THE CELLULAR ORIGIN OF HUMAN IMMUNOGLOBULINS (γ_2, γ_{1M}, γ_{1A})*

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PLATES 40 TO 44

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During the last few years mainly in conjunction with other investigations in human immunopathology (1–4), we have had occasion to study in several thousand sections of lymphoid tissues the families of cells which contain and apparently form immunoglobulins and antibodies. In keeping with general interest in this cellular aspect of immunology, as shown by recent publications and reviews (5–16), we are presenting cumulative and new observations on the cellular origins of each of the three types of human immunoglobulins (γ_2, γ_{1M}, and γ_{1A}), especially the generally less abundant γ_{1M}- and γ_{1A}-globulins.

The γ_2-, γ_{1M}-, and γ_{1A}-globulins are the three antigenically related immunoglobulins found in the electrophoretic region which extends from the slow γ_2-globulins to that of the β-globulins (17, 18). The common origin of the immunoglobulins, as shown in this communication, further justifies placing them in one family. The nomenclature employed here (rather than the synonyms γ, β_{2A}, and β_{2M}) has been recommended by Waldenström (19).

Methods and Materials

Antigens.—

γ_2-Globulins: Serum fractions rich in γ_2-globulin were prepared either by zone electrophoresis on starch (20), DEAE cellulose ion exchange chromatography (21), or cold alcohol fractionation (22). The γ_2-globulins obtained by DEAE cellulose chromatography contained only 7S γ_2-globulins; the other preparations also contained small amounts of γ_{1A}- and γ_{1M}-globulins.

γ_{1A}-Globulins: γ_{1A}-Globulins were prepared by zone electrophoresis on starch, using γ_{1A} myeloma sera as starting material. The protein in the γ_{1A} myeloma peak was precipitated with ammonium sulfate at 40 per cent saturation and consisted primarily of γ_{1A}-globulin.

γ_{1M}-Globulins: γ_{1M}-Globulins were isolated from sera of patients with macroglobulinemia by zone electrophoresis on starch followed by dialysis against distilled water in which they are insoluble. In a few instances cryo-γ_{1M}-globulins were prepared by repeated precipitation

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in the cold and resolution in saline at 37°C. The proteins so obtained were contaminated by trace amounts of γ_2-globulins.

Antisera.—Rabbits were inoculated with γ_2-globulins, γ_{1A} myeloma globulins, and γ_{1M}-globulins. All antigens were incorporated in Freund's adjuvant and injected intramuscularly. The resulting antisera were evaluated by immunoelectrophoresis and double gel diffusion according to Ouchterlony. Antisera against γ_2-globulins were absorbed with β-globulins to make them specific for γ_2-globulins and γ_{1M}-globulins. Antisera against γ_{1A}-globulins were absorbed with γ_2-globulins. The resulting antisera reacted only with γ_{1A}-globulins when tested with whole serum.

Antisera against γ_{1M}-globulins were absorbed with γ_2-globulin (Cohn fraction II) and occasionally with albumin (fraction V) to make them specific for γ_{1M}-globulins. The specificity of the antisera was ascertained by immunoelectrophoresis and double gel diffusion.

Fluorescent Antibodies.—The antibody-active fractions of the antisera were separated by cold ethanol fractionation (Cohn fraction II) or by DEAE cellulose ion exchange chromatography. These fractions were conjugated at a protein concentration of 20 mg per ml with fluorescein isothiocyanate; in some instances either tetramethylrhodamine isothiocyanate or lissamine rhodamine B200 were used. The proportion of fluorescein to protein was 1:40

TABLE I

Reactivities of Fluorescent Antibodies with Human Immunoglobulins (γ_2, γ_{1M}, γ_{1A})

Antigen	Fluorescent antibody to		
	γ_2	γ_{1M}	γ_{1A}
γ_2	+	−	−
γ_{1M}	+	+	−
γ_{1A}	−	−	+

(weight ratio) in the usual reaction mixture and was approximately 4:1 (molecular ratio) in the fully dialyzed conjugate. The fluorescent antibodies to γ_2-, γ_{1M}-, and γ_{1A}-globulins were evaluated by immunoelectrophoresis and immunodiffusion and found to have the same specificities, Table I, as the antisera from which they were prepared. The first mentioned was specific for γ_2- and γ_{1M}-globulins, the second for γ_{1M}-globulins, and the third for γ_{1A}-globulins.

Immunofluorescence.—γ_{1M}-Globulins were identified in the tissue sections by direct staining with fluorescein-labeled antibody to γ_{1M}-globulins. This staining reaction was blocked with unlabeled antiserum to γ_{1M}-globulins. γ_{1A}-Globulins were detected by direct staining with fluorescein-labeled antibody to γ_{1A}-globulins. This staining reaction was blocked with unlabeled antiserum to γ_{1A}-globulins. γ_2-Globulins were identified by a two-step procedure: incubation with antiserum to γ_{1M}-globulin followed, after washing with isotonic buffered saline, by fluorescein- or rhodamine-labeled antibody to γ_2-globulin. In many instances γ_{1M}- and γ_2-globulins were identified simultaneously in contrasting yellow-green and orange-red colors by a two-step procedure: incubation with fluorescein-labeled antibody to γ_{1M}-globulins followed, after washing, by rhodamine-labeled antibody to γ_2-globulins. The selectivity of this staining sequence was regularly evaluated with lymphoid tissue sections in which there was a large or exclusive population of γ_{1M}-containing cells (as occurs for example in Waldenström's macroglobulinemia or rheumatoid arthritis) and also in those with a large predominance of γ_2-containing cells. The former cells had yellow-green fluorescence; the latter were

orange-red; rare cells with homogeneous color mixtures were yellow. Immunoglobulin-containing cells failed to stain with fluorescent antibodies to human albumin or to human fibrinogen.

