

Absence of Detectable IgM in Enzymatically or Biosynthetically Labeled Thymus-Derived Lymphocytes

(B lymphocyte/lymphocyte receptor)

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Communicated by Baruj Benacerraf, June 21, 1973

ABSTRACT Surface proteins of mouse thymus and spleen cells were radioiodinated with lactoperoxidase. After solubilization, the labeled proteins were precipitated by antibodies directed against mouse immunoglobulin chains; the precipitates were analyzed by radioautography after Na dodecyl sulfate-gel electrophoresis. Radioactive μ and L chains were absent from thymocyte extracts and conspicuous in spleen-cell extracts. The following cells were biosynthetically labeled for 4 hr with [35 S]methionine or 24 hr with [14 C]leucine: (1) Thymocytes, (2) cortisone-resistant thymocytes [both treated with rabbit antisera cytotoxic to bone marrow-derived (B) lymphocytes and IgM-containing plasma cells, to kill possible contaminating nonthymus-derived cells], (3) "activated thymocytes" (allogeneic cell cultures of cortisone-resistant thymocytes), (4) human Daudi cells (a B lymphoblastic cell line), and (5) purified mouse B spleen lymphocytes devoid of plasma cells. Again no μ and L chains could be detected in thymocyte or thymus-derived cell extracts by immune precipitation and gel electrophoresis, while these chains were conspicuous in B-cell extracts. "Educated thymocytes," obtained from spleens of lethally irradiated mice injected with syngeneic thymocytes and antigen, synthesized μ and L chains under similar conditions; this synthesis resulted from contamination of these cells by IgM-containing plasma cells.

The nature of thymus-derived (T) lymphocyte receptors is controversial (1). By immunofluorescence, immunoglobulin cannot be demonstrated on the surface of T lymphocytes, while it is easily demonstrable on thymus-independent (B) lymphocytes (2, 3). More sensitive tests, using radioactively labeled antibodies to detect surface immunoglobulin, showed that if thymocytes or T lymphocytes bear immunoglobulin accessible to the antibodies, it is much less abundant than on B lymphocytes (2, 4) and is in the range of what is detected on erythrocytes (4). Using radioiodination of cell-surface proteins, however, Marchalonis *et al.* found IgM molecules on human and mouse thymocytes (5). They suggested that T lymphocytes were more deeply buried in the cell membrane than B lymphocytes, thus explaining the failure of antibodies to detect surface immunoglobulins. Furthermore, Cone *et al.* found surface IgM on T lymphocytes engaged in a graft-versus-host reaction, and showed that this surface IgM had the anti- H_2 specificity expected for a T-cell receptor (6). However, Vitetta *et al.* (7) and Grey *et al.* (8), using similar techniques, failed to detect immunoglobulin chains on the surface of thymocytes or of various T lymphocytes.

Abbreviations: B lymphocyte, bone marrow-derived, thymus-independent lymphocyte; T lymphocyte, thymus-dependent lymphocyte.

In the present work, we attempted to detect immunoglobulin on or in thymocytes, various thymus-derived lymphocytes, spleen cells, and B lymphocytes, using cell-surface radioiodination as well as biosynthetic labeling procedures. The labeled proteins were precipitated by antibodies directed against mouse immunoglobulin chains and the precipitates were analyzed by radioautography after Na dodecyl sulfate-gel electrophoresis (9), a sensitive procedure with high resolving power.

