CELL SURFACE IMMUNOGLOBULIN

IV. Distribution among Thymocytes, Bone Marrow Cells, and Their Derived Populations*

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Bone marrow and bone marrow–derived (B)[1] cells have immunoglobulin (Ig) on their surface which presumably acts as an antigen-specific receptor. The evidence consists of the binding of specific antigen by subpopulations of cells (1, 2), the specific deletion of such subpopulations by binding to antigen immunoadsorbents (3) or radioactive antigen-induced suicide (4), the demonstration of surface Ig on B cells (5–9), and the blocking of antigen binding or antigen stimulation of B cells by anti-Ig (10).

Thymocytes and thymus-derived (T) cells also have antigen-specific receptors as demonstrated by radioactive antigen-induced suicide (4), specific absorption by target cells (11), and binding of antigen (12–14). In contrast to B cells, however, the evidence demonstrating Ig directly on the surface of thymocytes and T cells is controversial (15–20).

We have previously reported the use of enzymatic radioiodination of spleen cells for the isolation and characterization of surface Ig (21). This method appeared to have advantages over those used in earlier studies of T cells because radiolabeled surface molecules can be isolated from the cells and examined directly. Moreover, the specific activity of the radiolabeled surface molecules is probably high. The purpose of the present study was to utilize this approach to search for surface Ig on thymocytes, bone marrow cells, and their derived populations. The results indicate that Ig is present on bone marrow and B cells, but it was not detected on thymocytes and T cells.

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[1] *Abbreviations used in this paper:* B cells, bone marrow–derived cells; BSA, bovine serum albumin; C, complement; CRL, complement-receptor B lymphocytes; Ig, immunoglobulin; MEM, Eagle's minimal essential medium; NP-40, Nonidet P-40 (Shell Chemical Corp.); PBS, phosphate-buffered saline; SDS, sodium dodecyl sulfate; T cells, thymus-derived cells.

Materials and Methods

Preparation of Iodinated Cells.—4–8-wk old BALB/c, CF_1, or AKR mice were killed by cervical dislocation and the spleens perfused and removed as previously described (22). Bone marrow cells were flushed from excised femurs with phosphate-buffered saline, pH 7.3 (PBS), using a syringe and 22 gauge needle. Thymuses were removed and freed of parathymic nodes, capsules, and blood vessels under × 4 magnification. Lymph nodes were removed and teased into cold PBS in the same manner as spleens and thymuses. S-49.1 lymphoma cells, a gift from Dr. K. Horibata, Salk Institute, La Jolla, Calif., were maintained in RPMI-1640 medium (Grand Island Biological Co., Grand Island, N. Y.) containing 10% horse serum. All cells were washed two times in PBS, filtered through stainless steel screens, and counted in 0.05% trypan blue–PBS. Preparations were not used unless cell viability was greater than 95%. Iodination of the cell surface was performed as previously described (22) and the reaction terminated by three washings in cold PBS. In several experiments, iodinated cells were fractionated on bovine serum albumin (BSA) gradients (22, 23). In other experiments, cell lysates prepared by detergent treatment and extensive dialysis were iodinated both enzymatically and by the chloramine-T method (24).

Isolation of Cell Surface Ig.—After radioiodination, cells were lysed with 0.5% Nonidet P-40 (NP-40; Shell Chemical Corp., New York), the nuclei removed by centrifugation, and Ig precipitated by "sandwich" techniques (22). Five types of antisera were used for the initial binding step: (*a*) anti-$\mu\lambda$ prepared in goats or rabbits against purified mouse IgM (murine myeloma MOPC-104E); (*b*) anti-$\gamma\kappa\lambda$ prepared in rabbits or goats against purified mouse IgG; (*c*) monospecific rabbit anti-μ prepared and adsorbed as previously described (22); (*d*) monospecific rabbit anti-γ, a gift from Dr. H. Grey, National Jewish Hospital and Research Center, Denver, Colo.; (*e*) rabbit anti-κ, a gift from Dr. R. Dutton, University of California, San Diego. This serum was shown to block T cell reactions in vitro (25). Control antisera consisted of normal rabbit or goat serum or rabbit anti-ϕX. Complexes were then precipitated with an excess of goat anti-rabbit Ig or rabbit anti-goat Ig. Precipitates were washed four times in PBS, solubilized in 1% sodium dodecyl sulfate (SDS)–8 M urea, pH 8.4, reduced, alkylated, and electrophoresed on SDS–acrylamide gels (22).