Fluorescent antibodies were diluted and used at a concentration of about 2 to 4 mg protein per ml. They were absorbed with saline-insoluble, acetone-extracted tissue powders (rabbit bone marrow mainly). Staining reactions were carried out at room temperature for 15 to 45 minutes.

Tissues.—These were surgical specimens and biopsies obtained from 279 patients: 107 males and 172 females, ranging in age from 2 to 74 years. Of these, 118 patients had rheumatoid arthritis and the remainder had various rheumatic and other non-neoplastic diseases. There were 375 tissue specimens: 39 lymph nodes, mainly hyperplastic (17 from patients with rheumatoid disease), 2 spleens (enlarged, from patients with acquired hemolytic anemia), 3 thyroids (the site of chronic autoimmune thyroiditis), 232 synovial membranes (76 from individuals with rheumatoid arthritis), and 99 other specimens of various kinds. In addition, two fetal thymus glands weighing respectively 7.5 and 2.5 gm were obtained at autopsy on two twins, born at the 30th week of gestation and succumbing after 2 days because of prematurity and with hyaline membrane disease. The results and the illustrations in this publication were obtained mainly from the groups with rheumatoid arthritis and autoimmune disorders in which the immunoglobulin-forming cell types were normal except for number and, in instances, location.

For the study by immunofluorescence unfixed tissue blocks were frozen at dry-ice temperatures, stored at −30−−70°C, and sectioned in a cryostat at −30°C. Sections (3 μ) were thawed at room temperature, fixed in acetone for 15 minutes, and washed in isotonic buffered saline before treatment with the appropriate fluorescent antibody.

For the customary histologic study tissue blocks were fixed in 10 per cent neutral formalin. Paraffin sections were stained mainly with hematoxylin and eosin and the periodic acid–Schiff reaction. Carnoy's was the fixative of choice for material stained with methyl green and pyronin.

Fluorescence Microscopy.—The procedures were similar to those described elsewhere (1, 3). For visual observation and full color photography the exciter filter was UG2 and the barrier filter was GG4, with Corning C3389 over the eyepiece.

Fluorescence Microspectroscopy.—An eyepiece pupillary spectroscope (Zeiss) was used to resolve the fluorescence spectrum of immunoglobulin-containing cells. Those stained with fluorescein-labeled antibody were yellow-green; with rhodamine-labeled antibody, orange-red; and with both antibodies, yellow, spectrally resolved into green, yellow, orange, and red. Cell backgrounds were mainly blue, blue-violet, or colorless. For the simultaneous identification of γ_{1M}- and γ_2-globulins in a single cell, a tissue section was first exposed to fluorescein-labeled antibody to γ_{1M}-globulins, followed by washing and exposure to rhodamine-labeled antibody to γ_2-globulins. Cells with homogeneous rich yellow color in the cytoplasm, spectrally resolved into green, yellow, orange, and red, were considered, with the proof of specificity previously cited, to contain both γ_{1M}- and γ_2-globulins simultaneously.

Other Procedures.—For comparative purposes, spleens and lymph nodes of rabbits were studied during the primary and the secondary responses to immunization with bovine serum albumin. Antibody-forming cells were identified in sections by staining with fluorescent antigen-excess rabbit immune complex (2, 3), a direct "sandwich" technique which in our experience has been superior to others of its kind in sensitivity and clarity. The spleens and the thymus glands of normal 6-week-old mice were stained with fluorescent rabbit antibody specific for mouse γ_2-globulin, with unlabeled homologous antibody serving as a blocking reagent.

RESULTS

Germinal Centers.—Each of the immunoglobulins (γ_2, γ_{1M}, γ_{1A}) was identified in the germinal centers, Figs. 1 to 5, of lymphoid nodules of lymph nodes and spleen. Only one type of immunoglobulin was localized in an individual germinal center, with rare exception (see Discussion). Centers containing γ_2-globulin usually comprised the majority; those containing γ_{1M}-globulin were less frequent (except in rheumatoid disease); γ_{1A}-globulin containing centers were the least numerous. Centers lacking detectable amounts of any immunoglobulin were occasionally seen. Centers forming each of the immunoglobulins, one type of immunoglobulin per center, were present in the same section of some hyperplastic lymph nodes (in active rheumatoid disease for example).

The immunoglobulin was localized almost exclusively in the cytoplasm of germinal center cells but also possibly was present in the immediate extracellular surroundings. The germinal center cells containing immunoglobulin corresponded in conventionally stained companion sections to spherical or elliptical aggregations of primitive cells (variously called: primitive reticular cells, primitive lymphocytes, stem cells, blasts) distinguished by a large pale nucleus with nucleoli, indistinct cell outline, basophilic and pyrinophilic cytoplasm, and elongated, seemingly continuous, cell processes which appeared as though attached to a network of reticular fibers. In enlarging centers some of these cells were round and undergoing mitotic division.

