THE IMMUNOGLOBULINS OF MICE

II. Two Subclasses of Mouse 7S γ_2-Globulins: γ_{2a}- and γ_{2b}-Globulins

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Four major classes of immunoglobulins have been identified in normal mouse serum (1). One of these classes was designated 7S γ_2-globulin and several myeloma proteins were identified as γ_2–myeloma proteins. The observation that two γ_2–myeloma proteins, 5563 and MPC-11, differ from one another in antigenic composition (2), although both are immunochemically identifiable as members of the 7S γ_2-globulin class (1), raised the possibility that the 7S γ_2-globulins might be further subdivided.

Studies were undertaken with myeloma and normal immunoglobulins to determine whether subclasses of 7S γ_2-globulins could be identified. These investigations provided evidence for two immunochemically distinct subclasses of the 7S γ_2-globulins, tentatively designated 7S γ_{2a}-globulins and 7S γ_{2b}-globulins. Normal and immune serums were investigated and γ_{2a}- and γ_{2b}-globulins were identified in these mouse serums. The γ_2-immunoglobulin subclasses were shown to have similar, as well as distinctive features.

Materials and Methods

Normal and hyperimmune mouse serums, normal immunoglobulin fractions, and myeloma protein preparations were obtained as described previously (1). Ultracentrifugal and electrophoretic technics and immunoelectrophoresis and Ouchterlony analyses procedures have been described (1, 3, 4).

Antiserums were prepared by standard procedures (1, 3). Antiserum specific for γ_{2a}-globulin was prepared by absorbing rabbit antiserum against 5563 γ_{2a}–myeloma protein (R14 or R23) with a γ_1–myeloma protein (MPC-25) and, if necessary, a γ_{2b}–myeloma protein (MPC-31 or MPC-37). Antiserum specific for γ_{2b}-globulin was prepared by absorbing rabbit antiserum against MPC-11 or MPC-37 γ_{2b}–myeloma protein (R49 and R126) with a γ_1–myeloma protein, and, if necessary, a γ_{2a}–myeloma protein (5563 or Adj.PC-5). Specificity was determined by Ouchterlony analysis using meyloma proteins as shown in Fig. 1, and by immunoelectrophoresis. Isoantiserum (anti–Iga-1) was similar to that used previously (5).

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In the course of the present work, the antigenic properties peculiar to an individual myeloma protein (6) and those specific to a class of myeloma proteins had to be distinguished. Determinants characteristic of a class of globulin were identified by immunodiffusion tests performed with normal gamma globulins and other myeloma proteins of the same class as the particular myeloma protein used as the immunizing antigen for each antiserum.

RESULTS

Identification of γ_{2a}- and γ_{2b}-Globulins.—Five γ_2-myeloma proteins were examined by Ouchterlony tests with a variety of rabbit antisera. Precipitin line formation, as shown in Fig. 1 (left), revealed an antigenic determinant (or group of antigenic determinants) shared in common by γ_2-myeloma proteins 5563 and Adj.PC-5 but which is not present on the other three γ_2-myeloma proteins. Immunoglobulins having this antigenic determinant are termed γ_{2a}-globulins. Another antigenic determinant (or group of determinants) is revealed by a different antiserum Fig. 1 (right). This determinant is present on γ_2-myeloma proteins MPC-11, MPC-31, and MPC-37, but is absent from the 5563 and Adj.PC-5 γ_2-myeloma proteins. Myeloma proteins having this determinant are termed γ_{2b}-globulins.