Labeling of Thymocytes with Tyrosine-^3H.—1–2 × 10^8 washed cells were suspended at a concentration of 1 × 10^7/ml in Eagle's minimal essential medium (MEM) lacking tyrosine and were labeled for 1–3 hr with 20 μCi/ml tyrosine-^{3}H (New England Nuclear Corp., Boston, Mass.). After 1 hr, the medium was supplemented with 10% fetal calf serum. Cells were then washed, lysed, and Ig precipitated as described for spleen cells (22).

In Vitro Purification of T and B Cells.—

Preparation of complement-receptor B lymphocytes (CRL) from spleen and lymph nodes: Lymph node cells and spleen cells were radioiodinated and CRL were detected by rosette formation with sheep erythrocytes coated with rabbit anti-Forssman antibodies and mouse complement (26). The depletion of CRL was performed by differential flotation of free and rosette-forming lymphocytes in a BSA gradient (27). After ultracentrifugation, the supernatant (CRL depleted) was examined for remaining CRL. The pellet constituted the CRL-enriched fraction. Each fraction was assayed for the presence of radiolabeled Ig. In controls, uncoated erythrocytes were substituted for sensitized sheep red cells.

Separation of θ^+-(T) and θ^--cells from the lymph nodes: Suspensions of 2–3 × 10^8 iodinated cells were treated with either anti-θ or normal mouse serum and guinea pig complement (C) as previously described (26). Cells were then layered on BSA and centrifuged (27). Pellets consisted of cells killed by anti-θ serum or control serum; their supernatants contained enriched populations of B cells (anti-θ treated) or mixed T and B cells (control), respectively. Cells were washed, lysed, precipitated, and cell surface Ig characterized on SDS–acrylamide gels.

In Vivo Purification of T Cells.—

Preparation of cortisone-resistant thymus cells: 25 6-wk old BALB/c mice were injected intravenously with 2.5 mg of hydrocortisone acetate in 0.85% NaCl. 2 days later, residual thymus tissue containing 5% of the normal number of lymphocytes was removed under × 4 magnification, and parathymic lymph nodes were discarded. To confirm the absence of B lymph node cells in the preparation, small aliquots of cells were assayed for the presence of θ-antigen by cytotoxic tests (26) using anti-θC3H and C. All cell preparations were > 95% θ^+.

Preparation of activated T cells: 100 adult BALB/c mice were irradiated with 700 rads and 50 were injected intravenously with 5×10^7 AKR thymus cells per recipient in 0.85% NaCl 1–2 hr later. Control groups consisted of either uninjected-irradiated or uninjected-normal animals. 5 days after treatment, animals were killed and spleens removed. 8.5×10^7 lymphocytes were iodinated, washed, lysed, and specific and control precipitates prepared. Washed

TABLE I

Recovery of Acid and Immunoprecipitable Radioactivity from Thymocyte Lysates

Lysing agent	Acid-precipitable radioactivity	% of acid-precipitable radioactivity			% of acid-precipitable radioactivity that is specifically precipitable*	
		Supernatant	Nuclear pellet	1 M NaCl extract of nuclear pellet	Supernatant	Extract of nucluar pellet
	cpm					
0.5% NP-40	6,682,850	89	11	0.1	0	0.05
5.0% NP-40	5,484,600	96	4	0.3	0	0.04
1.0% Triton X	5,805,850	84	16	0.1	0	0.05
0.9 M urea + 0.2 M 2-ME,‡ pH 4.0 (33)	3,858,900	20	80	1.3	0.02	0.09
H_2O	4,557,410	15	85	3.4	0.02	0.09

