

Analysis of Fd Antigen in Gamma and Mu Paraproteins with Shared Idiotype¹ (37140)

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(Introduced by A. J. Steigman)

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Immunoglobulin heavy and light chains can each be divided into a variable and a constant region. The variable portion of the heavy chain is the amino-terminal half of Fd plus the Fc fragment and contains three zones. Each zone is centered on an intrachain disulfide bond: C_{H1} is in the carboxy half of Fd and C_{H2} and C_{H3} are in Fc (1). Recent amino acid sequence analyses have focused on the heavy chain variable region, particularly on subgroup V_{HIII} of the three subgroups common to the gamma, alpha and mu chains (2-4). Antigenic determinants that differentiate the immunoglobulin classes and the subclasses of IgG are located in the Fc fragment; Fab is required solely for detection of the Gm₄ determinant close to the interheavy chain disulfide bonds of IgG₁ (5). This report further defines and localizes a C_{H1} antigen common to IgG.

Rabbit antisera were raised to polypeptides from myeloma protein He (IgG_{1-κ}). One of the antisera was made against cyanogen bromide fragment He₄ which extends from residue Nos. 88 to 256 and consists principally of the carboxy half of Fd. Antibody to this polypeptide reacts with purified IgG myeloma proteins of all subclasses, but not with IgA, IgM or κ and λ light chains (6). The antisera also do not react with two γ heavy chain disease (HCD) proteins, CRA (IgG₁) and GIF (IgG₂) (7). CRA lacks all but the amino terminal 11 residues of Fd and GIF contains the first 100 amino acids of V_H followed by a deletion to amino acid No.

216 (8). The failure of antibody to a fragment including residue Nos. 88 to 256 to react with the HCD proteins suggests that the 40 amino acids extending into Fc (Nos. 216 to 256) are not involved in the recognized antigenic determinant(s) mentioned above (7).

The immunoglobulins Til IgG_{2-κ} and Til IgM-κ from a patient with biclonal gammopathy have identical light chains and share the same idiotypic determinants (2, 9). The sequence of the first 40 amino acids of each heavy chain was found to be identical (10). It was of interest to determine whether the IgG Fd antigen could be detected in both Til IgG and in Til IgM and whether the amino acids extending into V_H of the γ chain were of serologic importance.

Materials and Methods. The Fd polypeptide He₄ and the Fc polypeptide He₅ + He₆ (amino acid Nos. 257 to 431) of the IgG₁ myeloma protein He were named after the ones of myeloma protein Eu published at the time (11). In a subsequent amino acid sequence study of Fd of He, the He₄ polypeptide was called He H₂ (12). Antibodies were raised in rabbits to these polypeptides.

Various dilutions of Til IgG_{2-κ} and Til IgM-κ, kindly provided by Doctors Wang and Fudenberg of the University of California School of Medicine, were tested against the antibodies made against the Fd and Fc polypeptides of He by the Ouchterlony immunodiffusion technique. The maximum protein concentration was 1 mg/0.2 ml. Two multiple myeloma proteins, IgG_{1-κ} and IgG_{3-λ} were treated with trypsin to prepare Fab(t) and Fc(t) fragments and their light chains were

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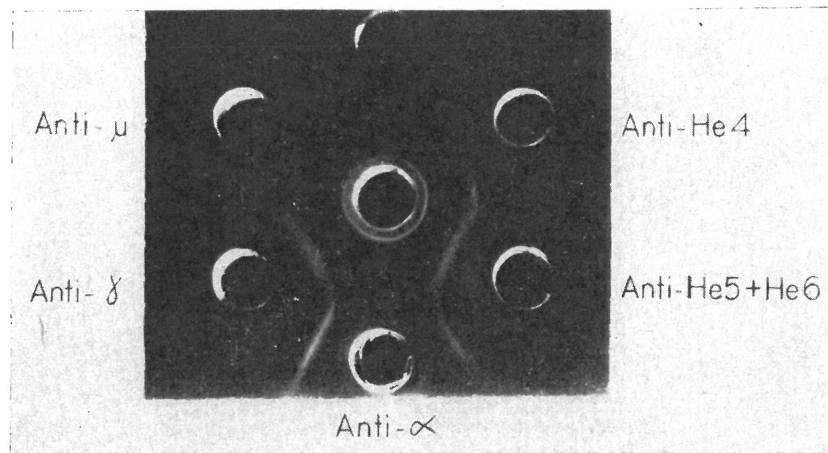


FIG. 1. Center well: Til IgG. Content of peripheral wells indicated. Top well empty.

separated by mild reduction and alkylation (13). The fragments and chains were studied with the antisera raised against He₄ and He₅ + He₆ by the Ouchterlony method.