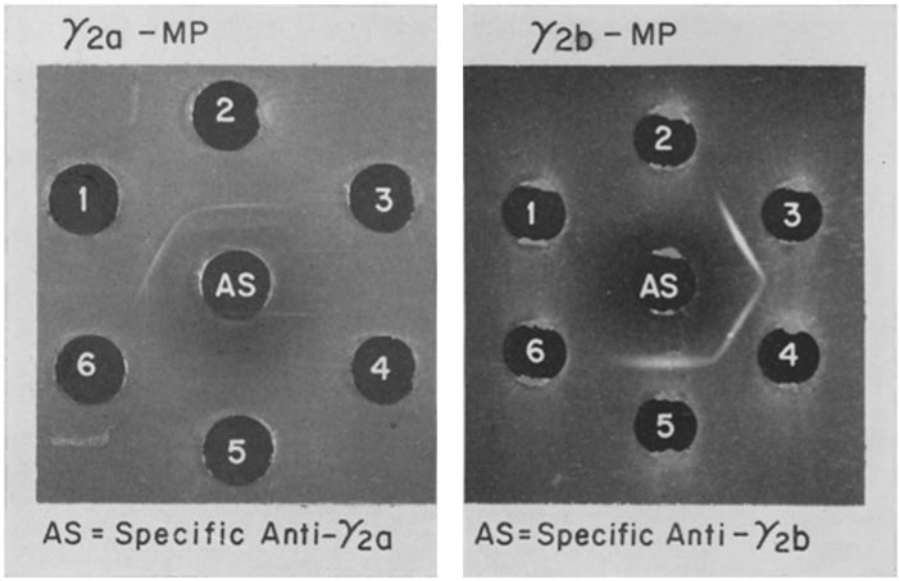


FIG. 1. Ouchterlony gel diffusion analyses demonstration of (left) specific antigenic determinants on γ_{2a}-myeloma proteins (*MP*), and (right) specific antigenic determinants on γ_{2b}-myeloma proteins. In all tests well *1* contained 5563 γ_2MP; *2*, Adj.PC-5 γ_2MP; *3*, MPC-11 γ_2MP; *4*, MPC-31 γ_2MP; *5*, MPC-37 γ_2MP; *6*, MPC-25 γ_1MP.

Rabbit antiserums used in center wells were (left) specific anti-γ_{2a}-globulin (R14Ā) and (right) specific anti-γ_{2b}-globulin (R49Ā).

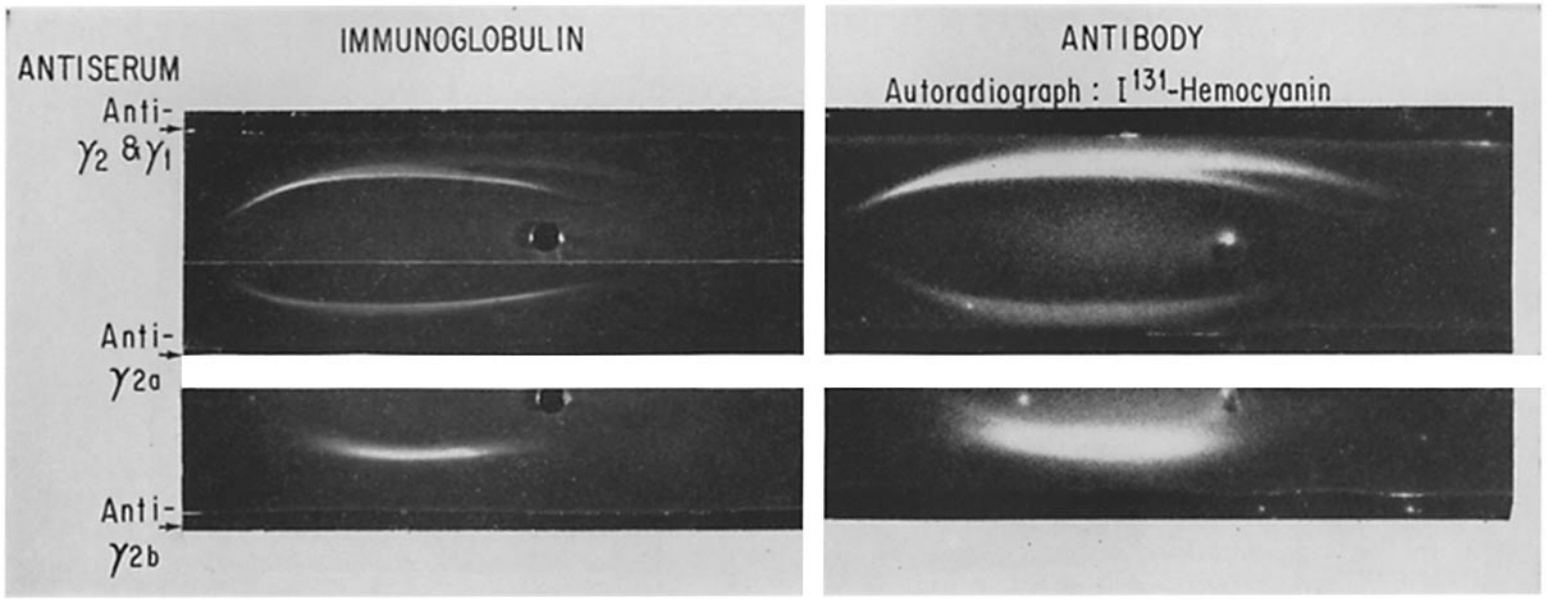
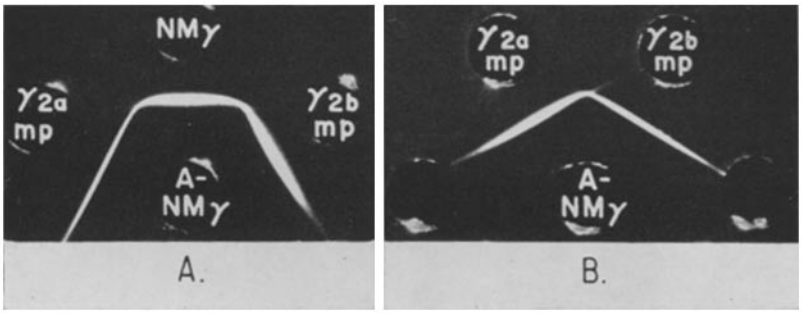