MATERIALS AND METHODS

Lactoperoxidase-Catalyzed Radioiodination of Cell-Surface Proteins. Thymocytes and spleen-cell suspensions were prepared as described (3), and used only when more than 95% of the cells were viable, as judged by trypan blue exclusion. Iodination was performed by the method of Marchalonis *et al.* (10) usually on 10^8 cells with 1 mCi of carrier-free Na- 125 I (80-140 mCi/ml, Amersham), 100 μ g of lactoperoxidase (Sigma, St. Louis, Mo.), and 30 μ l of 8.8 mM H_2O_2 . After a 5-min incubation at 30°, the cells were centrifuged at 4°. They were washed once with 5 mM L-cysteine·HCl in phosphate-buffered saline (pH 7.4), twice with 5 mM L-cysteine·HCl-10 mM KI in phosphate-buffered saline, and once with 10 mM KI in phosphate-buffered saline. Trypan blue exclusion showed no loss of viability. Three procedures were used to solubilize the labeled cell-surface proteins. (a) Cell homogenization in Nonidet P 40 (NP 40, Shell Oil Co., New York), 1% in 10 mM Tris·HCl (pH 7.4)-25 mM KCl-5 mM MgCl₂. The homogenates were spun for 60 min at $140,000 \times g_{max}$, the supernatants were passed onto Sephadex G-25 (Pharmacia, Uppsala, Sweden) equilibrated with 0.05% NP 40 in phosphate-buffered saline, and the excluded macromolecular radioactive material was collected. (b) Cell solubilization in 9 M urea-1.5 M acetic acid followed by gel filtration of the macromolecular radioactive material on Sephadex G-25 in 6 M urea-1 M propionic acid, and extensive dialysis against 50 mM Tris·HCl (pH 8.0)-150 mM NaCl (5). (c) Incubation of the washed labeled cells for 150 min at 37° in cell-culture conditions (RPMI 1640 medium, Microbiological Assoc. Inc., Bethesda, containing 5% fetal-calf serum), followed by extensive dialysis against phosphate-buffered saline of the culture medium containing released labeled proteins. The extracts obtained after the various procedures were analyzed for total, as well as trichloroacetic acid-precipitable, radioactivity with a Packard Autogamma spectrometer. Usually 10-30% of the radioactivity was not acid-precipitable.

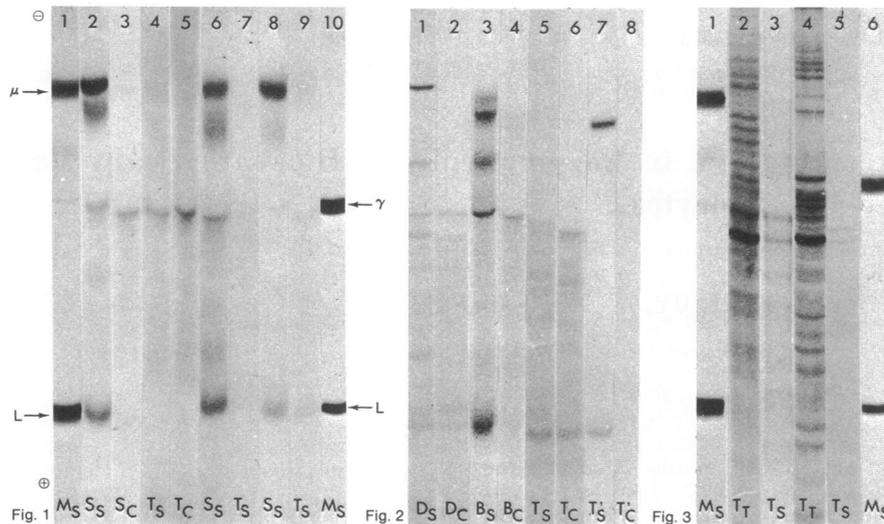


FIG. 1 (left). Radioautographs of Na dodecyl sulfate gels of immune precipitates: ^{125}I -labeled surface proteins of spleen and thymus. (1) Specific precipitate of the incubation medium of MOPC 104 E cells after 4 hr of culture in the presence of ^{14}C leucine, showing the position of extracellular μ and L chains. (2-5) Proteins from spleen and thymus released by NP 40 lysis. (6 and 7) Proteins from spleen and thymus solubilized in urea-acetic acid. (8 and 9) "Metabolically" released proteins from spleen and thymus. S_s and T_s : spleen- or thymus-specific precipitates. S_c and T_c : spleen or thymus control precipitates. (10) Specific precipitate of the incubation medium of MOPC 70 cells after 4 hr of culture in the presence of ^{14}C leucine, showing the position of extracellular γ and L chains. The radioactivity loaded on the gels varied from 1,000-11,000 cpm and exposure time from 8-42 days. On the radioautographs where few or no bands are seen, the radioactivity was spread throughout the gel or concentrated on the phenol blue front.