The mantle of small (mature) lymphocytes, Figs. 1 to 3, which characteristically surrounds the germinal center never containen detectable quantities of immunoglobulins although conceivably trace amounts might have been overlooked on occasion.

Primitive Reticular Cells.—Each of the immunoglobulins (one type per cell) was identified on occasion in the cytoplasm of primitive cells of large, Fig. 6, intermediate, Fig. 7, and small size, Fig. 8, occurring in lymphoid tissue of the medullary cords of lymph node, the red pulp of spleen, or elsewhere. These cells were solitary or in small groups. The large primitive cells resembled those seen in germinal centers (primitive reticular cells, primitive lymphocytes, stem cells, blasts). We have chosen to call them primitive reticular cells. Those of intermediate size conceivably were undergoing transition to the much more numerous plasma cells with which they were commonly associated. The cells of small size conformed to the morphology of small reticular cells. Primitive reticular cells containing immunoglobulin (γ_{1M} mainly) were present in the cortex of the fetal thymus gland, Fig. 8, in very limited number, in a proportion roughly estimated as one to many thousand small lymphocytes (thymocytes) which did not contain detectable immunoglobulin. The cells were of intermediate and, more commonly, small size. Cells of similar primitive reticular appearance, location, and number and forming mouse γ_2-globulin were present in the thymus gland of 6-week-old mice, Fig. 15.

Plasma Cells.—Each of the immunoglobulins (one type per cell, with rare

exception) was identified in the cytoplasm of plasma cells of immature and mature types. Those with least maturity corresponded morphologically to large oval or round primitive cells (blasts; plasmablasts), Fig. 9; those with most maturity included Marschalko and other types of plasma cells, Figs. 10 to 12; those in largest number were usually intermediate between these extremes; those having the most distinctive appearance contained Russell bodies in the cytoplasm (Russell-body plasma cells; "grape" cells), Figs. 13 to 14. Plasma cells containing γ_2-globulin usually comprised the majority; those containing γ_{1M}-globulin were less numerous (but not in rheumatoid disease). γ_{1A}-Globulin containing cells were the least numerous of all. Some plasma cells apparently did not contain detectable amounts of any immunoglobulin.

In companion sections conventionally stained, the Marschalko-type plasma cells conformed to the classical description: oval or round shape, deeply basophilic or pyrinophilic cytoplasm, distinct cell outline, eccentric nucleus with coarse clumps of chromatin, and, commonly, a juxtanuclear pale zone. The Russell-body plasma cell contained one or commonly many discrete, or confluent, spherical acidophilic cytoplasmic bodies which tended to overlie, obscure, or displace the pyknotic nucleus.

Very rarely, immunoglobulin was detected in the cytoplasm of small round or nearly oval cells, Fig. 16, which resembled small *lymphocytic* plasma cells (23), a nomenclature however that may be confused with the larger *lymphoid* plasma cells of Waldenström's macroglobulinemia (24, 25).

DISCUSSION

The results indicated that there are two closely related families of cells forming immunoglobulins (and antibodies) in human lymphoid tissue: germinal (reticular) centers (6) and plasma cells. The function of the plasma cell has been fully documented by the pioneer studies of Fagraeus (26) and those which followed (for reviews, references 12–15). The investigations by Fagraeus indicated that the cellular formation of antibody in the rabbit took place side by side with, and during, the development of reticular cells into plasma cells. Studies by Coons, Leduc, and Connolly (5) with immunofluorescence and by Nossal (7, 14) and Attardi and associates (9) with single-cell techniques showed that the immature plasma cell was the main source of antibody in the rabbit. Antibody (autoantibody to thyroglobulin) was recently demonstrated in human plasma cells (4).

Studies with immunofluorescence also indicated that germinal centers were the sites of formation of γ-globulins in man (6) and antibodies in the experimental animal (8, 10), possibly even in the very early stages of the immune response (27). Germinal centers are so named because they presumably germinate lymphocytes; and yet lymphocytes can be formed in the absence of germinal centers, as in the fetal thymus gland. As has been commented upon elsewhere (3), a germinal center arising as from a center of growth, enlarging as a spherical

nodule, and forming usually only one type of immunoglobulin (or one type of rheumatoid factor) appears in form and substance to fulfill some of the requisites for a clone (13),—a family of cells arising from one parent cell. Since germinal centers, like plasma cells and unlike small lymphocytes, contain immunoglobulins readily detectable by immunofluorescence, the question arises as to whether germinal center cells might also be precursors of plasma cells (28). The view that germinal center cells are on the way to becoming members of the plasma cell family has been expressed by Nossal (15) and supported by the histologic findings of Congdon and Makinodan (29). Their histologic studies (29) favored the idea that the antibody-forming cells of the plasma cell family (in the red pulp of mouse spleen) were derived from proliferating cells in the white pulp and these in turn seemed to be derived from dissociated germinal centers. We have drawn attention in the present work to the presence of immunoglobulins in large primitive cells (large reticular cells) which resemble the large cells of germinal centers but are separate from them. Primitive immunoglobulin-containing reticular cells of intermediate and, to a lesser extent, small size were also seen, conceivably undergoing transition to the much more numerous plasma cells with which they were commonly associated. The developmental history of plasma cells and their relation to reticular and other cells have been recently studied and discussed by McMillan and Engelbert (23), as well as by authors previously cited.

