THE SITE OF SYNTHESIS OF THE 19S γ-GLOBULINS IN DYSGAMMAGLOBULINEMIA*

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The cellular origin of the 19S γ-globulins has not been definitely established. Several investigators have employed the fluorescent antibody technique in an attempt to identify the origin of certain of these globulins in patients with rheumatoid disease and Waldenström's macroglobulinemia.

Mellors *et al.* have found rheumatoid factor, a 19S γ-globulin, in the plasma cells of patients with rheumatoid arthritis (1). Dutcher and Fahey have shown by this method that the origin of the pathologic macroglobulins found in Waldenström's disease is a "lymphocytoid plasma cell" associated with this disease (2). Kritzman, Kunkel, McCarthy, and Mellors studied a patient with Waldenström's disease whose macroglobulin had rheumatoid factor activity (3); studies with fluorescein-labeled aggregated γ-globulin on spleen and lymph node sections from this patient revealed specific staining of small lymphocytoid cells and larger cells, resembling plasma cells, which they called "atypical lymphocytes." No intranuclear fluorescence was seen in these cells in contrast to the observations of Dutcher and Fahey. Burtin was not able to find intracellular macroglobulin in the spleen of a patient with Waldenström's disease but he did observe specific extracellular fluorescence (4); subsequently he described fluorescent cells in the lymph nodes of patients with Waldenström's disease which resembled plasma cells and lymphocytes (5). Curtain and O'Dea have given suggestive evidence that an abnormal macroglobulin found in a patient with Mikulicz' syndrome and another macroglobulin found in a patient with myeloma originated from plasma cells and their precursors (6).

Two patients were recently observed who had a dysgammaglobulinemia characterized by an elevation in the serum concentration of 19S γ-globulins, re-

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ferred to as β_2M-globulins by our European colleagues, a marked deficit in the 7S γ_2-globulins, and an absence of 7S γ_1-globulin or β_2A-globulin. These children had high titers of isohemagglutinins, Forssman antibody, bactericidal antibody against *Escherichia coli* and *Salmonella typhosa*, and in one case, anti-O typhoid agglutinins which were shown to be 19S γ_1-globulins (7). Preliminary studies on biopsy material obtained from these children revealed an absence of plasma cells in their lymph nodes, spleen, and bone marrow. In the study reported here, it was found possible to demonstrate 19S γ_1-globulins in cells resembling transitional cells in splenic tissues by using the fluorescent antibody method.

Materials and Methods

Tissues.—An inguinal lymph node was obtained from one patient with dysgammaglobulinemia (D. B.) and from a patient with congenital agammaglobulinemia (J. S.). Abdominal lymph nodes were obtained from another patient with dysgammaglobulinemia (F. E.) at the time of splenectomy, performed because of a severe hemolytic anemia. The spleen of this patient (F. E.) was also studied as well as splenic tissue from four other children who had undergone splenectomy for congenital spherocytosis. In each instance, a portion of the tissue was immediately frozen in a dry vial immersed in acetone and dry ice; the frozen samples were stored at −20°C until sectioning. Other portions of the tissues were fixed in absolute alcohol or Zenker's solution.

Antibodies.—Goat antihuman 19S γ-globulin (Waldenström type) was obtained from Hyland Laboratories, Los Angeles. Upon immunoelectrophoresis with whole human serum, the goat antiserum gave a number of lines of specific precipitation. This antiserum was adsorbed with serum from a patient with congenital agammaglobulinemia so that upon immunoelectrophoresis only three bands of precipitation remained; these corresponded to the 7S γ_2-globulins, the 7S γ_1-globulins (β_2A) and the 19S γ_1-globulins (β_2M). The antiserum was further adsorbed with purified human 7S γ_2-globulin; the antiserum then yielded only a single band of specific precipitation, corresponding to the 19S γ_1-globulin (β_2M), when reacted with normal sera or with sera from the two patients with dysgammaglobulinemia. Rabbit antihuman 7S γ_2-globulin and antihuman serum albumin were prepared in our laboratories using as antigens purified proteins separated by low temperature ethanol-water fractionation.