FIG. 2 (center). Radioautographs of Na dodecyl sulfate gels of immune precipitates: ^{35}S methionine-labeled proteins of Daudi cells (D), spleen B lymphocytes (B), thymocytes (T), and "educated thymocytes" (T'). (1 and 2) Daudi cells, specific (D_s) and control (D_c) direct precipitates. The amount of protein loaded on the gels was different from that used for the indirect precipitates and migration of the μ chain is slightly slower. (3 and 4) Spleen B cells, specific (B_s) and control (B_c) precipitates. (5 and 6) Thymocytes, specific (T_s) and control (T_c) precipitates. (7 and 8) "Educated thymocytes" (contaminated by IgM-containing plasma cells), specific (T'_s) and control (T'_c) precipitates. The amount of radioactivity loaded on the gels varied from 3400-7000 cpm (except for T'_c : 1400 cpm) and exposure time from 13-27 days.

FIG. 3 (right). Radioautographs of total cell lysates and their immune precipitates: ^{14}C leucine-labeled proteins of activated cortisone-resistant thymocytes and cortisone-resistant thymocytes. (1) Specific precipitate of intracellular MOPC 104 E proteins, labeled as described in legend of Fig. 1, showing the position of intracellular μ and L chains. (2 and 3) Activated thymocytes, total lysate (T_l), and its specific precipitate (T_s). (4 and 5) Cortisone-resistant thymocytes, total lysate (T_l), and its specific precipitate (T_s). (6) Specific precipitate of intracellular MOPC 70 proteins, labeled as described in legend of Fig. 1, showing the position of intracellular γ and L chains. The amount of radioactivity loaded on the gels varied from 1600-15,000 cpm and the exposure time from 20-31 days.

Biosynthetic Labeling of Cell Suspensions with ^{35}S Methionine or ^{14}C Leucine. (a) Thymocytes from (CBA x C57BL/6) F_1 mice were used after incubation with antibodies against mouse e-specific B-lymphocyte antigen (3) and against mouse-specific plasma-cell antigen (11), prepared in rabbits, in the presence of rabbit complement (3) to kill possible contaminating B lymphocytes and IgM-producing plasma cells, respectively. The antiserum against mouse-specific plasma-cell antigen used, prepared as described (11), recognized almost 100% of spleen IgM-containing cells, by selective immunofluorescence with two fluorochromes (3). (b) Cortisone-resistant thymocytes from C57BL/6 mice were prepared as described (12) and treated with the same antisera and complement. (c) Activated thymocytes were obtained *in vitro*, by culturing 2.5×10^6 cortisone-resistant thymocytes from DBA/2 mice (H_2d) together with 2.5×10^6 cortisone-resistant thymocytes from C57BL/6 mice (H_2b), in flat-bottom tubes containing 3 ml of RPMI 1640 medium with 5% fetal-calf serum. The cells were used for biosynthetic labeling after 3 or 4 days of culture, when they show marked proliferation, as judged by ^3H thymidine incorporation. (d) "Educated" thymocytes were obtained *in vivo* (13). 10-Week-old CBA/ca

mice were injected intravenously with 10^8 syngeneic thymocytes and 5×10^8 sheep erythrocytes within 4 hr after receiving 800 R total body irradiation. The mice were killed 6 days later. Spleen-cell suspensions were prepared and passed through small glass-wool columns to remove most of the dead cells. Cell smears were fixed in acetone and stained with a rhodamine-labeled rabbit antibody against mouse μ chain (Ig fraction) (3); since immunofluorescence showed an important contamination by IgM plasma cells, some of the cell suspensions were further treated with rabbit antibody against mouse-specific plasma-cell antigen and complement. (e) Spleen lymphocytes devoid of plasma cells were obtained from Swiss mice by centrifuging 200×10^6 spleen cells for 10 min at $200 \times g$ through a continuous 5-15% sucrose gradient prepared in dilute Hank's balanced salt solution containing 20% fetal-calf serum (14). A homogeneous population of lymphocytes sedimented as an intermediary band, while most of the other cells, including plasma cells, were found in the pellet. The lymphocytes were incubated with antibody against mouse-specific plasma-cell antigen and complement, and in some experiments simultaneously with rabbit antibody against mouse-specific lymphocyte antigen and complement

