Brief Definitive Reports

CELL SURFACE IMMUNOGLOBULIN*

VIII. Synthesis, Secretion, and Cell Surface Expression of Immunoglobulin in Murine Thoracic Duct Lymphocytes

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(Received for publication 2 January 1974)

For reasons not relevant to the present studies, thoracic duct lymphocytes from young BALB/c mice were observed to synthesize an H chain with a different mobility from μ-chain. This finding was unexpected because the H chains synthesized by and on the surface of splenocytes from similar animals are almost exclusively μ-chain (1). To investigate this finding further, thoracic duct lymphocytes and splenocytes of young Balb/c mice were labeled with [^{3}H]tyrosine or were surface radioiodinated with ^{125}I. The classes of Ig synthesized and secreted by these cells and attached to their cell surfaces were determined.

Materials and Methods

Preparation of Cell Suspension.—Male Balb/c mice of 2½–3½ mo of age were purchased from Jackson Laboratories, Bar Harbor, Maine. Cell suspensions were prepared from perfused spleens and thoracic duct lymphocytes were obtained (2) from the first 18–24 h of drainage. 6-wk old germ-free mice (C3H) were obtained from Sprague Dawley, Madison Wis.

Radiolabeling of Cells.—Cells were iodinated as previously described (1) at a concentration of 10^8 cells/ml with 2 mCi of ^{125}I, 50 μg of lactoperoxidase (Sigma Chemical Co., Inc., St. Louis, Mo.) and 25 μl of 0.03% hydrogen peroxide. The reaction was terminated after 5–10 min by the addition of 25 volumes of cold phosphate-buffered saline pH 7.3 (PBS). Cells were centrifuged, washed once in PBS and once in Eagle's Minimal Essential Medium (MEM) (Grand Island Biological Corp., New York).

For labeling with [^{3}H]tyrosine, cells were washed in MEM containing 10% fetal calf serum and 2% antibiotic-antimycotic mixture and lacking tyrosine. Cells were suspended at 5×10^6/ml. 40–100 μCi/ml of L-tyrosine (3,5-^{3}H) (New England Nuclear, Boston, Mass.) were added and the cultures were incubated at 37°C in a moist CO_2 environment.

Immunoprecipitation of Radiolabeled Ig from Cell Lysates.—After incubation of radiolabeled cells, incubation medium and cells were separated by centrifugation. Cells were lysed in 0.5% Nonidet P_{40} (NP_{40}, Shell Chemical Corp., New York) for 10 min at 4°C. Media and lysates were dialysed for 16 h at 4°C against 2–4 liters of PBS. After dialysis, lysates were centrifuged at 10,000 *g* for 30 min and small aliquots of the lysates were precipitated in 10% trichloroacetic acid (TCA) and counted (1). Ig was precipitated from the lysates as previously described, using a "sandwich" procedure: rabbit antimouse Ig and goat antirabbit Ig (1). Four rabbit antimouse Ig sera were employed: a multispecific sera with specificities to μ, γ, λ, and κ, and three sera monospecific to μ, γ, or α. These three antisera were made by immunizing

* This work was supported by U. S. Public Health Service Grant Al-11164.

rabbits with isolated H chains from murine myelomas MOPC 104E, mouse serum IgG (Pentex Biochemical, Kankakee, Ill.) and TEPC 15 respectively. These monospecific antisera were a gift from Doctors McWilliams and Lamm, Dept. of Pathology, New York University Medical Center. The specificity of these sera was established by showing that the antisera did not bind radioiodinated myeloma protein of other classes (using sandwich immunoprecipitation and analysis by sodium dodecyl sulfate [SDS]-acrylamide gel electrophoresis). Rabbit anti-$\phi\chi$ was used as a control for nonspecific precipitation. Immune precipitates were washed 3–4 times in cold PBS and counted. The value for the control immunoprecipitate was subtracted from the experimental value to determine the amount of radioactive Ig that was specifically immunoprecipitated. Immunoprecipitates were dissolved in 2% SDS, reduced and alkylated and electrophoresed on SDS-5% acrylamide gels (1). Radioiodinated γ- and L-chains were electrophoresed on companion gels as molecular weight markers.

RESULTS

In five experiments, thoracic duct cells were labeled with [^{3}H]tyrosine and the lysates were acid- and immunoprecipitated. An average of 7.8% of acid precipitable radioactivity in the cell lysate was specifically precipitated as Ig. Fig. 1 shows analysis of the immunoprecipitates after acrylamide gel electrophoresis in a representative experiment. As can be seen, when anti-α was used for immunoprecipitation large α- and L-chain peaks of predicted mobility were observed whereas anti-μ and anti-γ precipitated no detectable H or L chains. In all immunoprecipitates including the control, a small peak of radioactivity was observed in fractions 28-32 corresponding to a protein of mol wt of about 35,000 daltons.

