THYMUS-DERIVED (T) CELL IMMUNOGLOBULINS

PRESENCE OF A RECEPTOR SITE FOR IgG AND ABSENCE OF LARGE AMOUNTS OF "BURIED" IG DETERMINANTS ON T CELLS*

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The presence of immunoglobulin receptors on thymus-derived (T) cells has been extremely difficult to document and is still a matter of considerable controversy. Most of the demonstrations have involved inhibition of known T cell functions with anti-Ig antisera (1–3). With one notable exception, experiments concerned with the direct demonstration of immunoglobulin on T cells all indicate that if there is any immunoglobulin associated with T cells, it is extremely small, i.e., two to three orders of magnitude lower than that present on bone marrow-derived (B) cells (in the range of a few hundred molecules per cell) (4, 5). In contrast to this, Marchalonis et al. have claimed that there are similar amounts of Ig associated with T and B cells (6). The purpose of the present paper was twofold: (*a*) to investigate the possibility that T cells had relatively large amounts of Ig that went previously undetected due to being "buried" in the membrane, and (*b*) to describe the capacity of certain theta-positive tumors to passively adsorb significant quantities of IgG onto their surfaces.

Materials and Methods

Source of Cells.—Thymic and splenic lymphocytes were obtained from normal CF_1, BDF_1, and AKR mice. Spontaneous thymomas that arose in AKR mice were maintained by serial passage. The DBA/2 lymphoma L-5178-Y (7) was maintained by serial passage either in vivo in ascitic form or in vitro in Dulbecco's modified Eagle's medium supplemented with asparagine, folic acid, 5% trypticase soy broth, and 10% fetal calf serum.

Anti-Immunoglobulin-Coated Plastic Bead Columns.—In order to deplete populations of surface Ig-positive cells as measured by the immunofluorescence assay, lymphoid cells were passed through Degalan bead columns which were previously coated with the IgG fraction of rabbit anti-mouse Ig antisera. The detailed procedure employed to coat the columns and elute the lymphoid cells is described elsewhere (8).

Quantitation of Immunoglobulins Associated with Mouse Lymphocytes.—Quantitation of immunoglobulins was performed on viable and disrupted cells. Disruption was accomplished by (*a*) repeated freeze-thawing of lymphocytes, (*b*) treatment with the nonionic detergent Nonidet P-40 (NP40; Shell Oil Co., New York) at a final concentration of 0.5% for 10 min at room temperature, and (*c*) treatment with 9 M urea-1.5 M acetic acid for 2 hr at 37°C and 16 hr at room temperature or at 37°C for 30 min (6). The supernatants of the treated cells were

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exhaustively dialyzed against phosphate-buffered saline. The quantitation of immunoglobulins on the intact cells and the disrupted cells was performed as previously described (4).

Lactoperoxidase-Catalyzed Labeling of Lymphocytes and Immunoprecipitation.—The iodination of lymphocytes was performed as described by Vitteta et al. (5). Cell lysates were prepared by treatment of the washed cells with 0.5% NP40 or with 9 M urea-1.5 M acetic acid as detailed above. Specific precipitation of radioiodinated Ig from the cell lysates was done by addition of 30 μg of carrier mouse Ig to 0.5 ml of lysate followed by the appropriate quantity of rabbit anti-mouse Ig. Control precipitations were made with chicken IgG or keyhole limpet hemocyanin and their respective antisera. The immune precipitates were dissolved in 0.5 M tris(hydroxymethyl)aminomethane buffer, pH 8.4, containing 1% sodium dodecyl sulfate (SDS) and 8 M urea, reduced and alkylated, and electrophoresed on 5% polyacrylamide gels which contained 0.1% SDS, 0.5 M urea.

TABLE I

Effect of Different Methods of Cell Disruption on Ig Quantitation

	Intact		Method of disruption					
			NP40		Freeze-thaw		Urea-acetic acid	
	κ	μ	κ	μ	κ	μ	κ	μ
			ng/10⁶ cells					
Spleen	7.5	5.6	22.5	13.1	15.6	9.4	8.8	1.9
Thymus	0.6	0.3	1.6	1.2	1.2	0.9	1.0	0.3
Spleen after anti-Ig column	0.7				2.3			
Thymus after anti-Ig column	0.2				0.8			

RESULTS

Quantitation of T Cell Immunoglobulins before and after Cell Disruption.—Previous studies indicated that extremely small amounts of Ig determinants could be detected on the surface of viable T cells: 0.04–0.09 ng/10^6 thymocytes and 0.4–0.6 ng/10^6 splenic or lymph node T cells compared with 30–90 ng/10^6 B lymphocytes (4). One possible explanation for this two to three order of magnitude difference is that although comparable amounts of Ig are present on B and T cells, most of the constant region antigenic determinants are buried in the membrane of T cells and are therefore not available to react with anti-Ig antisera (6). To study this possibility, Ig quantitation was performed on lymphoid cells before and after disruption of cell membranes by either freeze-thawing, treatment with NP40, or treatment with 9 M urea-1.5 M acetic acid. Lymphoid cells studied were (*a*) thymic lymphocytes, (*b*) splenic lymphocytes, and (*c*) splenic and thymic lymphocytes eluted from a plastic bead column coated with rabbit anti-mouse Ig antisera. The thymic lymphocytes and cells eluted from anti-Ig columns were used as T cell populations. They contained less than 1% of cells with easily detectable Ig and over 90% of cells had detectable theta antigen by immunofluorescence. Normal spleen cells were used as a source of B cells. Approximately 40% of these cells had readily detectable Ig. As seen in Table I, regardless of the source, there was a two- to fourfold increase in measurable Ig when disrupted lymphocytes were