(3) to kill the T lymphocytes. Dead cells were removed by repeated washings, and aliquots of the cell preparation were used for smears to ascertain by immunofluorescence the absence of IgM-containing plasma cells. (f) Daudi lymphocytes, an established cell line from a human Burkitt lymphoma (kind gift of Dr. G. Klein, Karolinska Institute, Stockholm), were maintained in Dulbecco's modified Eagle's medium with 20% fetal-calf serum. (g) IgM and IgG plasma cells were obtained, respectively, from mouse myelomas MOPC 104 E and MOPC 70 grown in Balb/c mice (kind gifts from Dr. M. Potter, N.I.H., Bethesda).

10 to 25×10^6 cells were suspended at 5×10^6 cells per ml (1 to 2×10^6 cells per ml for Daudi cells) in Eagle's medium containing 10% fetal-calf serum and lacking either methionine or leucine, and were incubated at 37° for 4 hr with 10 μ Ci/ml of [³⁵S]methionine (Amersham, 140 Ci/mmol), or for 24 hr with 2 μ Ci/ml of [¹⁴C]leucine (New England Nuclear Corp., 327 Ci/mol). After the cells were washed, lysates were obtained by homogenization in 1% NP 40 and centrifugation, and filtered on Sephadex G-25 in phosphate-buffered saline or extensively dialyzed against phosphate-buffered saline. Small samples, used to determine total and trichloroacetic acid-precipitable radioactivity, were dissolved in 50 μ l of NCS (Nuclear Chicago), added to 8 ml of Omnifluor solution (New England Nuclear Corp.), and counted in a Beckman LS-250 liquid scintillation counter. With [³⁵S]methionine labeling, about 30% of the radioactivity or more was not acid-precipitable; with [¹⁴C]leucine, all radioactivity was acid-precipitable.

Immune Precipitation. Aliquots of the soluble fractions containing radioactive proteins were treated for 30 min at 37° with either rabbit immunoglobulin fractions containing antibodies directed against mouse μ chains and mouse whole immunoglobulins (3), or a similar amount of normal rabbit immunoglobulin (controls). Rabbit immunoglobulin was then precipitated by incubation with an excess of sheep immunoglobulin directed against rabbit immunoglobulin for 2 hr at 37° followed by 16 hr at 4°. To avoid any possible cross-reactivity, all normal rabbit immunoglobulin and sheep immunoglobulin against rabbit immunoglobulin had been passed onto columns of Sepharose-bound mouse IgG and IgM (3) and all antisera were absorbed with mouse thymocytes. In some instances, direct precipitation was performed, using rabbit antisera and carrier mouse IgG and IgM (3); rabbit antibody against ovalbumin and ovalbumin were used as controls. With Daudi cells, rabbit antibody against human IgM and carrier human IgM (gift of Dr. M. Seligmann, Paris) were used. The precipitates were washed 3 times in phosphate-buffered saline containing 0.1% NP 40, 5 mM KI, and 1 mM methionine or 1 mM leucine, as appropriate, followed by 10% trichloroacetic acid, ethanol-ether, and ether. The dried precipitates were dissolved and reduced in the sample buffer used for gel electrophoresis, 3% Na dodecyl sulfate-5% 2-mercaptoethanol-10% glycerol-62.5 mM Tris-HCl (pH 6.8). The dissolved precipitates were heated for 1 min at 100°, and small samples were counted.

Acrylamide-Gel Electrophoresis. The immune precipitates and samples of whole-cell lysates dissolved in sample buffer, were subjected to electrophoresis in tube gels by the discontinuous Na dodecyl sulfate-gel procedure (9), with 10% acrylamide in the running gel. The gels were stained with

Coomassie blue, destained, sliced longitudinally, dried, and used for radioautography (Kodirex X-Ray film, Kodak). In the figures, the relative alignment of gels was made possible by comparison of the mobilities of immunoglobulin chain standards (obtained from [¹⁴C]leucine-labeled MOPC 104 and MOPC 70 proteins). The amount of radioactivity from thymocytes or thymus-derived cells placed on the gels was in the same range as that from spleen or Daudi cells. Whenever radioautographs of specific and control precipitates were to be compared, the radioactivity loaded on the two gels was of the same order of magnitude. The radioautographs were exposed for up to 6 weeks to insure that even the less radioactive bands were detected.