The secretions from these cells gave similar results to the lysates. Fig. 2 compares the immunoprecipitates of multispecific anti-Ig with anti-α in a representative experiment. After reduction and alkylation, identical H-chain peaks consisting entirely of α-chains were obtained. In this and the other experiments, the L-chain peak was moderately higher when the multispecific antiserum was used suggesting that "free" excess L chains were synthesized and secreted (3). This possibility was confirmed by immunoprecipitation by multispecific anti-Ig

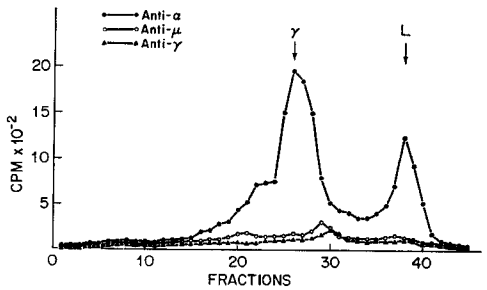

FIG. 1. Ig synthesized by thoracic duct cells. [^{3}H]tyrosine-labeled lysates were immunoprecipitated with antiserum specific to either μ, α, γ, or to an unrelated antigen, bacteriophage $\phi\chi$ (control). Dissolved precipitates were reduced and alkylated before electrophoresis on SDS-5% acrylamide gels.

of the supernate of the anti-α immunoprecipitate (Fig. 2). An L-chain peak but no H-chain peak was observed. The peak in fraction 28-32 referred to above which is nonspecifically immunoprecipitated is again evident.

The above results contrast with those obtained when splenocytes from other similar mice were examined. In two experiments, it was shown that the Ig synthesized and secreted by splenocytes was IgM (1) with a trace amount of IgG.

Our earlier studies also reported that IgM was the dominant Ig on the surface of murine splenocytes even when substantial amounts of IgG were being synthesized (4). Three experiments were performed in which thoracic duct cells were enzymatically radioiodinated and the resultant cell lysates acid- and immuno-precipitated. An average of 2.3% of acid precipitable radioactivity was specifically precipitated as Ig. As can be seen in Fig. 3, the only class of Ig that could be definitely detected was IgM. There may be a small amount of IgA. The small peak between H and L chains is again observed, suggesting that this unidentified protein has a surface phase.

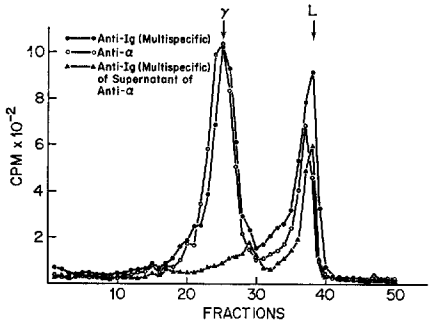

FIG. 2. Ig secreted by thoracic duct cells. [^{3}H]tyrosine-labeled secretions were immunoprecipitated with either multispecific anti-Ig or anti-α. The supernatent of the anti-α precipitate was then reprecipitated with multispecific anti-Ig. See Fig. 1.

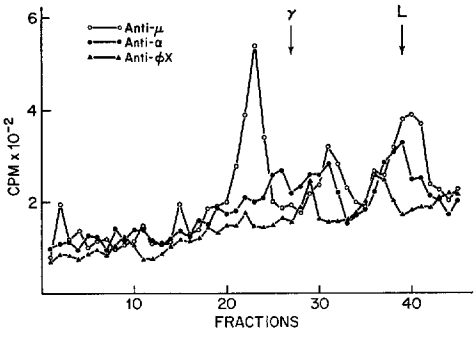

FIG. 3. Ig on the surface of thoracic duct cells. Cells were surface labeled with ^{125}I and lysates were immunoprecipitated with anti-μ, anti-α, or anti-$\phi\chi$ (control). See Fig. 1.

Recently, it was shown that splenocytes and lymph node cells of germ-free mice synthesize and secrete only IgM without detectable IgG or IgA (5). It was, therefore, of interest to determine which class of Ig, if any, would be synthesized by thoracic duct cells from such mice.

After labeling with [^{3}H]tyrosine, lysates and secretions were immunoprecipitated. 0.8% of the 2.3×10^6 acid precipitable cpm that had been incorporated by 10^7 cells was specifically precipitated as Ig. Analysis of the reduced and alkylated immunoprecipitates on SDS-acrylamide gels gave markedly different results from those obtained when thoracic duct cells from normal mice were used: IgM synthesis was readily demonstrated and no IgA synthesis was detected.

