Subclasses of Human γ_2-Globulin Based on Differences in the Heavy Polypeptide Chains

Abstract. *Three subclasses of human γ_2-globulin (IgG) molecules were detected in normal human serum with antiserums prepared in monkeys. These subclasses, designated γ_{2a}-, γ_{2b}-, and γ_{2c}-globulins, have antibody activity. The distinguishing antigenic characteristics of each subclass were associated with the heavy polypeptide chains and the F (fast) fragments resulting from treatment of γ_2-globulins with papain.*

The γ_2-globulin (IgG) (*1*) molecules are composed of two types of polypeptide chains—the heavy chains and the light chains (*2*). Two antigenically different types of light chains (type I and type II), (or κ and λ), (*1*) have been identified in normal human γ_2-globulin (*3*). Antigenic heterogeneity of the heavy chains (γ-chains) (*1*) in γ_2-globulin molecules was investigated in this study.

Rhesus monkeys were immunized with pooled normal human γ_2-globulin emulsified in Freund's complete adjuvant. Some of the resulting antiserums produced three precipitin arcs in the γ-globulin electrophoretic region when they were used to develop the immunoelectrophoretic pattern of normal human serum. Similar immunoelectrophoretic findings have been described (*4*). The three precipitin arcs are referred to as γ_{2a}-, γ_{2b}-, and γ_{2c}-globulins (*5*). In immunoelectrophoresis of normal human serum the γ_{2b}-globulins migrate further toward the cathode than the γ_{2a}-globulins (Fig. 1*A*). Both are electrophoretically heterogeneous. In contrast, the γ_{2c}-globulin precipitin arc is less heterogeneous electrophoretically and is further from the antiserum trough than the arcs of either the γ_{2a}- or γ_{2b}-globulins (Fig. 1*A*).

Several observations indicate that the γ_{2a}-, γ_{2b}-, and γ_{2c}-globulins are members of the immunoglobulin family, separate from γ_{1A}-globulin (IgA) (*1*), γ_{1M}-globulin (IgM) (*1*), or the recently described γ_{1J}-globulin (*6*), and that they constitute subclasses of the γ_2-globulin class. The γ_{2a}-, γ_{2b}-, and γ_{2c}-globulin precipitin arcs differ in electrophoretic mobility and immunoelectrophoretic configuration from those of γ_{1A}-, γ_{1M}-, and γ_{1J}-globulins. In addition, monkey antiserum specific for the γ_{2a}-, γ_{2b}-, and γ_{2c}-globulins does not react with purified preparations of γ_{1A}-, γ_{1M}- or γ_{1J}-globulins. On the other hand, specific antiserum detects all three subclasses in chromatographically purified normal γ_2-globulin. The γ_{2a}-, γ_{2b}-, and γ_{2c}-globulins are present in all normal human serums that have been tested and are absent, or markedly diminished, in serums from patients with agammaglobulinemia or severe hypogammaglobulinemia.

Radioimmunoelectrophoresis (*7*) was used to demonstrate that molecules of these three subclasses may have antibody activity. Immunoelectrophoresis was performed on a normal human serum and on serums from five patients with chronic thyroiditis and circulating antithyroglobulin antibodies (*8*). The γ_{2a}-, γ_{2b}-, and γ_{2c}-precipitin arcs were developed with monkey antiserums (Fig. 1, *A* and *B*). After exposure of these precipitin arcs to I^{125}-labeled (*9*) human thyroglobulin (*10*), the immunoelectrophoretic plates were placed in contact with x-ray film. The resulting autoradiograph (Fig. 1, *C* and *D*) clearly demonstrates binding of the thyroglobulin by the γ_{2a}-, γ_{2b}-, and γ_{2c}-precipitin bands of the thyroiditis serum, but not by the control serum. Similar findings are described by Lichter (*11*).

Further study of γ_{2a}-, γ_{2b}-, and γ_{2c}-molecules required that they be separated from one another. Techniques are not available for isolating these three normal human serum components. However, some γ_2-myeloma proteins are antigenically related to either the γ_{2a}- or the γ_{2b}-globulin molecules of normal human serum (*4*). γ_2-Myeloma proteins were therefore isolated and tested by Ouchterlony analysis with monkey antiserum. Precipitin bands of some, but not all, myeloma proteins intersected one another, indicating antigenic differences. Absorption of the monkey antiserum with a γ_2-myeloma protein, shown to be antigenically related to normal human serum γ_{2a}-globulin, yielded an antiserum that precipitated γ_{2b}- and γ_{2c}- but not γ_{2a}- globulin molecules (Fig. 1*A*, lower; Fig. 1*B*, upper). Similarly, absorbing the antiserum with a γ_{2b}-myeloma protein resulted in an antiserum that precipitated the γ_{2a}- and γ_{2c}- but not the γ_{2b}-molecules in serum (Fig. 1*A*, upper; Fig.