* $\frac{\text{Specific precipitate (anti-}\mu, \gamma, \lambda, \kappa) - \text{control precipitate}}{\text{Total acid precipitate}} \times 100.$

‡ 2-ME, 2-mercaptoethanol.

precipitates were processed for acrylamide gel electrophoresis in the usual manner. In addition, pooled cell suspensions were assayed for donor (anti-θC3H) and recipient (anti-θAKR) T cells by cytotoxic tests using appropriate anti-θ, or normal mouse sera and guinea pig C′.

RESULTS

Thymocytes and Bone Marrow Cells.—Our surface radiolabeling technique involves the evaluation of molecules soluble in NP-40; cell surface Ig, not soluble in detergent, is lost in the nuclear pellet. Because of the possibility that T cells might have detergent-insoluble Ig, we evaluated several different conditions for lysing thymocytes with regard to loss of radiolabeled protein, including Ig, in the nuclear pellet. In addition, the radioactivity pelleting with the nuclei was extracted with 1 M NaCl, specifically precipitated, and analyzed by acrylamide gel electrophoresis.

As seen in Table I, the least radioactivity was lost by detergent lysis. To

TABLE II

Cell Surface and Intracellular Ig Precipitable from Lysates of Radiolabeled Cells

Cell source	Radiolabel	Precipitating antiserum	% of acid-precipitable radioactivity that was specifically precipitable*
Thymus			
Total	^{125}I cell surface	Anti-γ, κ, λ	0.5, 0.1, 0.1, 0‡
		Anti-μ, λ	0.1, 0.15, 0.05, 0.1
		Anti-κ (Dutton)	0, 0.1
	^{125}I cell lysate	Anti-γ, κ, λ	0.05
		Anti-μ, λ	0.2
		Anti-κ (Dutton)	0.1
	tyrosine-^{3}H–labeled lysate	Anti-γ, κ, λ	0.2
		Anti-μ, λ	0.8
A + B§ subpopulation	^{125}I cell surface	Anti-μ, γ, κ, λ	0.1, 0.1
C + D§ subpopulation	^{125}I cell surface	Anti-μ, γ, κ, λ	0.5, 0.05
P§ subpopulation	^{125}I cell surface	Anti-μ, γ, κ, λ	0.15, 0.1
θ^{+}–Lymphoma cells	^{125}I cell surface	Anti-μ, γ, κ, λ	0, 0, 0.1
Bone Marrow			
Total	^{125}I cell surface	Anti-μ, γ, κ, λ	1.8, 1.6
		Anti-μ	1.0, 1.1
		Anti-γ (Grey)	0.9, 0.7
	tyrosine-^{3}H–labeled lysate	Anti-μ, γ, κ, λ	3.5
A + B§ subpopulations	^{125}I cell surface	Anti-μ, γ, κ, λ	0.2, 0.3
C + D§ subpopulations	^{125}I cell surface	Anti-μ, γ, κ, λ	1.4, 1.3

* $\frac{\text{Specific precipitate} - \text{control precipitate}}{\text{Total acid precipitate}} \times 100$.

‡ Each number represents an individual experiment. When control values were greater than experimental values, the difference is expressed as 0.

§ A + B = top of BSA gradient, C + D = bottom of BSA gradient, P = pellet from BSA gradient (23).

determine how much of this was "free" Na-^{125}I bound to nuclear material, duplicate aliquots of cells were radiolabeled with and without lactoperoxidase. Cells were lysed in NP-40, the nuclear pellet and cytoplasm prepared, and acid-precipitable radioactivity determined. 37% of the radioactivity in the nuclear

pellet appeared to be accounted for by ^{125}I bound to nuclear material. The remainder probably consisted of radiolabeled surface proteins made insoluble by the labeling procedure. 2–9% of this insoluble radiolabeled material could be extracted with 1 M NaCl and 3–8% of the extract was specifically precipitable. None of these reduced and alkylated precipitates gave identifiable H and L chain peaks after electrophoresis on acrylamide gels.