RESULTS

Surface Immunoglobulin Detected after Radioiodination of Lymphoid Cells. With spleen-cell extracts, specific precipitates usually contained 1-3% of the trichloroacetic acid-precipitable radioactivity. This represented 2- to 3-times as much radioactivity as in control precipitates. In thymus-cell extracts, the amount of radioactivity present in specific and control precipitates was similar and included 0.2-0.8% of the acid-precipitable radioactivity. Radioautographic analysis of the immune precipitates after Na dodecyl sulfate-gel electrophoresis (Fig. 1) was necessary to determine which labeled proteins were present in the various precipitates. Radioactive bands with the same mobility as μ and L chains were conspicuous in specific precipitates and absent in control precipitates from spleen-cell extracts; they were absent from both specific and control precipitates of thymus-cell extracts. Such results were obtained under the three conditions used to solubilize the labeled proteins. In addition to radioactive bands corresponding to μ and L chains, a band migrating slightly faster than μ chains was consistently observed in spleen-specific precipitates (Fig. 1, gels 2, 6, and 8).*

In addition to the labeling patterns described above, several other bands were observed. For example, a radioactive band corresponding to the front of the γ -chain band on the gel was found in specific precipitates of spleen extracts (Fig. 1, gels 2 and 6), and occasionally also in control precipitates from spleen (Fig. 1, gel 3) and in specific and control precipitates from thymocyte extracts (Fig. 1, gels 4 and 5). In a few cases, a faint band in the region of L chains was also seen in control precipitates from spleen cells, or in specific (Fig. 1, gel 9) and control precipitates from thymus-cell extracts. Since most of these bands were usually of similar intensity in specific and control precipitates, we think they are artifacts. In order to load enough radioactivity on the gels, we performed immune precipitations on rather concentrated cell extracts containing large amounts of radioactivity, not all of it being acid-precipitable (see *Methods*). This procedure might lead to nonspecific trapping of labeled proteins in the

* Migration of spleen-surface μ chains was identical to that of unlabeled μ chains and of labeled extracellular μ chains obtained after incubation of MOPC 104 E myeloma cells with [¹⁴C]leucine (Fig. 1, gel 1). This finding was ascertained on the same gel by addition to the specific precipitates of unlabeled IgM or [¹⁴C]-labeled MOPC 104 E extracellular proteins. Intracellular [¹⁴C]-labeled MOPC 104 E μ chains, on the other hand, migrated slightly faster than extracellular MOPC 104 E μ chains, but usually a little more slowly than the band seen ahead of μ chains on Fig. 1.

precipitates and also to artifactual binding of soluble radioactive material, thus explaining the diffuse background in some gels and the concentration of radioactivity at the front edge of the bands of unlabeled immunoglobulin used for precipitation; these chains indeed represent the bulk of the protein in the gels. Such an interpretation was reinforced by the observation of similar bands in immune precipitates from lysates of radioiodinated erythrocytes.

Cellular Immunoglobulin Detected after Biosynthetic Labeling of Lymphoid Cells. Human lymphocytes of the Daudi cell line, which bear surface IgM but do not secrete it in significant amounts (15), were incubated for 4 hr with [³⁵S]methionine. After cell lysis in NP 40, electrophoresis of the specific immune precipitates showed a radioactive band corresponding to μ chains (Fig. 2, gel 1), which was absent from control precipitates (Fig. 2, gel 2). Several faint bands were also frequently seen in specific and control precipitates. Some of them appeared to represent proteins that were nonspecifically trapped in the precipitates. However, since lysates of cells incubated with [³⁵S]methionine usually contained, in spite of extensive dialysis, significant amounts of trichloroacetic acid-nonprecipitable radioactivity, the possibility of artifactual labeling of immune precipitates also exists. In fact, faint labeling in the region of γ chains or of L chains was occasionally seen with similar intensity in specific or control precipitates (Fig. 2, gels 1 and 2). No well-labeled band corresponding to L chains was ever seen in the specific precipitates. Several human κ chains do not contain methionine (16), and it is not known whether the Daudi κ chains contain methionine.