The view has been expressed by Nossal (15) in his comprehensive review of evidence pro and con that "it is difficult to escape the conclusion that most small lymphocytes do not appear to form antibody." The present study is in agreement with that statement; the mantle of small lymphocytes which surrounds a germinal center does not contain detectable quantities of immunoglobulins.

A peculiarity of the fetal thymus gland, aside from possessing a small number of immunoglobulin (mainly γ_{1M})-forming cells of small and intermediate size and primitive appearance, was the presence of immunoglobulin within Hassall's corpuscles, Fig. 17. A similar localization of γ-globulin (30) and antibody (31) has been described in other studies.

While the human immunoglobulins were mainly cytoplasmic, as are antibodies, Figs. 19 and 20, and gamma globulins, Fig. 21, of other mammalian species, a rare focus of immunoglobulin (γ_{1M}) was localized also in the nucleus, Fig. 18. This has been described by others (24).

It was recently stated that the cellular origin of γ_{1M}-globulins has not been definitely established (32) or has remained a baffling problem (33). Several publications mainly concerned with rheumatoid arthritis (1–3) and with Waldenström's macroglobulinemia and other disorders (34, 24, 35–38, 33) have dealt with this problem. These and the present study should leave little doubt that γ_{1M}-globulins are formed in the same families of cells—germinal centers, plasma cells, and primitive reticular cells—which produce γ_2-globulins. γ_{1A}-

Globulins likewise have a similar origin, as shown for the first time in this study.

The final point concerns the occurrence of more than one type of immunoglobulin in one cell. γ_{1M}-Globulin and γ_2-globulin were identified on occasion in the same plasma cell, Fig. 16, large or small, immature or mature, and in the same germinal center in lymphoid tissue containing an abundant "polyclonal" (19) distribution of cells forming immunoglobulins of all types (γ_2, γ_{1M}, γ_{1A}) but, with the exceptions noted, only one type per cell. The identification of two immunoglobulins in one cell was based upon the occasional, yet undoubted, presence of cells with yellow fluorescence, spectroscopically resolved into a continuum (green, yellow, orange, red), in tissue sections that were first exposed to yellow-green (fluorescein-labeled) antibody to γ_{1M}-globulin, followed by washing and exposure to orange-red (rhodamine-labeled) antibody to γ_2-globulin. The vast majority of immunoglobulin-containing cells were either one color (yellow-green) or the other (orange-red) but occasional cells were rich mixtures of each (yellow). γ_{1M}-Globulins with rheumatoid-factor activities of two specificities have been detected in a single cell by immunofluorescence (3). More sensitive methods of assay (7, 9, 15) have demonstrated the occurrence of two different antibodies in the same cell in experimental animals.

Since this manuscript was completed the publication by Solomon, Fahey, and Malmgren (39) has appeared. These authors studied the cellular localization of γ_{1M}-globulins, γ_2- and γ_{1A}-myeloma proteins, and Bence Jones proteins in multiple myeloma and macroglobulinemia. Their observations in multiple myeloma revealed: a diversity of morphologic forms of malignant cells containing each type of myeloma protein; only one type of myeloma protein in individual cells; coexistence of Bence Jones protein and myeloma protein in the same cell; presence of the same type of protein in all or almost all of the malignant cells in a given patient. Their study and the present one dealing with normal cells appear to be mutually consistent and to supplement each other.

SUMMARY AND CONCLUSIONS

A study was made of the cellular origin of human immunoglobulins (γ_2, γ_{1M}, γ_{1A}). The results indicated that two closely related families of cells form immunoglobulins in human lymphoid tissue: germinal (reticular) centers and plasma cells. Thus their cellular origin in addition to their known antigenic relations further justifies placing the immunoglobulins in one family of proteins.

Immunoglobulins were also formed to a small extent in primitive reticular cells which resembled those of germinal centers but were separated from them. Possibly such cells were undergoing transition to the much more numerous plasma cells with which they were commonly associated.

The mantles of small lymphocytes which surrounded germinal centers did not contain detectable quantities of immunoglobulins.

While in general only one type of immunoglobulin was present in an indi-

vidual cell or germinal center, γ_2- and γ_{1M}-globulin were identified on occasion in the same plasma cell and germinal center.

A peculiarity of the fetal thymus gland was the presence of immunoglobulin, mainly γ_{1M}, in a small number of cells of small and intermediate size and primitive reticular appearance and in Hassall's corpuscles.