When lysates of ^{125}I-labeled thymus cells were allowed to stand for even short periods of time, radiolabeled material, containing up to 1% of the total acid-precipitable radioactivity, precipitated spontaneously (without the addition of antiserum). This material consisted primarily of a protein, molecular weight approximately 55,000 daltons. The possibility was considered that this material represents insoluble heavy chain. Therefore, tryptic digestion and cofinger-

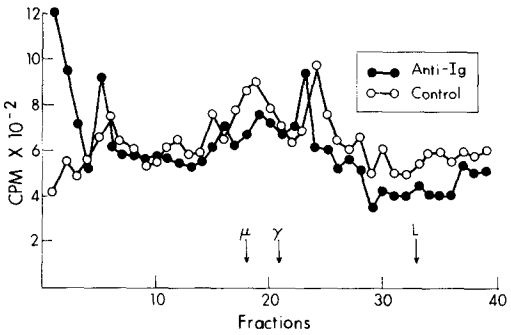

FIG. 1. Electrophoresis on SDS–acrylamide gels of anti-Ig and control precipitates obtained from lysates of surface-radiolabeled thymocytes. Precipitates were reduced and alkylated before electrophoresis. The positions of μ-,γ-, and L chains electrophoresed on companion gels are noted.

printing with mouse IgM and IgG were performed; the results failed to show structural similarity.[2] Therefore, before immunoprecipitation, this material was removed from the lysate by standing for 18 hr at 4°C or by standing in the presence of an unrelated antigen-antibody system ("cleared"lysates).

As seen in Table II, immunoprecipitation with anti-Ig of cleared lysates from either (*a*) total thymus cell population (10^8–10^9 cells), (*b*) subpopulations of small or large thymocytes prepared on BSA gradients, or from (*c*) θ^+–lymphoma cells, resulted in recoveries of 0.05–0.5% of the acid-precipitable radioactivity (5–40 $\times$ 10^6 cpm). The ratio of radoactivity of anti-Ig to control precipitates ranged from 0.7 to 1.2. When reduced and alkylated precipitates were electrophoresed on SDS–acrylamide gels, no H and L chain peaks were seen above the control levels (Fig. 1). In contrast, using 10^7–10^8 bone marrow cells, iodinated in the identical manner, approximately 2% of the acid-precipitable radioactivity was immunoprecipitated with antisera against μ-, γ-, κ-, and λ-chains. After reduction and alkylation of such precipitates (and electrophoresis on

[2] Schenkein, I., E. S. Vitetta, and J. W. Uhr. Unpublished result.

acrylamide gels), μ-, γ-, and L chain peaks were obtained (Fig. 2). Control precipitates of the bone marrow lysates contained less than 0.5% of the acid-precipitable radioactivity and gave no discernible peaks on the gels. To confirm that both μ- and γ-peaks were present, monospecific antisera were employed for immunoprecipitation. The results were entirely consistent with the

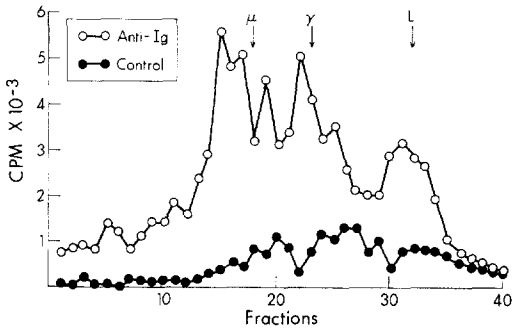

FIG. 2. Electrophoresis on SDS–acrylamide gels of anti-Ig and control precipitates obtained from lysates of surface-radiolabeled bone marrow cells. See Fig. 1.