Since the Daudi cells represent a malignant clone, the turnover of surface IgM may be faster than in normal B cells. Normal spleen lymphocytes were therefore prepared, and immunofluorescence with rhodamine-labeled antibody against mouse immunoglobulin was performed to ascertain the absence of plasma cells. After 4 hr of incubation with [³⁵S]methionine, strong radioactive bands of μ - and L-chain size were easily detected in the specific, and absent in the control, precipitate (Fig. 2, gels 3 and 4). The μ -chain band had the same mobility as extracellular μ chains. A band migrating between μ and γ chains was also observed in specific precipitates (Fig. 2, gel 3), as was seen after surface labeling with ¹²⁵I. The radioautograms were similar whether the incubated cells were only B lymphocytes obtained after killing of T lymphocytes with antibody against mouse-specific lymphocyte antigen and complement, or total spleen lymphocytes. These labeling conditions are therefore adequate to detect IgM synthesis by normal B lymphocytes.

Thymus cells were incubated under the same conditions, after exposure to rabbit antibodies against mouse-specific B-lymphocyte and plasma-cell antigens in the presence of complement to eliminate possible contamination by a small number of B lymphocytes and IgM plasma cells. Specific precipitates from cell lysates never showed even faint radioactive bands corresponding to μ chains (Fig. 2, gels 5 and 6). Several bands were occasionally seen in both specific and control precipitates, notably some faint bands corresponding to the front edge of migration of unlabeled γ and L chains of the immune precipitates (Fig. 2, gels 5 and 6).

Since no synthesis of IgM by thymocytes could be detected in these conditions, cortisone-resistant thymocytes and cortisone-resistant thymocytes activated *in vitro* were incu-

bated for 24 hr in the presence of [¹⁴C]leucine. Even after this prolonged labeling of T cells of known immune competence, specific and control precipitates of the lysates never showed, after electrophoresis, any radioactive band corresponding to μ and L chains, but only a few bands of radioactive proteins apparently nonspecifically trapped in the precipitates (Fig. 3, gels 3 and 5).

Spleen-cell suspensions of "educated" thymocytes, produced *in vivo* by injection of thymus cells and antigen into irradiated hosts, were also studied. Since they contained large numbers of damaged and fragile cells, they were incubated for only 4 hr with [³⁵S]methionine as the precursor. Electrophoretic analysis of the specific precipitates showed strong radioactive bands of μ and L chains, absent in control precipitate (Fig. 2, gels 7 and 8). Immunofluorescence study of these cell suspensions revealed that they contain 2-3% of large IgM-containing plasma cells. After treatment of the cell suspensions with antibody against mouse-specific plasma-cell antigen and complement, which was shown by immunofluorescence to kill or damage most of these plasma cells, the immune precipitates did not show any major bands of μ and L chains. Only various bands of less intensity were seen and if μ chains were still present, they were much less obvious than before. It is clear that with the highly sensitive technique used, contamination by a few remaining plasma cells can lead to detectable amounts of labeled μ chains.

DISCUSSION

The present experiments failed to detect IgM chains on the surface of thymocytes, using lactoperoxidase-catalyzed radioiodination, under conditions where the presence of these chains on the surface of spleen cells was easily demonstrable. This failure was not due to some peculiarity of the method used to obtain the surface proteins in a soluble form after their labeling, since similar results were observed with three different techniques, including those used by Marchalonis *et al.* (5). Our observations are therefore in agreement with those of Vitetta *et al.* (7) and Grey *et al.* (8), and do not confirm the results of Marchalonis *et al.* (5, 6).