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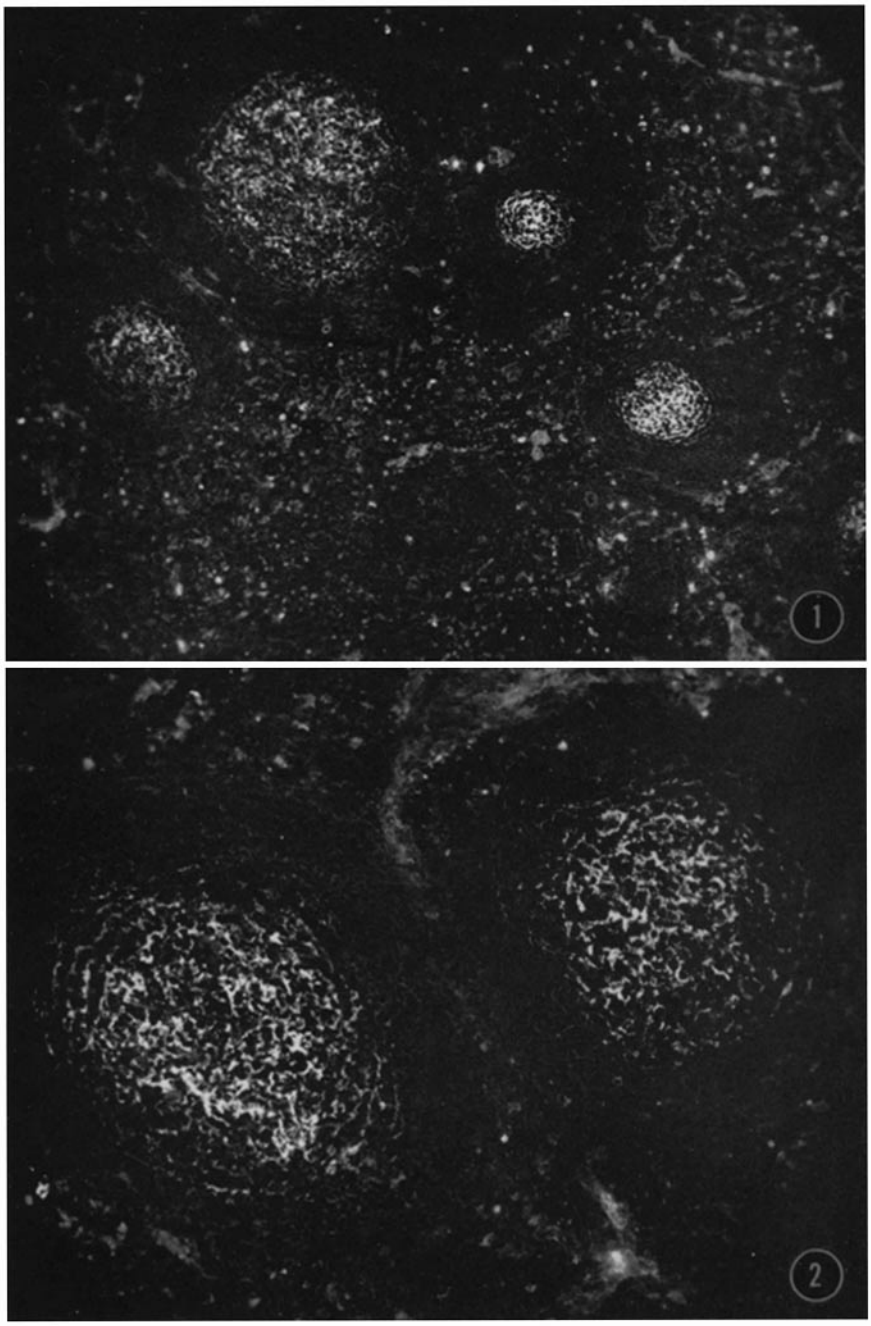
EXPLANATION OF PLATES

The illustrations are immunofluorescence photomicrographs of frozen sections. Except where otherwise indicated, tissues are human lymph nodes and magnifications are × 1500.

PLATE 40

FIG. 1. Four germinal centers forming γ_2-globulin. × 100.

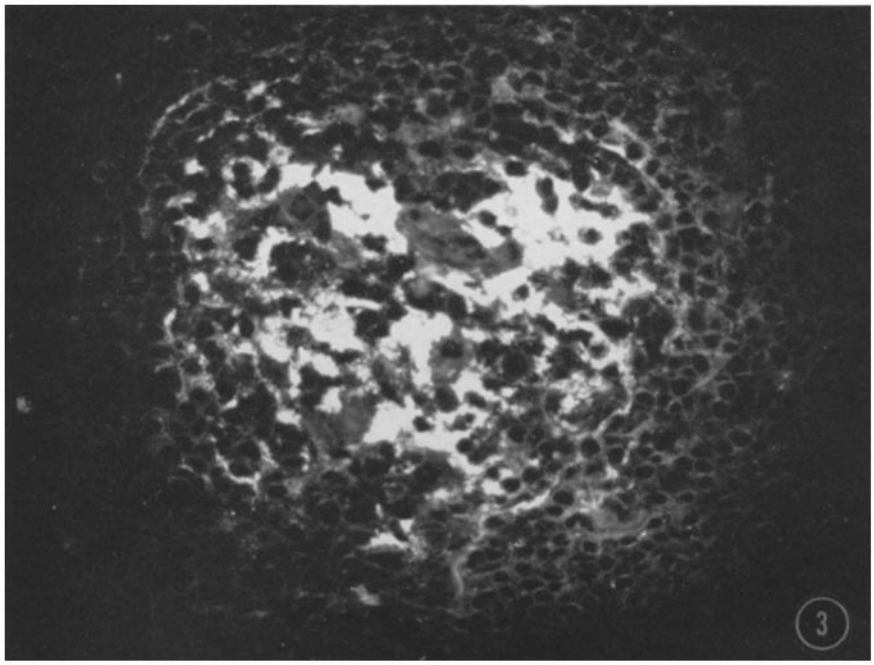
FIG. 2. Two germinal centers forming γ_2-globulin. × 170.