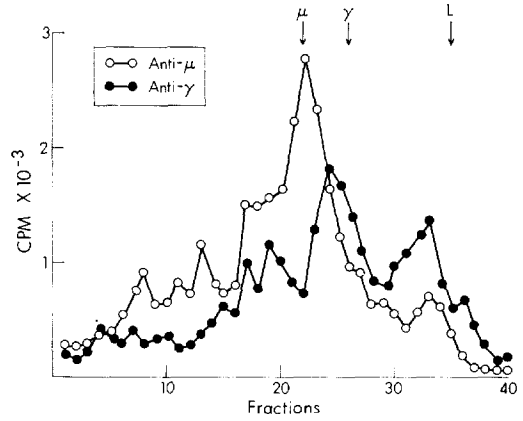

FIG. 3. Electrophoresis of anti-μ and anti-γ precipitates obtained from lysates of surface radiolabeled bone marrow cells. See Fig. 1.

previous findings, i.e., anti-μ precipitated 1% and anti-γ 0.8% of the acid-precipitable radioactivity, and each precipitate gave peaks at the expected positions after acrylamide gel electrophoresis (Fig. 3). Virtually all of the cell surface Ig was associated with the small lymphocytes from the bottom layers of the BSA gradients (Table II). Cells from the top layer containing the majority of plasma cells and macrophages gave no detectable peaks.

Several explanations for a failure to label Ig on thymus cells were considered: (*a*) Cell surface Ig might be removed by washing before iodination. To test this

possibility, washed thymocytes were incubated for 120 min in MEM with 10% fetal calf serum, in order to reconstitute surface molecules. Cells were then pelleted and iodinated without further washing. No Ig was recovered from the surfaces of such cells.

(*b*) Ig on the cell surface could be "hidden" and unavailable to lactoperoxidase. Two types of experiments were performed to investigate this possibility. In the first, lysates were prepared by detergent extraction, dialyzed, and the intracellular proteins labeled with ^{125}I by either enzymatic iodination or the chloramine-T method. Less than 0.5% of the 1–2 $\times$ 10^8 acid-precipitable cpm were obtained in the specific precipitate, and no H and L chain peaks were seen after electrophoresis of reduced and alkylated precipitates on acrylamide gels. In addition, thymocytes were labeled for 1–3 hr in vitro with tyrosine-^3H, the lysates prepared, and specifically precipitated. Of the 2.8 $\times$ 10^7 acid-precipitable cpm obtained in the lysate, less than 0.3% of the radioactivity was specifically immunoprecipitated. Again, no H and L chain peaks were obtained after electrophoresis of reduced and alkylated precipitates on acrylamide gels.

(*c*) Is the cell concentration necessary for detecting Ig on T cells different from that required to detect Ig on B cells? Preliminary experiments had shown that the ability to label Ig on B cells was a function of cell concentration (10^7–10^8/ml is optimal). Thymocytes were therefore radiolabeled at concentrations ranging from 10^5 to 10^9 cells/ml. Lysates were then precipitated and dissolved precipitates examined by acrylamide gel electrophoresis. No cell surface Ig was demonstrated in any of the cell concentrations used.

Immunocompetent (Cortisone-Resistant) Thymocytes.—After cortisone treatment in vivo, approximately 5% of thymocytes remained, which is consistent with previous reports (28, 29). Such cells, which have been characterized as the immunocompetent thymocytes (28, 29), were found to be >95% θ^+ by cytotoxic tests and therefore were considered to be essentially free of B lymph node cells. After radioiodination and preparation of cell lysates, 0.1–0.5% of the acid-precipitable radioactivity was recovered in immunoprecipitates with anti-Ig. These precipitates had no discernible H or L chain peaks when reduced and alkylated precipitates were electrophoresed on SDS–acrylamide gels.

T and B Cells Purified In Vitro.—It was possible that T cells might have more antigen-specific receptors (and hence cell surface Ig) than thymocytes. Therefore preparations of T cells were examined for cell surface Ig.