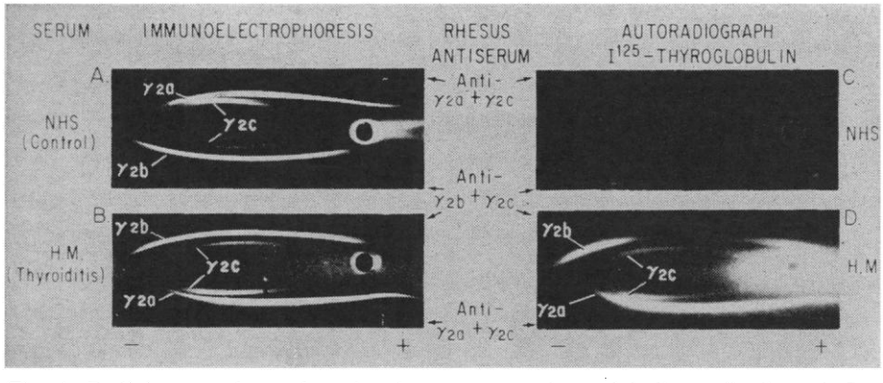

Fig. 1. Radioimmunoelectrophoresis of γ_{2a}-, γ_{2b}-, and γ_{2c}-globulin antibodies. *A*, Immunoelectrophoretic precipitin arcs identifying γ_{2a}-, γ_{2b}-, and γ_{2c}-globulins in normal human serum (NHS). *B*, Immunoelectrophoresis showing the same proteins in serum HM from a patient with chronic thyroiditis and circulating antibodies to thyroglobulin. *C*, Autoradiograph showing no nonspecific binding of I^{125}-thyroglobulin by NHS precipitin arcs. *D*, Autoradiograph showing I^{125}-thyroglobulin is specifically bound by γ_{2a}-, γ_{2b}-, and γ_{2c}-globulin antibodies in HM serum.

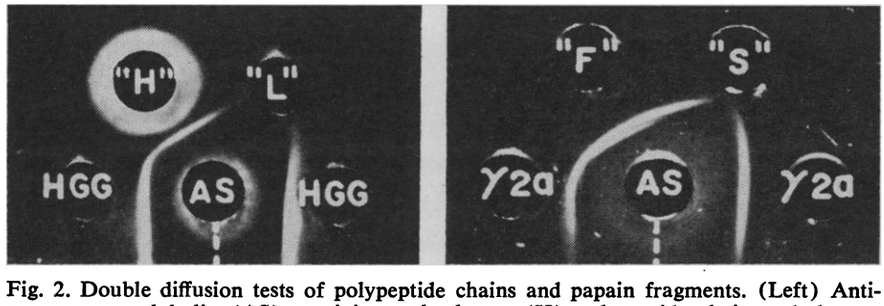

Fig. 2. Double diffusion tests of polypeptide chains and papain fragments. (Left) Antiserum to γ_{2a}-globulin (AS) precipitates the heavy (H) polypeptide chain and the unreduced pooled human γ-globulin (HGG) from which it was derived. The light (L) polypeptide chain is not precipitated. (Right) The same antiserum (AS) precipitates the intact γ_{2a}-globulin myeloma protein and the fast (F) fragment of papain-digested γ_{2a}-globulin myeloma protein. The slow (S) fragment is not precipitated.

1*B*, lower). Absorption with both γ_{2a}- and γ_{2b}-myeloma proteins yielded an antiserum specific for γ_{2c}.

γ_2-Myeloma proteins can be identified as γ_{2a}-, γ_{2b}-, or γ_{2c}-globulins with the use of these absorbed antiserums. Of 72 γ_2-myeloma proteins tested, approximately 15 percent are γ_{2a}-, 65 percent are γ_{2b}-, and 7 percent are γ_{2c}-globulins. The remaining 13 percent of γ_2-myeloma proteins are antigenic variants of still other types, indicating further complexity that will not be considered in this report.