FIG. 2. Immunoelectrophoretic identification of γ_{2a}- and γ_{2b}-globulins. Immunoelectrophoresis was carried out with serum from mice hyperimmunized with hemocyanin. The separate immunoelectrophoretic samples were developed with antiserum to 7S γ_2- and 7S γ_1-globulins, antiserum specific for γ_{2a}-globulin, and antiserum specific for γ_{2b}-globulin. *Left:* Photo of the serum protein precipitin lines apparent after 48 hours of diffusion. *Right:* Autoradiographs prepared after covering the immunoelectrophoresis plates with I^{131}-hemocyanin to allow antihemocyanin antibodies to fix radio-labeled antigen.

γ_{2a}- and γ_{2b}-Globulins in Normal Serum.—Immunoelectrophoresis and Ouchterlony tests were used to identify γ_{2a}-globulins and γ_{2b}-globulins in normal serum and serum from hyperimmunized mice. Rabbit antiserums against γ_{2a}–myeloma proteins and γ_{2b}–myeloma proteins (made specific by absorption) were used in the immunoelectrophoresis of normal mouse serum. Each of the antiserums reacts to form a precipitin arc in the gamma region of normal serum (Fig. 2), showing that both antigenic determinants and, presumably, two classes of protein are present in this region of normal serum. The γ_{2a}– and γ_{2b}–antigenic



FIGS. 3 *A* and 3 *B*. γ_{2a}- and γ_{2b}-globulins in normal serum demonstrated by Ouchterlony gel diffusion tests.

FIG. 3 *A*. γ_{2a} (5563)– and γ_{2b} (MPC-31)–myeloma proteins (*mp*) are compared to a normal 7S γ_2-globulin population using antiserum R75 (*A*-NMγ). Spur formation of the normal mouse globulin (NMγ) over both γ-myeloma proteins (γMP) occurred but is not readily seen on the photographic reproduction.

FIG. 3 *B*. Antiserum (R75) prepared in rabbits against normal mouse 7S γ_2-globulin reacts with γ_{2a}– and γ_{2b}–myeloma proteins to form intersecting precipitin bands, showing that normal γ_2-globulins had elicited antibody response against both γ_{2a}– and γ_{2b}–antigenic determinants. The dense precipitin line common to both proteins indicates that both proteins share common antigenic determinants.

determinants of normal serum were studied further. Rabbit antiserum against normal γ-globulin reacted with specific determinants of both γ_{2a}– and γ_{2b}–myeloma proteins. Intersection of precipitin lines formed by adjacent γ_{2a}– and γ_{2b}–myeloma proteins (Fig. 3 *B*) indicates that the antiserum contains antibodies against both types of specific determinants. An Ouchterlony test comparison of normal γ_2-globulins with γ_{2a}–myeloma proteins and γ_{2b}–myeloma protein is shown in Fig. 3 *A*. Rabbit antiserum against normal γ-globulin showed that both γ_{2a}– and γ_{2b}–myeloma proteins are antigenically deficient in comparison to the normal γ_2-globulin population. These observations indicate that the normal serum γ-globulins used to immunize the rabbits contained the specific γ_{2a}- and γ_{2b}-globulin determinants.