θ^+–T cells: When iodinated lymph node cells were treated with anti-θ serum and C, approximately 60% of the cells were lysed and their ghosts pelleted by centrifugation through a BSA gradient; in the control, containing normal serum and C, there were approximately 10% dead pelletable cells. A comparison of the two pellets indicated that the θ^+-cells, representing 60% of the original population, contained the same amount of labeled cell surface Ig as the dead cells (10%) in the control (Fig. 4). It was concluded, therefore, that dead B cells which were pelleted in both tubes contained all of the cell surface Ig and that the T cells were negative.

CRL⁻ T cells: In these experiments, splenic lymphocytes which formed rosettes with EAC were separated by centrifugation over BSA into a pellet of CRL⁺ B cells and a supernatant containing the CRL⁻ T cells (27). The two populations were then examined for the presence of θ-antigen (by cytotoxic tests) and cell surface Ig (by direct radioiodination). In one representative experiment, (Table III) in which the CRL⁻ cell population contained 86.2% θ⁺-cells, the CRL⁺ B cells contained 4–5-fold as much surface Ig. In control

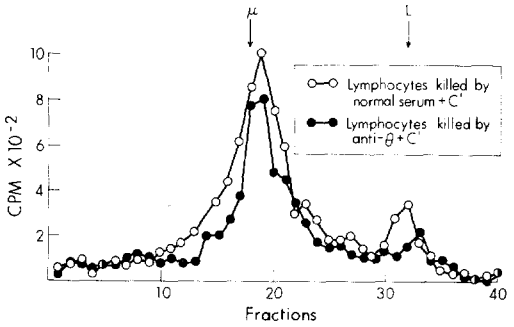

FIG. 4. Electrophoresis of anti-Ig precipitates obtained from lysates of surface-radiolabeled lymph node cells. Prelabeled cells were treated with either anti-θ serum or normal serum + C and dead cells isolated after centrifugation on BSA gradients. See Fig. 1.

TABLE III

Cell Surface Ig in CRL and Non-CRL from Mouse Spleen

Treatment of* cell preparation*	Characteristics of cells remaining in the supernatant		Ratio of cpm in cell surface Ig in pellet/ supernatant
	% CRL	% θ-positive	
EAC (CRL-depleted)	3.7	86.2	4.4
E (control, nondepleted)	18.2	48.4	0.8

* Cells were incubated with appropriate erythrocyte suspension to allow formation of EAC-CRL rosettes and submitted to differential flotation by centrifugation in a BSA gradient. The supernatant contains the free lymphocytes and the pellet, rosettes, and free erythrocytes. The controls were incubated with E and treated similarly.

experiments in which rosettes were not formed before centrifugation, both θ⁺- and Ig⁺ cells were evenly distributed between the pellet and supernatant in the gradient. It was concluded that CRL⁻ T cells did not have detectable Ig.

Activated T Cells.—Spleens of mice undergoing graft-*versus*-host reactions (AKR thymocytes injected into irradiated BALB/c mice) were harvested 5 days after transfer. The spleens contained 74% donor T cells and 13% recipient T cells as determined by cytotoxic testing with anti-θC3H and anti-θAKR antisera. Equal numbers of splenocytes from normal, irradiated, and reconstituted mice were iodinated. It was found that differences between control and specific

precipitates of the lysates were 4.0, 0, and 0.7%, respectively. Electrophoresis of such precipitates on SDS–acrylamide gels (Fig. 5) indicated cell surface Ig only on the splenocytes from the normal mice.

Mixture of Thymocytes and B Cells.—What is the minimum number of Ig molecules that can be detected in a lymphoid cell population using the method

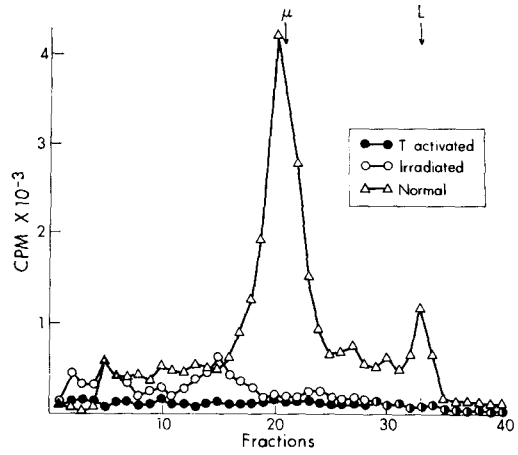

FIG. 5. Electrophoresis of anti-Ig precipitates obtained from lysates of surface-radiolabeled T-activated spleen cells and control cells (from normal and irradiated mice). See Fig. 1.