In addition, these observations emphasize the possibility of artifactual labeling of immunoglobulin chains used to form immune precipitates in cell lysates or supernatants. Some of the present experiments might have led to the erroneous conclusion that γ chains, or γ and L chains, are present on thymocytes, if the comparison between control and specific precipitates, showing the same faint radioactive bands in both, had not been made. It is therefore essential to perform control precipitations under conditions that mimic as closely as possible conditions for specific precipitation, and to analyze both by gel electrophoresis. Indirect precipitation should be favored over direct precipitation since: (a) all the reagents can be used in the same amounts and be identical, except that rabbit immunoglobulin with specific antibody activity is replaced in controls by normal rabbit immunoglobulin and (b) the presence of large amounts of unlabeled carrier μ chains can be avoided. It seems likely that the occasional artifactual labeling of unlabeled chains results from the presence of trichloroacetic acid-nonprecipitable radioactivity, which was still found, despite extensive precautions, in the cell lysates or supernatants submitted to immune precipitation (see also ref. 8). Even washing of the immune precipitates with trichloroacetic acid was not effective in preventing the occasional occurrence of these artifactual radioactive bands.

Only normal thymocytes were used for surface labeling in order to avoid another source of erroneous interpretation, namely the use of T lymphocytes bearing on their surface passively adsorbed immunoglobulins. There is good evidence for the existence of a receptor for immune complexes or immunoglobulin on activated (17) or malignant (8) thymocytes.

In experiments involving biosynthetic labeling with radioactive amino acids, it was easy to demonstrate synthesis of μ chains by a human clone of B lymphocytes and by normal mouse-spleen B lymphocytes, while no labeled μ chains could be detected in cells of thymus origin. Even after 24 hr of incubation with [14 C]leucine, no labeled μ or L chains were found in cortisone-resistant thymocytes, i.e., immunocompetent thymocytes, or in cortisone-resistant thymocytes activated in a mixed lymphocyte culture. In these experiments, thymus cells were incubated with rabbit antibodies against mouse-specific B-lymphocyte and plasma-cell antigens in the presence of complement before incubation with the radioactive precursors, in order to decrease the possibility of contamination by B lymphocytes and IgM plasma cells. That this precaution was necessary is suggested by the report of Vitetta *et al.*, who found synthesis of immunoglobulin by a minor fraction (less than 2% of the total cell population) of mouse-thymus cells; this fraction did not bear the θ antigen and probably consisted of plasma cells and/or B lymphocytes (18). This observation points out another possible major source of artifact—the possible heterogeneity of the cell population used. For example, the activated thymocyte population synthesized IgM very actively, but this synthesis resulted from contamination by immature IgM-containing plasma cells. It is commonly accepted that lethally irradiated mice cannot mount an immune response and that cells recovered from their spleens after transfer of syngeneic thymocytes are only T cells, yet it is obvious that this concept needs to be revised. In fact, the occurrence of marked plasma-cell proliferation in the lymphoid organs of sublethally (19) and lethally (20) irradiated rats has already been well described.

The present experiments do not rule out the possibility that the T-cell receptors are immunoglobulin molecules. If they are, however, T-cell immunoglobulin receptors must differ from B-cell surface immunoglobulin in one of the following respects: (a) their amount is much lower and perhaps their turnover is much slower, or (b) they have a different relationship to the cell membrane (since they are not iodinated in conditions where B-cell immunoglobulin is, or since, after labeling and solubilization, they cannot complex with antibodies against immunoglobulin though B-cell immunoglobulin can), or (c) they are a different type of immunoglobulin chain. If, for instance, T-lymphocyte immunoglobulin receptors were incomplete chains consisting mostly of the variable region, they would be difficult to demonstrate, unless antibodies reacting with the variable part were used and attention was focused on detection of molecules smaller than conventional chains. Another possibility is that the T-cell receptor is

not an immunoglobulin molecule at all. In this case, the products of the Ir genes would be obvious candidates (21).

Finally, a word should be said about the μ chains of B lymphocytes. With surface labeling, as well as with biosynthetic labeling, of spleen lymphocytes, at least two bands moving more slowly than γ chains were specifically precipitated by antibody against μ chains. The first of these was a major well-defined band with the same mobility as μ chains of secreted IgM, i.e., moving somewhat slower than intracellular μ chains of MOPC 104 E myeloma. A less sharp band, which migrated faster than the intracellular μ chains of MOPC 104, was also found. This observation suggests that these are several types of μ chains in or on the surface of B lymphocytes, differing perhaps in their carbohydrate content (22, 23).

We thank Misses Anne Zurn and Monique Poincelet for their excellent technical assistance. This work was supported by Grant no. 3.408.70 of the Fonds National Suisse de la Recherche Scientifique.

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