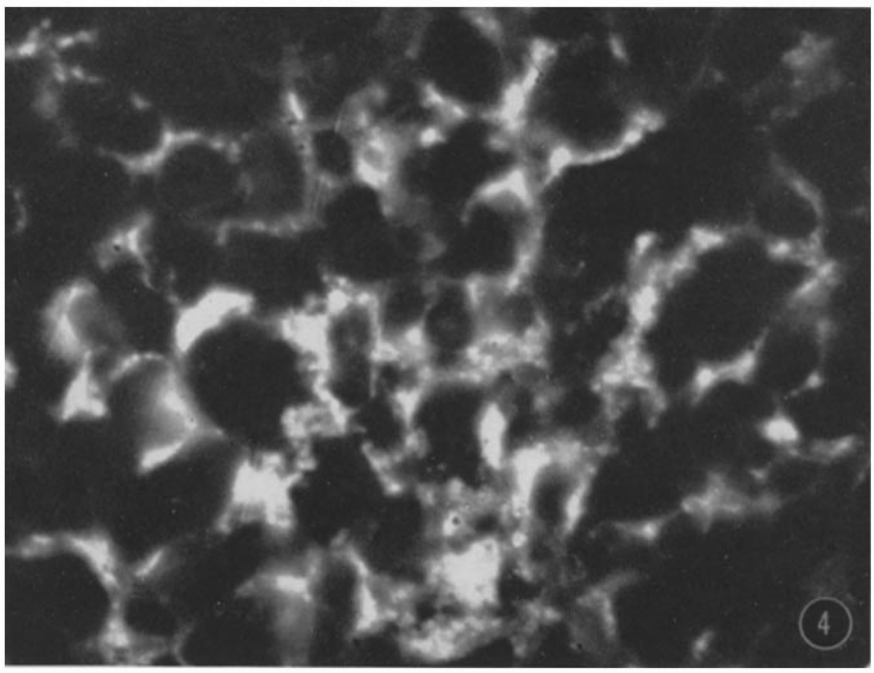


(Mellors and Korngold: Cellular origin of human immunoglobulins)

PLATE 41

FIG. 3. Germinal center forming γ_{1M}-globulin. × 400.
FIG. 4. Portion of germinal center forming γ_{1M}-globulin.





(Mellors and Korngold: Cellular origin of human immunoglobulins)