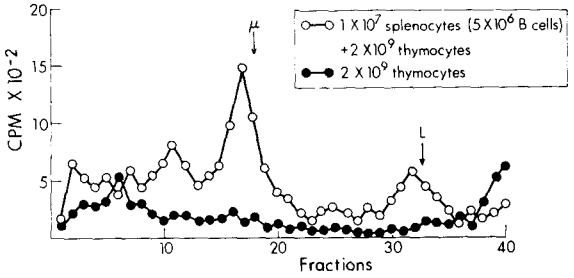

FIG. 6. Electrophoresis of anti-Ig precipitates obtained from lysates of surface-radiolabeled thymocytes and a mixture of thymocytes and splenocytes. See Fig. 1.

of cell surface radioiodination? Preliminary experiments had shown that μ- and L chain peaks could be detected on acrylamide gels after electrophoresis of reduced and alkylated precipitates from as few as 10^7 iodinated spleen cells. Accordingly, 10^7 spleen cells were mixed with 2×10^9 thymocytes (from 30 mice) and the mixture was radioiodinated. The control consisted of 2×10^9 thymocytes alone. In these experiments μ- and L chain peaks were detected in reduced and alkylated precipitates from the cell mixture, but not in the thymocytes alone (Fig. 6). Assuming that 50% of the spleen cells are B cells (16) and

that the B cell surface has 1×10^5 molecules of Ig (6, 30, 31), the radioiodination method can detect 5×10^{11} molecules of surface Ig. If surface Ig molecules are evenly distributed among the thymocytes and the density of Ig per cell does not affect the iodination reaction, there are fewer than 250 molecules of Ig per cell. If only cortisone-resistant thymocytes (5%) contain Ig, there are less than 5000 molecules of Ig per cell.

DISCUSSION

The significant finding to emerge from these studies is that immunoglobulin (Ig) has not been detected on T cells from a variety of sources: thymocytes, cortisone-resistant thymocytes, θ^+– lymph node cells from normal mice, CRL^- spleen cells from normal mice, θ^+–spleen cells sustaining a graft-vs.-host reaction, and an established line of θ^+-mouse lymphoma cells. In contrast, bone marrow cells or B cells in the spleen (CRL^+ or θ^--cells) have easily detectable Ig. In the case of spleen cells, the predominant Ig is IgM in its monomeric form, whereas bone marrow cells also have IgG on their surface.

We considered several possibilities other than the absence of Ig to explain the negative results with T cells: (*a*) Surface Ig on T cells might be insoluble in detergent. Investigation of radiolabeled material pelleting with the nuclei and material spontaneously precipitating from the detergent-treated lysate failed to reveal evidence of H or L chain. (*b*) Surface Ig on T cells might be hidden and thus escape radioiodination. However, radiolabeling of extracts of thymocytes either enzymatically or by the chloramine-T method and labeling of thymocytes with tyrosine-^{3}H failed to reveal Ig. (*c*) The conditions of iodination might be suboptimal for labeling Ig. The concentration of cells is an important variable for the labeling of Ig on B cells; 10^7–10^8 cells/ml is optimal. However, no cell surface Ig was detected on thymocytes radioiodinated in concentrations ranging from 10^5 to 10^9/ml. (*d*) Ig might be "loosely" bound to the surface of the T cell and thus be removed by washing. However, iodination of unwashed cells or washed cells that had been incubated in MEM with fetal calf serum in order to reconstitute surface molecules also gave negative results. (*e*) The specificity of the antiserum used in the above experiments might not detect the Ig chains on T cells. In the majority of experiments, a pooled antiserum with specificities to μ, γ, κ, and λ was used. In addition, an anti-κ serum that had been successfully used to block T cell functions initiated by antigen in vitro (25) was also employed. This antiserum also gave negative results. (*f*) The surface radioiodination technique might be less sensitive than other techniques that have been used to detect Ig on T cells. However, by mixing spleen cells and thymocytes, we demonstrated that a contamination of one B cell per 400 thymocytes (i.e. $5 \times 10^6/2 \times 10^9$) could be detected. If one assumes 10^5 molecules of Ig per B cell (6, 30, 31), no effect of the density of Ig/cell on the iodination reaction, and equal numbers of receptors/cell, we could have detected 250 molecules of Ig/cell.

