkylated proteins by gel filtration on Sephadex G200 in 5 M guanidinium chloride.

Enzymatic fragments of γ2G. Four hour papain digests of γ2G were equilibrated with a tris buffer by passage through Sephadex G25 and fractionated on DE22 by gradient elution with tris buffers to separate Fab (i.e. fragment containing light chain + Fd) and Fc. These fragments were further purified by gel filtration on Sephadex G200 [6].

Antisera were raised against γ2 Fab and γ1 Fab in Freund's complete adjuvant by injecting rabbits on days 1 and 8 in multiple subcutaneous sites. Further injections of the alumina-absorbed proteins were given at intervals.

3. Results

The antiserum to γ2 Fab reacted strongly on gel diffusion with γ2G and light chains and weakly with γ2 Fc. This antiserum, after absorption with isolated light chains and γ2 Fc, gave reactions of identity with γ2G and γ2-chains. The latter were prepared by dissociation of reduced γ2G in 5 M guanidinium HCl and

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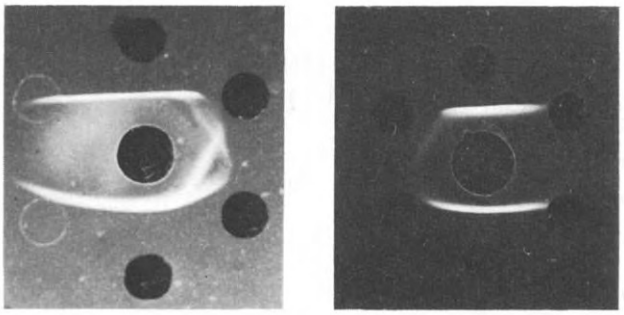

Fig. 1. Reaction of anti-γ2 Fab (*left*) and anti-γ2 Fd (*right*) with (1) γ2G, (2) γ2-chain (5 M guanidinium chloride preparation), (3) L-chain, (4) γ2 Fab.

shown by immunological tests to be free of L-chains. Although relatively insoluble, the isolated γ2-chains gave a satisfactory reaction with the absorbed anti-Fab when used at a concentration of 2 mg/ml in an agar gel containing 0.1 M tris buffer pH 8.2 (fig. 1). These findings indicate that the absorbed anti-γ2 Fab antiserum is directed against H-chain determinants located in the Fd section of the γ2-chain and expressed in the absence of an associated L-chain. The antiserum resembles that described by Nussenzweig and Benacerraf [5] and is referred to as anti-γ2 Fd.

On double immunodiffusion the anti-γ2 Fd reacted strongly with γ2 Fab, γ1G and γM (fig. 2) indicating the presence of common determinants on the 3 classes

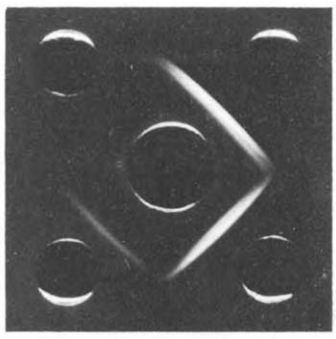

Fig. 2. Detection of common determinants on guinea-pig immunoglobulins. Well (1) L-chains, (2) γM, (3) γ2G, (4) γ1G. Centre well anti-γ2 Fd.

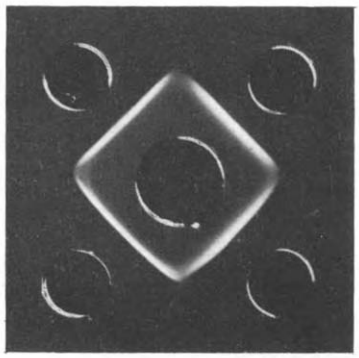

Fig. 3. Reaction of anti-γ2 Fd with γ1G (3), γ2G (2) and their Fab fragments – γ1 Fab (4) and γ2 Fab (1).

of heavy chains. This could not be directly confirmed, since the γ1 and μ-chains isolated in 5 M guanidinium chloride were poorly soluble in aqueous solution and failed to react with the anti-γ2 Fd serum. However, as mentioned above, the antiserum did react with γ2-chains isolated in the same way, so it is reasonable to regard the common determinants shown in fig. 2 as