The submolecular localization of the subclass (γ_{2a}, γ_{2b}, γ_{2c}) specific antigenic determinants was investigated in several ways. The heavy and light polypeptide chains of normal γ_2-globulin and those of γ_{2a}-, γ_{2b}-, and γ_{2c}-myeloma proteins were prepared by reduction and alkylation, and the polypeptide chains were separated on columns of Sephadex G-100 (*12*). Ouchterlony analysis with specific antiserums revealed that the specific γ_{2a}-, γ_{2b}-, and γ_{2c}-antigenic determinants were present only on the heavy polypeptide chains (Fig. 2*A*). The light chains did not react with the specific antiserums. Additional evidence that the light chains do not take part in the γ_{2a}-, γ_{2b}-, and γ_{2c}-globulin differences was obtained by examining type I and type II myeloma proteins. The subclass (γ_{2a}-, γ_{2b}-, γ_{2c}-) distribution was the same in 48 γ_2-myeloma proteins with type I light chains and 24 γ_2-myeloma proteins with type II light chains. Also, absorption of the antiserum with type I and type II Bence Jones proteins did not significantly alter immunoelectrophoretic or Ouchterlony precipitin patterns identifying γ_{2a}-, γ_{2b}-, and γ_{2c}-globulins, which provides additional evidence that the subclass specific antigenic determinants are not located on the light chains.

Further studies of the submolecular localization of the subclass specific determinants were made with molecular fragments obtained by papain digestion (*13*). γ_{2a}- and γ_{2b}-Myeloma proteins were treated with papain in the presence of cysteine and the S (Fab) (slow) (*1*) and F (Fc) (fast) (*1*) fragments were isolated (*3*). The F fragment and the intact γ_2-myeloma protein from which it was prepared were precipitated by the antiserum while the S fragment was not (Fig. 2*B*). Thus, the distinctive antigenic characteristics of these subclasses are located on the F fragment of the heavy chain.

The F fragments of the heavy chains of γ_2-globulins determine many biologic properties of the molecules, such as complement fixation (*14*), reactivity with rheumatoid factors (*15*), placental transfer (*16*), and skin fixation (*17*). They also are the site of genetically controlled factors detected by the Gm (hereditary γ-globulin factor) hemagglutination-inhibition test system (*18*). Preliminary studies of reverse passive cutaneous anaphylaxis indicate that γ_{2b}- and γ_{2c}-myeloma protein molecules can bind to guinea pig skin, while γ_{2a}-myeloma protein molecules cannot. Differences in other biologic and genetic characteristics of γ_{2a}-, γ_{2b}-, and γ_{2c}-globulin molecules are under investigation.

After this paper was submitted, two reports (*19*) were published describing two populations of γ_2-globulin molecules detected with antiserums prepared in rabbits. Studies in our laboratory of the myeloma proteins Cr and Zu (*20*) used in these reports show that the Cr protein falls in the group of γ_2-myeloma proteins not identifiable as γ_{2a}-, γ_{2b}-, or γ_{2c}-, and that the Zu protein is antigenically related to γ_{2c}. Apparently, the rabbit antiserums used (*19*) did not distinguish the differences between γ_{2a}-, γ_{2b}-, and γ_{2c}-globulins described in our report.

Note added in proof: Another recent paper (*20*) describes four subgroups of γ_2-globulins. γ_{2b}- and γ_{2c}-Globulins are antigenically related to the We and Vi subgroups of these authors.

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8 July 1964

Valence and Affinity of Equine Nonprecipitating Antibody to a Haptenic Group

Abstract. *A nonprecipitable antibody to a haptenic group has been purified from equine serum. It is a 7S β_{2A} immunoglobulin with high affinity for the specific hapten and two binding sites per antibody molecule. These results rule out univalence and low affinity as explanations of the nonprecipitability of this antibody.*

Since the early descriptions of nonprecipitating equine antibody by Pappenheimer (*1*) and Heidelberger *et al.* (*2*), many explanations have been proposed for the inability of this antibody to precipitate with antigen. The two most popular explanations have been that the antibody is either of low affinity or is incomplete in the sense that it has only one antigen-combining site per molecule. In a recent review of the subject of "incomplete antibodies" (*3*) these two interpretations are discussed and several others are mentioned. Recent studies have demonstrated the frequent occurrence of nonprecipitating antibody in antiserums to protein and have indicated that these antibodies migrate on electrophoresis as β-globulins (*4*). These investigations have not, however, shed any new light on the reason for its nonprecipitability. We now describe a nonprecipitable antibody component which has been detected in a population of equine antibody molecules directed against the *p*-azophenyl-β-lactoside (Lac) haptenic group. After isolation and purification of this immunoglobulin, it has been