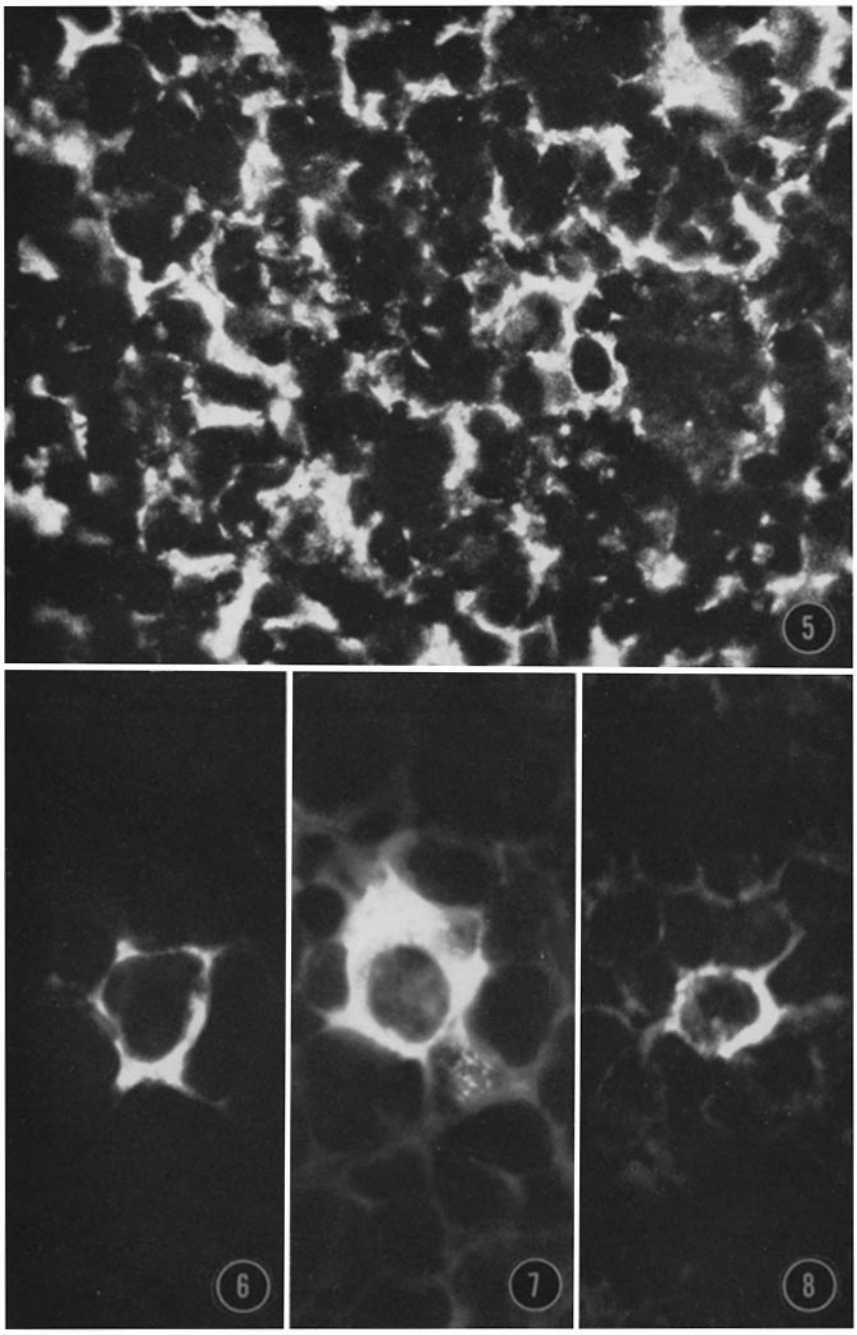
PLATE 42

FIG. 5. Germinal center forming γ_{1A}-globulin. × 750.

FIG. 6. Large primitive (reticular) cell forming γ_{1M}-globulin.

FIG. 7. Primitive (reticular) cell forming γ_{1M}-globulin.

FIG. 8. Small primitive (reticular) cell forming γ_{1M}-globulin in fetal thymus gland.



(Mellors and Korngold: Cellular origin of human immunoglobulins)

PLATE 43

FIG. 9. Plasmablast forming γ_{1M}-globulin.

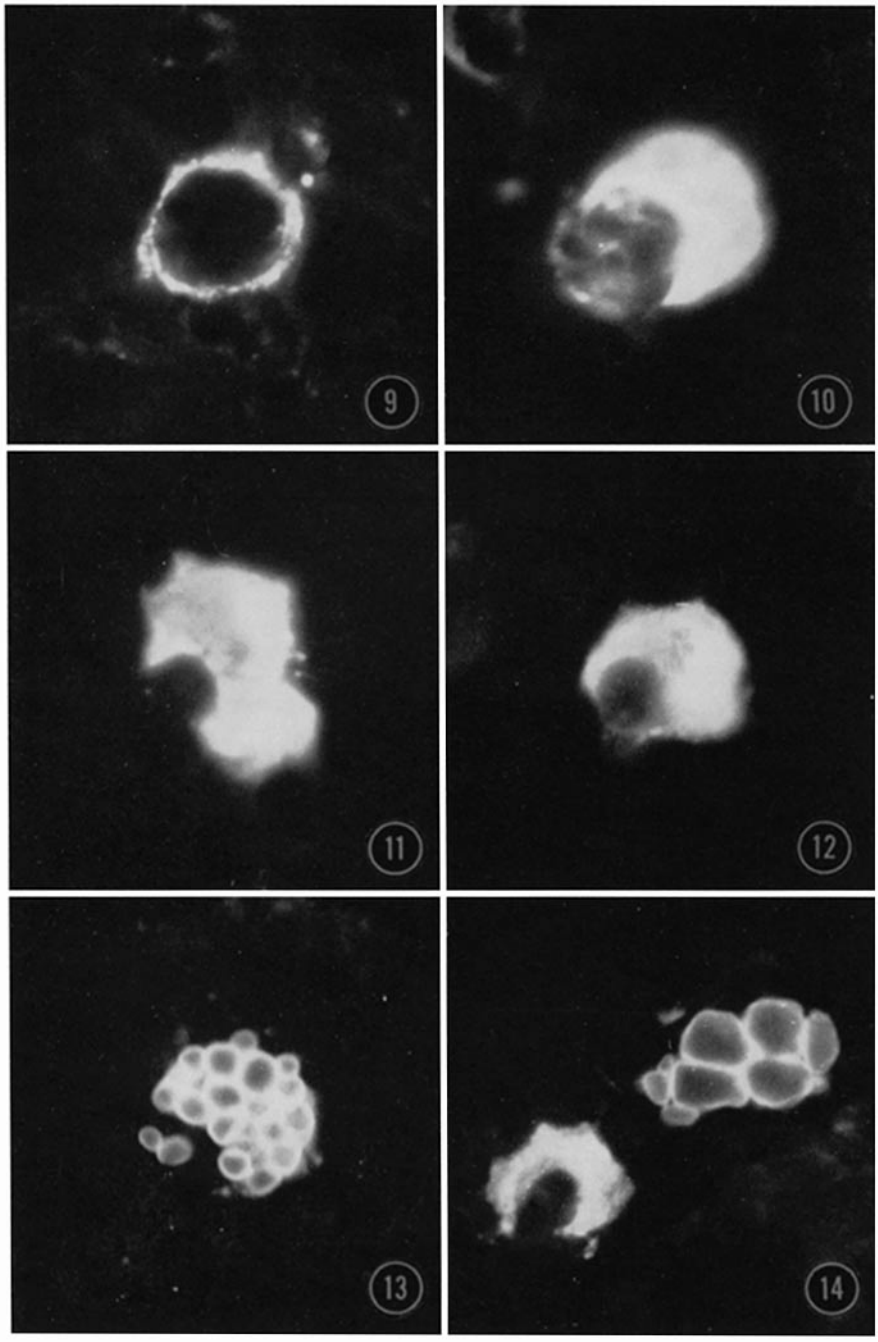
FIG. 10. Mature plasma cell forming γ_2-globulin.