Saturated $(NH_4)_2SO_4$ was added to an equal volume of either goat or rabbit antiserum at room temperature. The precipitates were taken up to their original volume in 0.15 M NaCl, dialyzed for 7 days against several changes of distilled water, dialyzed against 0.15 M NaCl, and then conjugated with fluorescein isothiocyanate in a ratio of 0.05 mg of dye per mg of protein according to the method of Coons (8), as modified by Riggs *et al.* (9). After removing excess fluorescein isothiocyanate by dialysis against 0.15 M NaCl for 5 days, the conjugated antisera were concentrated by lyophilization and stored at −20°C. Prior to use, the antisera were passed through a Dowex-50 ion-exchange column to remove non-specific fluorescence.

Localization of Antigens.—The tissues were sectioned in a cryostat (8) at a thickness of 4 to 6 μ. After mounting on a dry slide, the sections were fixed in acetone for 10 minutes. Some were then washed for 20 minutes in saline which was buffered to pH 7.2 with sodium phosphate. Both the washed and the unwashed sections were stained with one of the following antisera: (*a*) fluoresceinated antihuman albumin; (*b*) fluoresceinated anti-7S γ_2-globulin; (*c*) fluoresceinated anti-19S γ-globulin; and (*d*) fluoresceinated anti-19S γ-globulin to which 7S γ_2-globulin had been added. In the last instance, 2.5 mg of 7S γ_2-globulin was added to each ml of antiserum so that any antibodies which reacted with 7S γ_2-globulin but remained

unprecipitated would be complexed far into the zone of antigen excess. The labeled antisera were applied for 40 minutes at room temperature in a moist chamber. The sections were then washed three times for 2 minutes each in 0.15 M NaCl buffered to pH 7.2 with sodium phosphate. A drop of buffered glycerine (pH 7.2) was placed on the tissue before putting on the coverslip and the sections were examined under the ultraviolet microscope. Subsequently, the coverslips were gently lifted from the tissues under saline and the tissues were stained with multi-purpose stain® (Paragon C & C, New York). The Zenker-fixed tissues were sectioned in paraffin and stained with toluidine blue and eosin; the alcohol-fixed tissues were likewise sectioned in paraffin, but stained with methyl green pyronine or Schiff's periodic acid–leukofuchsin.

RESULTS

Lymph Nodes.—The lymph nodes of the two patients with dysgammaglobulinemia (F. E. and D. B.) could not be distinguished from the lymph node taken from an agammaglobulinemic child (J. S.). They lacked germinal centers and had poor structural organization (10). These nodes exhibited an apparent reticuloendothelial hyperplasia as is also seen in the lymph nodes of children with congenital agammaglobulinemia. No specific intracellular fluorescence could be discerned in these nodes and no periodic acid–leukofuchsin (PAS) stained cells could be found. Extracellular fluorescence was observed in all the unwashed sections; this fluorescence was eliminated by washing.

Spleen.—The spleen of the patient (F. E.) with dysgammaglobulinemia had a meager white pulp which consisted only of mature lymphocytes; germinal centers were not found. The sinuses of the red pulp were lined with hypertrophic reticuloendothelial cells in which considerable hemosiderin deposition was evident as a result of the hemolytic anemia from which the patient was suffering at the time of splenectomy (7). Lymphocytes and histiocytes were present in the red pulp but no plasma cells were seen. In sections stained with fluoresceinated anti-7S γ_2-globulin, fluoresceinated anti-19S γ-globulin and fluoresceinated anti-19S γ-globulin to which 7S γ-globulin had been added in a quantity sufficient to inhibit the cross-reaction of this antiserum with cells containing 7S γ-globulin, specific fluorescence was found in small numbers of isolated cells present in the red pulp; these cells were never encountered in clusters. No fluorescence was seen in the washed sections stained with fluorescent anti-human albumin antiserum. No intranuclear fluorescence was found in the washed sections. The cytoplasm of those cells reacting with labeled anti-γ-globulin antisera fluoresced homogeneously and no Russell's bodies were seen. Prior washing of the sections in buffered saline eliminated the extracellular fluorescence which was seen in all the unwashed sections.