The simplest but not unique interpretation of our findings is that conventional Ig is not the antigen-specific receptor on T cells. Our data is consistent with many previous reports which failed to detect Ig on T cells using other techniques such as immunofluorescence (5–7, 16) and rosette formation with θ^+-lymphocytes (32). It is difficult to reconcile these negative results with those of others claiming that binding of specific antigen by T cells can be blocked by anti-κ or anti-μ (9, 15) and that Ig can be demonstrated on the surface of thymocytes using direct (17) or sandwich (19) binding of anti-Ig to cells. One possibility is that the antisera that give positive results have additional specificities against cell membrane components. This has been suggested by Takahashi et al. (32) who demonstrated that cytotoxicity of anti-μ and anti-κ sera for thymocytes could not be adsorbed with serum but was removed by thymocytes or lymph node cells. Studies by Lesley et al. (25) indicate that those anti-κ sera which blocked T cell functions in the presence of C (presumably by cytotoxicity), did not kill B cells, suggesting that anti-cellular antibody might be present along with a low level of anti-κ antibody. Nossal et al. (19) have shown that anti-thymocyte antibody is far more effective than anti-Ig antibody in binding to T cells; hence, contamination with small amounts of such cellular antibody might be sufficient to cause the antiserum in question to bind to and/or kill T cells. In fact they also demonstrated the binding of anti-Ig sera to erythrocytes. Anti-cellular antibodies could be "natural" ones or might result from immunization, particularly if complete Freund's adjuvant is employed.

A second possibility is contamination of thymocyte suspensions with B cells from parathymic nodes or blood vessels. This is particularly important when large numbers of thymocytes are employed and only several hundred Ig molecules are found per cell.

Our present data, as well as studies by others, indicate a maximum cell concentration of Ig that is so low that its function as a receptor becomes questionable. Moreover, there appears to be no increase of this Ig on activated T cells (19). We therefore suggest that the nature of the receptor remain an open question. Possible candidates include portions of Ig (e.g. V regions of L or H chains) that may have lost antigenicity (9), "IgX," or an antigen-recognition unit which is unrelated to Ig.

SUMMARY

Thymocytes, bone marrow cells, and their derived T and B cell populations were examined for the presence of Ig by the cell surface radioiodination technique. Both IgM and IgG were identified on bone marrow cells. Thymocytes and T cells had no detectable cell surface Ig. Radiolabeling of mixtures of B cells and thymocytes suggest that the method may detect as little as 250 molecules of Ig per cell. Based on these findings, we suggest that the T cell receptor for antigen is not a conventional tetrameric Ig.

Note Added in Proof.—Since the submission of this manuscript, two studies by Marchalonis *et al.* (1972. *Nat. New Biol.* **235:**240 and 1972. *J. Exp. Med.* **135:**956) have described results which are in direct contradiction to our own. Using the cell surface radioiodination technique, they claim that there are *equal* numbers of Ig molecules on thymocytes, T cells, and B cells. Our studies, although not excluding minute amounts of Ig on T cells, offer compelling evidence against large amounts of Ig. We suggest that the differences are due to B cell contamination in their thymocyte preparations and inadequate quantification, e.g., lack of suitable controls for non-specific immunoprecipitation.

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