Results. Til IgG reacts with antiserum against IgG and the antisera against the carboxy half of Fd of IgG (anti-He₄) and against the principal antigenic portion of Fc of IgG (anti-He₅ + He₆) (Fig. 1). Til IgM reacts only with antibody against mu (Fig. 2). The antibody against Fd (Fig. 3) reacts with Til IgG and another IgG myeloma but not with Til IgM; the reactions with the antibody against the Fc polypeptide are similar (Fig. 4). Anti-He₄ reacts with the Fab(t) fraction of the IgG₁ and IgG₃ myeloma proteins but not with their light chains or Fc(t) fragments (Fig. 5).

Discussion. An antigenic determinant in

Fd of 50 IgG proteins but not in IgA or IgM immunoglobulins has been previously reported (6). The results of a serologic study of Til IgG and Til IgM, with shared idiotype (Figs. 1-3), show the presence of the Fd antigenic determinant in Til IgG but not in Til IgM. It was not expected that Til IgG and Til IgM would have common Fc antigenic determinants (Figs. 1, 2 and 4). The antibody to He₄ gave precipitation lines to the Fab(t) fragments of the IgG₁ and IgG₃ myeloma proteins; it failed to react with the Fc(t) fragments or the κ and λ light chains of these proteins (Fig. 5). The antibody's lack of reaction with Fc from other IgG subclasses and light chains (6) (Fig. 5) points to specificity for antigenic determinants common to the C_{III} region of all human γ chains.

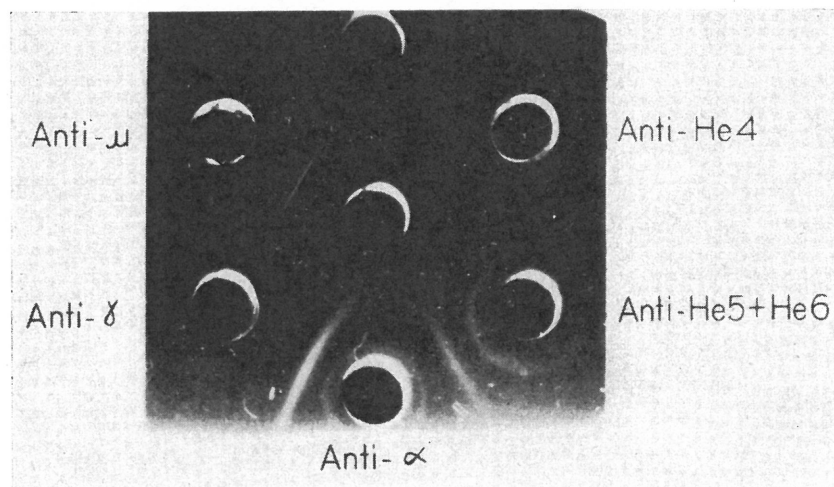


FIG. 2. Center well: Til IgM. Peripheral wells: see Fig. 1

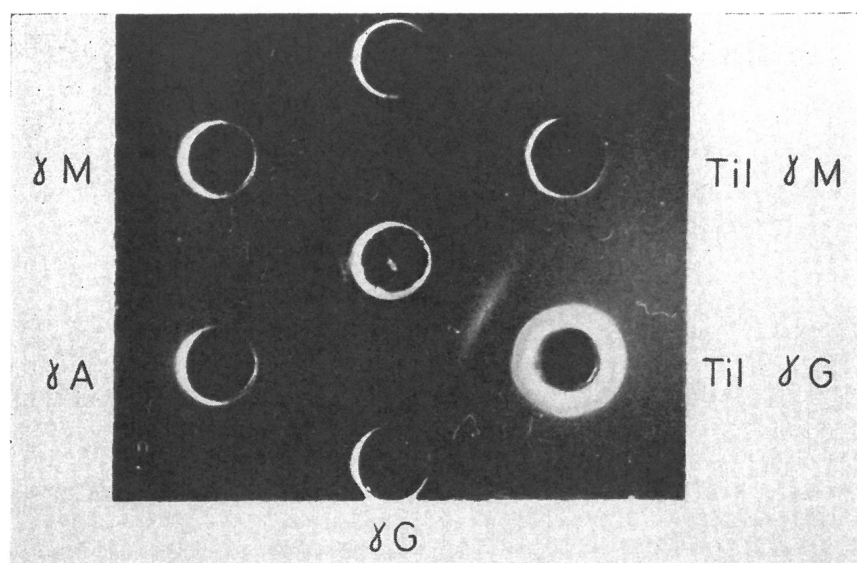


FIG. 3. Center well: Antibody to peptide comprising carboxy half of Fd of IgG₁ (anti-He₄). Content of peripheral wells indicated and described in text and Fig. 1. Top well empty.