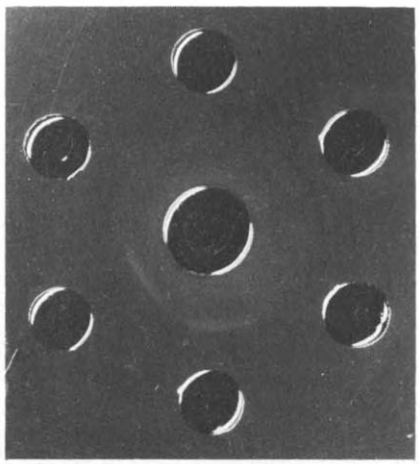

Fig. 4. Reaction of anti-γ2 Fd absorbed with γM (centre) with (1) γ2G, (2) γ2 Fab, (3) γ1 Fab, (4) γ1G, (5) γM, (6) γ2 Fc.

indicative of a common structure in the Fd regions of γ2, γ1 and μ-chains.

As shown in fig. 3, the anti-γ2 Fd serum produced slight spurring between γ1 Fab and γ2 Fab, but not between either fragment and its parent immunoglobulin or between γ1G and γ2G themselves. In accordance with this observation, the anti-γ2 Fd antiserum, when repeatedly absorbed with γM (fig. 4) no longer precipitated γM, γ1G or γ1 Fab, but still reacted with γ2 Fab and γ2G (but not with γ2 Fc).

4. Discussion

The Fd specificity of the absorbed γ2 Fab antiserum was shown by: (i) its reaction with γ2-chains isolated in 5 M guanidinium chloride and free of detectable light chains; (ii) its failure to react with γ2 Fc monomer which consists of the *C*-terminal half of the γ2-chains (molecular weight 29,000 cf. 53,000 for the whole γ2-chains [6]). Although this γ2 Fd antiserum did not react with γ1 or μ-chains isolated in guanidinium chloride, its reaction with γ1G and γM indicates the presence of common Fd determinants on the 3 classes of guinea-pig heavy chains. Oriol and Binaghi [7] have recently shown that a similar anti-Fd serum precipitates about 65–95% of all 3 classes of guinea-pig Ig. When absorbed with γM the anti-γ2 Fd serum used in the present study still reacts with γ2G and γ2 Fab but no longer with γ1G or γ1M (or γ2 Fc) showing that there are class specific determinants in the Fd section of the γ2 heavy chain. Using similar techniques class specificity was also demonstrated on γ1 Fd.

Comparative sequence studies have established that Fd has a variable region which extends to 114–116 residues from the *N*-terminus [8, 12]. The allotypic markers Aa1, Aa2 and Aa3 which are located on rabbit heavy chains, are common to γG, γA and γM [9] and appear to be determined by multiple sequence differences in this *N*-terminal region of the heavy chain [10]. The inference of these studies – namely that the variable region of Fd is common to all Ig classes in the rabbit is supported by the studies of Koshland et al. [11] who have shown that (i) amino acid compositional differences between Aa1 and Aa3 rabbit antibodies of given specificity are identical whether γM or γG are compared and (ii) the amino acid differences between pairs of γ or μ-chains isolated from 2 distinct antibodies are also identical. The presence of a shared Fd variable region in human immunoglobulins is indicated by the closely similar *N*-terminal sequences of monoclonal γ1 and μ-chains [12, 13]. The *C*-terminal half of Fd on the other hand, appears to have a constant sequence in chains of a given isotypic and allotypic specificity. Study of peptides adjoining intra-chain disulphide bridges in the *C*-terminal half of Fd in human γG monoclonal proteins has revealed differences specific for subclasses in about a third of the 30 residues examined [14].

These detailed studies on rabbit and human Igs suggest that the common determinants of guinea-pig heavy chains are present on the variable *N*-terminal sections of Fd from γ1, γ2 and μ-chains, while the class specific determinants demonstrated above are probably on the constant *C*-terminal section of Fd. The presence of both common and isotypically distinct structural features on single polypeptide chains has genetic implications which have recently been discussed [11, 15].

Acknowledgements

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