Serums from six inbred mouse strains (C3H/He, BALB/c, DBA/2JN,

C57BL/6JN, AL/N, and STR/N) were tested for γ_{2a}- and γ_{2b}-globulins (determinants) by Ouchterlony analyses with specific (absorbed) antiserums. Both subclasses were identified in normal serum from all strains of mice by precipitin formation with specific antiserums.

Antibody Activity.—Serums from mice immunized with hemocyanin were used to identify antibody activity in the γ_{2a}- and γ_{2b}-globulins. Radioimmunoelectrophoresis, as shown in Fig. 2, revealed that the γ_{2a}- and γ_{2b}-globulins in hyperimmune serums contained antibody activity. After immunoelectrophoresis using specific antiserum, the agar plates were covered with labeled antigen (I^{131}-hemocyanin). Precipitin lines containing antibody-fixed labeled antigen were detected as dense arcs in autoradiographs prepared from the agar immunoelectrophoretic plates (Fig. 2).

Immunochemical Characteristics Revealed by Heterologous Antiserum.—Antigenic determinants common to the γ_{2a}-globulins, the γ_{2b}-globulins, and the other three classes of mouse immunoglobulins were sought. Antiserums prepared in rabbits by immunization with purified mouse myeloma proteins, normal γ_2-globulin, or normal γ_{1M}-globulin reacted with each immunoglobulin class. Representative examples of all five classes (or subclasses) were compared on a single hexagonal Ouchterlony test and found to form precipitin lines which fused with the precipitin lines formed by adjacent examples of other immunoglobulin classes (not illustrated). These findings (presented in part in reference 1) indicated that γ_{2a}- and γ_{2b}-globulins, 7S γ_1-globulins, γ_{1A} (β_{2A})-globulins, and γ_{1M}-globulins share common antigenic configurations.

Antigenic determinants were identified that were common to both γ_{2a}- and γ_{2b}-globulins but were not shared with the other three classes of immunoglobulins; *i.e.*, not with 7S γ_1-, γ_{1A} (β_{2A})-, or γ_{1M}-immunoglobulin classes. All five γ_2–myeloma proteins, including both γ_{2a}- and γ_{2b}-globulins, share a common precipitin line revealed by appropriate antiserum (Fig. 4, left). This last antiserum did not react with 7S γ_1-globulin (Fig. 4) or γ_{1A} (β_{2A})- or γ_{1M}-globulins when tested separately (not seen here). The antigenic determinants in Fig. 4 (left) are specific for γ_2-globulins.

Antigenic determinants specific for γ_{2a}-globulins and specific for γ_{2b}-globulins have been demonstrated above (Figs. 1 and 2). γ_2–Myeloma proteins were found to have one or the other specific antigenic configurations but not both.

These immunochemical studies illustrate the complex antigenic nature of mouse 7S γ_2–myeloma proteins. These immunoglobulins have three separate categories of antigens: (*a*) those characteristic of γ_{2a}- or γ_{2b}-globulins: (*b*) those shared by both subclasses of 7S γ_2-globulins; and (*c*) those common to all immunoglobulin classes.