He₄ includes the carboxy half of Fd plus approximately 30 amino acids of the variable region of Fd and 40 amino acids placed in Fc. Studies with γ HCD proteins lacking amino acids proximal to No. 216 showed that the interheavy chain disulfide bridge and the amino acids in Fc do not influence the antigenic determinants of the Fd polypeptide (7). The present report suggests that the amino acids proximal to residue No. 120 in the variable region, which includes the idiotypic zone, similarly lack antigenic significance. Changes in conformation may mask the above sites and make them inaccessible

to the antibody (14) but the reaction of the antibody with 50 IgG proteins of all subclasses makes this unlikely. Common antigenicity in this region may be lacking because He₄ includes the hypervariable region between residue Nos. 101 and 109 and two thirds of the hypervariable residues between Nos. 86 and 91 (15). Further studies such as adsorption of the antibody with mu chains, indirect precipitation or binding, have not been possible due to lack of Til protein and of antibody.

The results support the proposal that variability in the heavy chain is limited to the

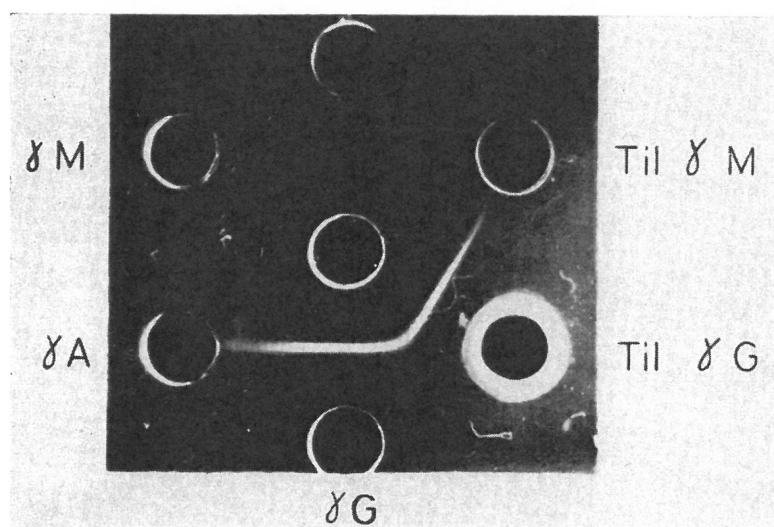


FIG. 4. Center well: Antibody to principal antigenic peptide in Fc of IgG₁ (anti-He₅ + He₆). Peripheral wells: see Fig. 3.

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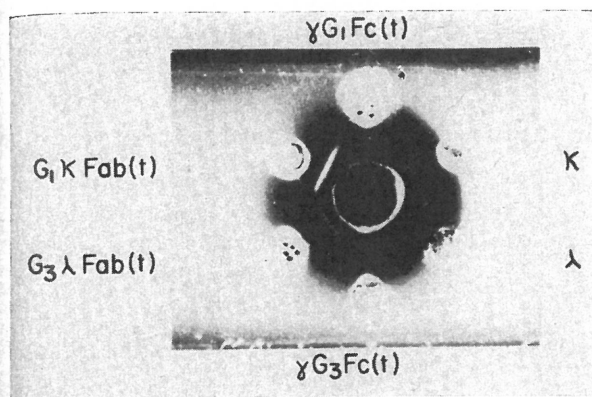


FIG. 5. Center well: Antibody to peptide comprising carboxy half of Fd of IgG₁ (anti-He₄). Contents of peripheral wells: fractions and chains of IgG₁-κ and IgG₃-λ myeloma proteins.

amino half of Fd and that the carboxy half of Fd is constant (1, 16). Common antigenicity among heavy chains was earlier reported in the Fd segment (17). The finding of similar subgroups in the variable region of all heavy chains indicates that the earlier finding probably refers to this region (2-4). No antigenic determinants common to a peptide overlapping the variable and constant regions were demonstrated. Their detection would have supported the idea of a cell changing from IgM to IgG synthesis. The "overlap" peptide theory suggests that V genes controlling the variable region of the immunoglobulin chain have nucleotides overlapping those of C genes regulating the synthesis of the common region, at the gene fusion site (18). The resulting peptide may not bear antigenic determinants, especially since it includes hypervariable region(s) at the carboxy end of the variable half of Fd.

The present report further localized the Fd antigenic determinants common to IgG to its C_{H1} zone. The similarity of the C_{H1} amino acid sequence in IgG₁ and IgG₄ (19), in addition to this serology common to all IgG subclasses, suggest that the amino acid sequences of C_{H1} of IgG₂ and IgG₃ will resemble the other subclasses closely. The amino acid sequence of C_{H1} of IgM has shown surprising structural differences from C_{H1} of IgG (20).

Summary. The carboxy half of Fd of IgG has previously been shown to be an antigen common to IgG proteins. In this report the antigenic determinants were not found in an

IgM paraprotein with a region of idiotype similar to that of an IgG paraprotein which contained them. The available data suggest that the antigenic determinants are contained between residue Nos. 120 and 216, the C_{H1} region of IgG.

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