DISCUSSION

The aforementioned studies indicate a dichotomy between the predominant class of Ig on the surface of thoracic duct cells of normal mice and the class of Ig synthesized and secreted by these cells. Thus, IgM was detected on their surface, whereas the Ig synthesized and secreted was IgA. Identification of the two classes of Ig was firmly established by the different mobilities of μ- and α-chain after electrophoresis on acrylamide gels in SDS (relative position of μ- and α-chains were also determined by the position of the L chains in the same gels and electrophoresing L- and γ-markers on companion gels) and by the use of monospecific antiserum to α, μ, or γ-chain.

Our studies extend earlier qualitative findings of Asofsky and Mandel (6) that thoracic duct cells of mice can synthesize in vitro IgA and IgM but not IgG_1 or IgG_2. Moreover, at selected concentrations of cells, IgA synthesis dominated as detected by radioautography, e.g., at 5×10^6 cells/ml, 7 of 12 cultures were positive for IgA, but only 1 of 12 for IgM. Our results are also consistent with the observations that the concentration of IgA in mesenteric lymph of several species of laboratory animals is substantially higher than its concentration in the sera (7–9), and that the major portion of serum IgA in mice is derived from synthesis by intestinal associated lymphocytes (10).

Our interpretation of the present finding is that IgA is synthesized and secreted by a small number of plasma cells in thoracic duct lymph which have emigrated from gut-associated lymphoid tissue and that IgM is on the surface of B lymphocytes (as the antigen-specific receptor of the unstimulated cell). The considerations that lead to this conclusion are: (*a*) a plasma cell can synthesize 100-fold or more Ig than a lymphocyte (11, 12), and (*b*) the cell surface represents a small compartment and turnover of cell surface IgM is relatively slow in unstimulated B cells (11, 12). Hence, in such lymphocytes little synthesis is necessary to maintain a steady state of IgM on the cell surface. Our studies do not exclude two alternative possibilities: (*a*) IgM was synthesized by and is on the surface of the same cells that are synthesizing and secreting IgA, or (*b*) IgM is bound passively to lymphocytes (13, 14, footnote 1). This possibility is rendered less likely by the demonstration in our studies that the cell

[1] Hudson, L., J. Sprent, and J. F. A. P. Miller, unpublished observations.

surface IgM was present as a monomer as determined by acrylamide gel electrophoresis of unreduced immunoprecipitates.

The absence of readily detectable cell surface IgA is provocative. Perhaps, IgA-bearing lymphocytes do not circulate through the thoracic duct. However, cell surface IgG also was not demonstrated on thoracic duct cells by surface radioiodination and only trace amounts are present on splenocytes of young Balb/c mice (1). These results appear to contrast with many studies of others in which IgG (e.g., 15) or IgA (e.g., footnote 2) was demonstrated on a significant proportion of intact lymphoid cells. There are several possibilities to explain this discrepancy: (*a*) Enzymatic radioiodination does not iodinate α- and γ-chains efficiently. (*b*) Memory cells may have IgM on their surface. (*c*) Memory cells may have predominently IgG or IgA on their surface but young mice may have had insufficient antigenic stimulation to create a sizable population of such cells (i.e. which have already switched). (*d*) α- and γ-chains may have less representation on the cell surface than μ, i.e., sufficient Ig to function as receptors but not to be demonstrated easily by a biochemical technique. The first possibility is not likely. Studies in this laboratory (16) and by Wernet and Kunkel[3] indicate that IgG can be readily iodinated on myeloma and human lymphoblastic cells respectively. The possibility that IgM is on the surface of memory cells destined to synthesize IgG or IgA is rendered unlikely by the findings that treatment of lymphocytes with anti-α or anti-γ together with complement selectively diminishes synthesis of the corresponding class of Ig (see 16). We favor the possibility that with further stimulation, classes of Ig other than IgM would be detected on murine lymphocytes by radioiodination.

Thoracic duct lymphocytes from germ-free mice were also studied because splenocytes from these axenic mice synthesize an unusually high proportion of IgM relative to total protein but no IgG or IgA (5). Electron micrographs of the spleens of these mice reveal a markedly high proportion of plasma cells, e.g., as many as 24% of splenocytes (5). In contrast to normal mice, thoracic cells from axenic animals synthesize and secrete substantial amounts of IgM, confirming that axenic animals do not effectively regulate their IgM synthesis and their lymphoid cells do not differentiate to produce IgA and IgG. Thus, IgM synthesis in their thoracic duct cells may be due to the emigration from intestinal lymphoid tissue of plasma cells that have not switched to IgA synthesis.

We acknowledge the excellent technical assistance of Mr. Yuen Chinn and Mr. Henry Siu. Mr. Chinn performed all the thoracic duct cannulations.

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