After photographing the fluorescent sections using a calibrated mechanical stage to fix the tissue area being observed, the coverslips were gently lifted and the sections were stained with multi-purpose stain. The cells which had been specifically fluorescent were localized on the same section after staining. These cells were large and triangular in shape, having an average diameter of 15 μ. The

nucleus was eccentric in every instance with a reticular chromatin pattern. The cytoplasmic to nuclear ratio was relatively high. These cells resembled the transitional cells of Fagraeus (11). PAS stains on other sections of the spleen revealed similar cells in the red pulp, which were morphologically identical to the specifically fluorescing cells and which contained coarse granules of positively staining material in the cytoplasm. No PAS positive material was found in the nuclei. These same cells were also pyroninophilic.

After reacting sections from the normal spleens with fluoresceinated anti-19S γ-globulin antiserum adsorbed with 7S γ_2-globulin, cells with specific fluorescence could be identified, but were fewer in number than in the spleen of the dysgammaglobulinemic patient (F. E.). These cells in the normal spleens were also pyroninophilic and contained PAS positive granules in the cytoplasm but not in the nucleus. When sections from normal spleens were reacted with conjugated anti-7S γ_2-globulin antiserum or with conjugated anti-19S γ-globulin antiserum, the plasma cells also exhibited fluorescence, but the plasma cells did not fluoresce in washed sections reacted with anti-19S γ-globulin antiserum to which 7S γ_2-globulin had been added.

DISCUSSION

Two children with dysgammaglobulinemia characterized by an elevation of the 19S γ-globulins and a marked deficiency of 7S γ-globulins in their sera offered a unique opportunity to study the cellular origin of the γ_1-macroglobulins (7). A count of 5000 nucleated cells from the bone marrow of one of these patients revealed only 1 mature plasma cell, and in the other case, no plasma cells were encountered among 5000 nucleated bone marrow cells (7). Sections of lymph nodes from both patients and of spleen from one of them contained no identifiable plasma cells. With the fluorescent antibody technique, cells containing γ-globulins could not be demonstrated in lymph node sections from either child. However, sections of splenic tissue did contain cells specifically reacting with fluorescein-labeled anti-19S γ_1-globulin antiserum. These cells, which were found in the red pulp, resembled the transitional cells described by Fagraeus (11).

Smith has shown that the administration of typhoid vaccine to 1-day-old infants results in the appearance of 19S anti-H antibodies rather than 7S antibodies. Lymph nodes from these infants who were synthesizing 19S anti-H antibody contained no plasma cells (12). It has been well established that during the first month of life the infant forms no mature plasma cells (10, 13). Craig has observed high titers of typhoid anti-O antibodies in rabbits' sera at 4 to 8 days after primary stimulation at which time only transitional cell types had increased and no increase in plasma cells was yet noted (14). As he was undoubtedly measuring 19S γ-globulin antibodies his findings correlate well with those of Smith, as well as with the findings in these patients with dysgammaglobulinemia. High titers of macroglobulin antibodies apparently can be formed

in the absence of plasma cells, or in the absence of any proliferative activity among these cells.

It has been presumed that the transitional cell is a precursor of the mature plasma cell (11). The data obtained in this study suggest that at least some of these cells may be mature cell forms rather than simply precursors of plasma cells. Fagraeus has pointed out the similarity of these cells seen in the lymph node cells of newborns, as studied by Smith, with the lymphocytoid plasma cells seen in Waldenström's macroglobulinemia (15). It is tempting to postulate that Waldenström's disease may be a myeloma of these cells which are responsible for the synthesis of 19S γ-globulins and resemble transitional cell types.

The anti-19S γ-globulin antiserum, after adsorption with agammaglobulinemic serum and 7S γ_2-globulin, gave a single band of precipitation upon immunoelectrophoresis with normal and dysgammaglobulinemic sera. After labeling this adsorbed antiserum with fluorescein isothiocyanate, labeled soluble complexes reacted with plasma cells in the normal spleen presumably due to specific interaction of 7S γ_2-globulin in the cells with antibodies from the soluble labeled complexes in the serum. The development of this apparently specific fluorescence could be eliminated by further addition of 7S γ_2-globulin to the tagged antiserum so that the cross-reacting antibody was bound far into the region of antigen excess. This points out the necessity for care in the interpretation of results obtained with adsorbed sera since soluble complexes do remain and the antigen-antibody reaction is a reversible one, so that labeled antibody may then react with antigen in the tissues unless this is inhibited by a great excess of specific antigen added to the antiserum after labeling (16).