Isoantigens.—Iga-1 and Iga-2 isoantigens (allotypes) occur only as properties of γ_2-globulins (1, 5), and are not found on the other classes of immunoglobulins. In order to determine the relationship of these isoantigens to the structural

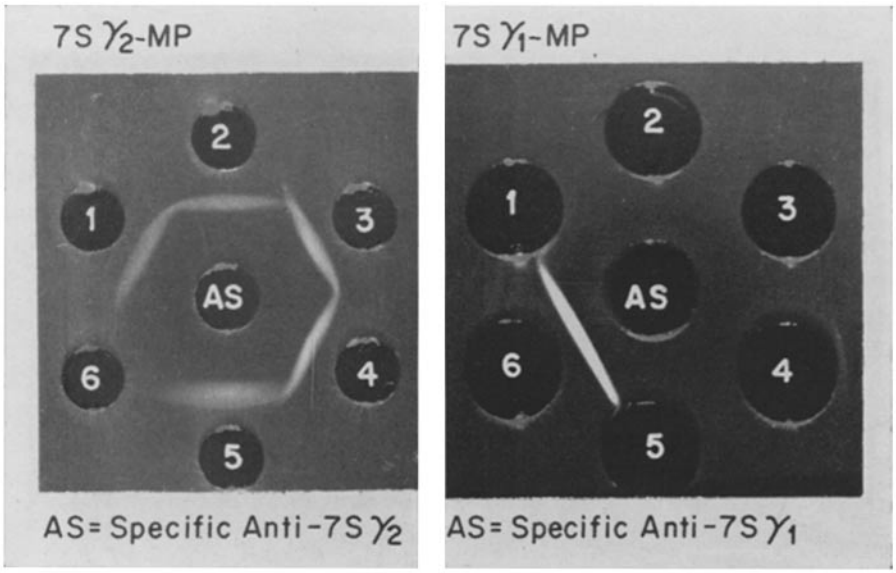


FIG. 4. Ouchterlony gel diffusion analyses demonstrating antigenic determinants specific for γ_2-globulins and shared in common by both γ_{2a}- and γ_{2b}-myeloma proteins. Antigens in all wells are the same as in Fig. 1; *i.e.*, wells *1* and *2* have γ_{2a} MP; *3*, *4*, and *5* have γ_{2b} MP, and *6* has a 7S γ_1 MP.

Rabbit antiserums used in center wells are (left) specific anti-γ_2-globulin prepared by immunization of rabbits with F pieces from papain digest of γ_2 MP (R110Ā) and (right) specific anti-7S γ_1-globulin (R121Ā).

TABLE I

Properties of γ_{2a}- and γ_{2b}-Myeloma Proteins

	Electrophoretic mobility*	Ultracentrifugation ($s_{20,w}$)	Isoantigen (Iga-1)
	mm	*S*	
Normal 7S γ_2-globulin	−10 to +7	6.5	+
γ_{2a}-Myeloma proteins			
5563	+5	6.6	+
Adj.PC-5	−6	7.0	+
γ_{2b}-Myeloma proteins			
MPC-11	+6	6.9	0
MPC-31	−10	7.1	0
MPC-37	−14	6.9	0

* Millimeters from site of application to paper strip. Electrophoresis carried out under standard conditions (2).

configurations responsible for γ_{2a}- and γ_{2b}-globulin specificity, five γ_2–myeloma proteins were tested with anti–Iga-1 isoimmune serum (Table I). Only the γ_{2a}–myeloma proteins had the Iga-1 isoantigens, whereas the three γ_{2b}–myeloma proteins lacked the Iga-1 isoantigens. This evidence indicates that only γ_{2a}-globulins carry the Iga-1 isoantigenic determinant.

Physicochemical Features.—The γ_{2a}-globulins and γ_{2b}-globulins of normal serum showed similar electrophoretic heterogeneity on immunoelectrophoresis (Fig. 2). γ_{2a}– and γ_{2b}–myeloma proteins migrated at both ends of the 7S γ_2-globulin spectrum (Table I).

Ultracentrifugal analysis of purified γ_{2a}– and γ_{2b}–myeloma proteins revealed that both groups of myeloma proteins sedimented in the same range; *i.e.*, 6.6S to 7.0S (Table I).

DISCUSSION

Two subclasses of mouse 7S γ_2-globulins, designated γ_{2a}-globulins and γ_{2b}-globulins, are identified and characterized in the present work. Although they share many common immunochemical and physicochemical properties, γ_{2a}-globulins and γ_{2b}-globulins can be distinguished with appropriate antiserums on the basis of distinctive antigenic determinants. They are separate from the 7S γ_1-globulins, γ_{1A} (β_{2A})-globulins, and γ_{1M}-globulins of the normal immunoglobulin system in mice.