FIG. 11. Mature plasma cell forming γ_{1M}-globulin.

FIG. 12. Mature plasma cell forming γ_{1A}-globulin.

FIG. 13. Russell-body plasma cell forming γ_{1M}-globulin in spleen.

FIG. 14. Two plasma cells, including Russell-body type, forming γ_{1A}-glob



(Mellors and Korngold: Cellular origin of human immunoglobulins)

PLATE 44

FIG. 15. Primitive (reticular) cell forming mouse γ_2-globulin in thymus gland of 6-week-old mouse.

FIG. 16. Small plasma cell forming γ_2-globulin and γ_{1M}-globulin in spleen.

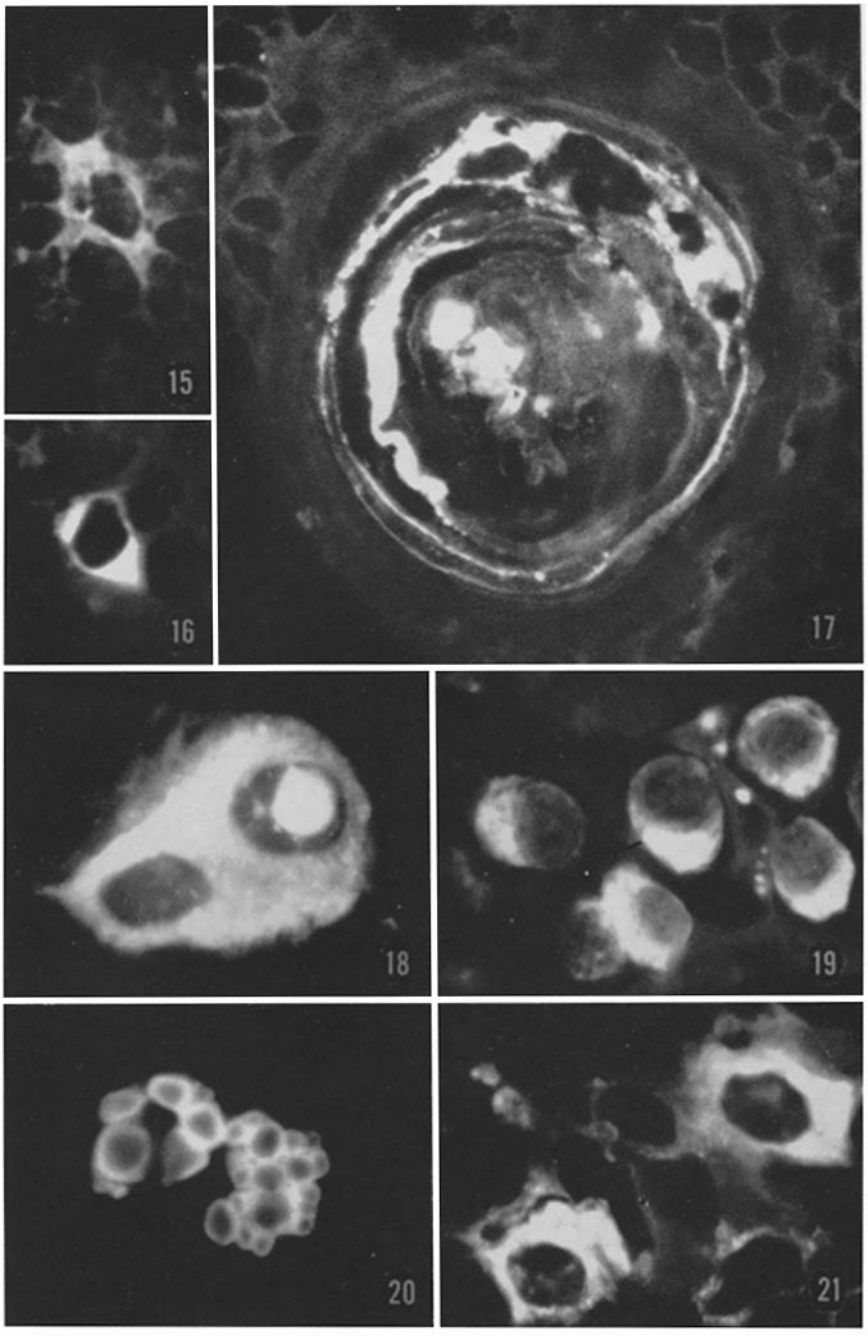
FIG. 17. Hassall's corpuscle containing γ_{1M}-globulin in fetal thymus gland. $\times$ 1000.

FIG. 18. Nuclear (as well as cytoplasmic) γ_{1M}-globulin in one of two plasma cells.

FIG. 19. Small plasma cells forming rabbit antibody to bovine serum albumin in rabbit spleen.

FIG. 20. Two Russell-body plasma cells forming rabbit antibody to bovine serum albumin in rabbit spleen.

FIG. 21. Two plasma cells forming mouse γ_2-globulin in mouse spleen.



(Mellors and Korngold: Cellular origin of human immunoglobulins)