SUMMARY

Lymph nodes and splenic tissue from patients with congenital agammaglobulinemia and dysgammaglobulinemia and from normal subjects were studied with the use of immunofluorescence and histochemical stains to determine the site of synthesis of the 19S γ_1-globulins. The two patients with dysgammaglobulinemia had high serum concentrations of the 19S γ_1-globulins and a marked deficit of the 7S γ-globulins. These patients, as well as agammaglobulinemic children, had only rare or no plasma cells in their tissues. Cells were identified in sections of spleen from a dysgammaglobulinemic child as well as from normal individuals which exhibited specific fluorescence with an anti-19S γ-globulin antiserum adsorbed with 7S γ_2-globulins and which stained positively with PAS and methyl green pyronine. These cells resembled the transitional cells described by Fagraeus.

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EXPLANATION OF PLATE 122

FIG. 1. Fluorescent cell in a section of normal spleen reacted with labeled specific anti-19S γ_1-globulin antibody (approximately × 800).

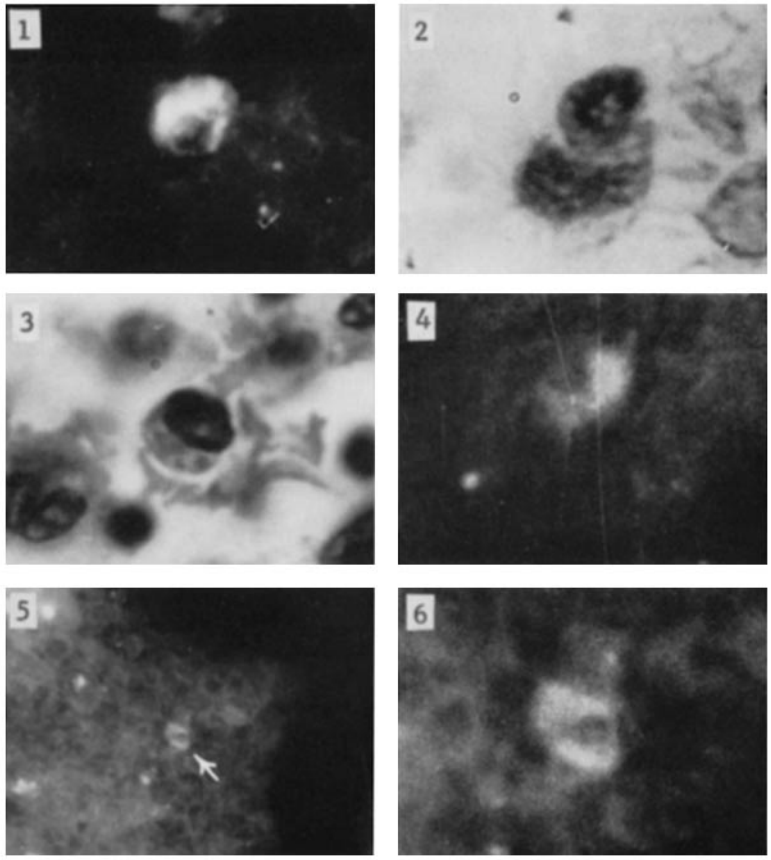
FIG. 2. Same area in section of Fig. 1 stained with multi-purpose stain® (approximately × 800).

FIG. 3. Cell in a section of normal spleen stained with Schiff's periodic acid–leukofuchsin. This section was *not* exposed to labeled antibody (approximately × 800).

FIG. 4. Cell in a section of spleen from dysgammaglobulinemia child (F. E.) exposed to labeled specific anti-19S γ_1-globulin antibody (approximately × 800).

FIG. 5. A section of spleen from dysgammaglobulinemic patient (F. E.) containing a fluorescent cell (arrow) which reacted with labeled specific anti-19S γ_1-globulin antibody (approximately × 100). Small white spots in the section to the left of the cell are artifacts.

FIG. 6. Central area of section in Fig. 5 showing the fluorescing cell at high magnification (approximately × 800).



(Cruchaud *et al.*: 19S γ-globulins in dysgammaglobulinemia)