The observations which we have conducted, so far, do not rule out the existence of more than two subclasses of γ_2-globulins. Indeed, there may be further subdivisions of the molecules within the γ_{2a}- and γ_{2b}-groups. The present observations, however, do indicate that immunochemical differences occur within a major immunoglobulin class of mouse serum. All of the inbred mice tested contained γ_{2a}- and γ_{2b}-globulin molecules.

Because the γ_{2a}- and γ_{2b}-immunoglobulins are similar in so many respects, it would be natural to wonder if they differ in L chain characteristics on a basis similar to the L chain differences between type I and type II γ_2-globulins of human serum (7). Additional studies (8), however, have shown that the differences between γ_{2a}– and γ_{2b}–mouse immunoglobulins are not due to differences in L polypeptide chains but are due to differences in the H polypeptide chains.

The terminology, γ_{2a}-globulins and γ_{2b}-globulins, is proposed on the basis that the antigenic differences are present in the H chain. (If the differences had proven to be in the L chains, then I and II designations would have been appropriate). γ_{2b}- and γ_2 -globulins are clearly subgroups of the major immunoglobulin class already termed 7S γ_2-globulins. Capital letters have been used in immunoelectrophoretic nomenclature (9) to designate immunochemically unrelated groups (β_{1A}, β_{1B}, etc.) or major classes of related systems (γ_{1M}, γ_{1A}, etc.). The γ_2 term of γ_{2a}- and γ_{2b}-globulins indicates a common immunochemi-

cal relationship in the H chains of these proteins, characteristic of γ_2-globulins and distinctive from the other three major immunoglobulin groups of mice. The lower case letters, a and b, were chosen to indicate subclasses of the major immunoglobulin class, the 7S γ_2-globulins.

Three subgroups of human γ_2-globulin were described by Dray (10) on the basis of differences revealed by primate antiserums against human γ-globulin. These human γ-globulin subgroups reflect differences in H chain composition (11), but further studies are needed to clarify the relationship between mouse γ_{2a}- and γ_{2b}-immunoglobulin subclasses and the human γ_2-immunoglobulin subgroups.

Subclasses of γ_2-globulins have not yet been identified in other species than mouse and man. Discrimination within major immunoglobulin classes is difficult without myeloma proteins, and in mice the availability of myeloma proteins has played an important role in the identification of mouse immunoglobulin classes and subclasses.

Structural similarities between γ_{2a}- and γ_{2b}-molecules were detected with heterologous antiserums, and structural differences were shown with isologous (and certain heterologous) antiserums. Similarities and differences of the γ_{2a}- and γ_{2b}-globulins may reflect similarities and differences in the genes determining the structure of the H chains of each type of protein. Smithies *et al.* (12) have suggested that chromosomal rearrangement may explain structural similarities and that unequal crossing over and independent mutation may account for differences in human haptoglobin molecules. Similar events could account for the genes controlling the H chains of γ_{2a}- and γ_{2b}-globulins.

The present studies indicate greater heterogeneity in immunoglobulins than had hitherto been appreciated. It may be hoped, however, that a clearer understanding of the heterogeneity within the immunoglobulin system will aid in the eventual correlation of structure with the functional roles of immunoglobulins and with the genetic factors determining their composition. Further studies are underway on the composition of γ_{2a}- and γ_{2b}-globulins, and possible differences in immune function are being investigated.

SUMMARY

Two subclasses of mouse 7S γ_2-globulins are identified, and are designated γ_{2a}- and γ_{2b}-globulins. They are distinguished from 7S γ_1-globulins, γ_{1A} (β_{2A})-globulins, and γ_{1M}-globulins of mouse serum. Antibody activity was detected among the γ_{2a}-globulins and γ_{2b}-globulins of hyperimmune mouse serum.

γ_{2a}– and γ_{2b}–myeloma proteins were identified. The genetically determined isoantigen, Iga-1, was present on γ_{2a}–myeloma proteins, but not on γ_{2b}–myeloma proteins.

These findings indicate a complexity among the 7S γ_2-globulins which must

be taken into account in structural, functional, and genetic studies of immunoglobulins.

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