compared with intact cells. Different methods of disruption yielded different results in that the greatest amount of Ig was detected after NP40 lysis and the least after urea-acetic acid treatment. Whether the observed increase was due to release of cytoplasmic Ig or uncovering of membrane-bound Ig is not known, but whatever the mechanism, it is clear that populations containing predominantly T cells and those containing a large number of B cells behave

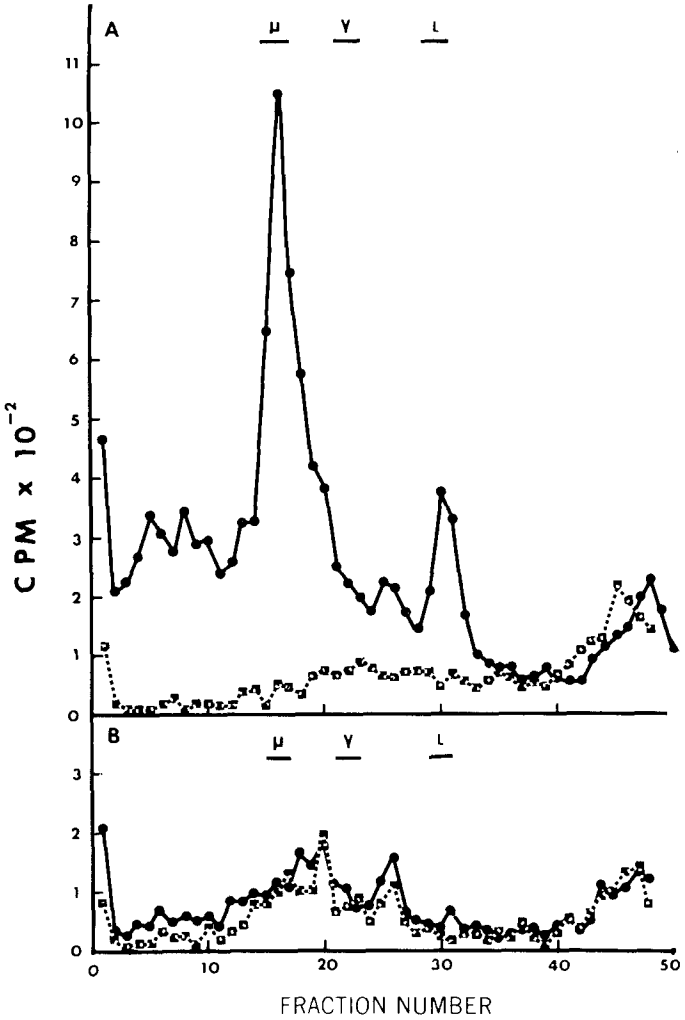

FIG. 1. SDS gel electrophoresis of anti-Ig and control precipitates obtained from NP40 lysates of radioiodinated splenic lymphocytes (*A*) and thymic lymphocytes (*B*). Precipitates were reduced and alkylated before electrophoresis. The positions of migration of μ, γ, and L chains are indicated. ●—●, anti-Ig; ■--■, control.

similarly, and that there is no disproportionate increase in the measurable T cell Ig after disruption of cell membranes.

Surface Immunoglobulins Detected by Lactoperoxidase-Catalyzed Iodination of Thymus and Spleen Cells.—Since by use of the quantitative radioimmunoassay we were unable to demonstrate large amounts of T cell Ig as was previously reported (6), it was decided that it was important to try and demonstrate this by the lactoperoxidase method. The amount of trichloroacetic acid (TCA)-precipitable radioactivity from cell lysates was generally in the range of 15–30%. From spleen cell lysates approximately 2–4% of the TCA-precipitable radioactivity was precipitated with antisera to mouse Ig, whereas 0.4–1% was precipitated with control antisera. In contrast, with thymus cell lysates only 0.3–0.8% of the protein-bound counts were precipitated by anti-mouse Ig, whereas in control precipitates, 0.2–0.7% was precipitated. Furthermore, as shown in Fig. 1, SDS polyacrylamide gel electrophoresis of the reduced and alkylated immune precipitates of spleen cell lysates revealed H and

TABLE II

Ig Associated with Theta-Positive Lymphomas

Tumor	Source	κ	$\gamma 2$	μ
			ng/10^6 cells	
T 10 P6	AKR solid tumor	1.44	0.88	0.13
T 1	AKR solid tumor	1.69	1.38	0.38
L-5178-Y	DBA 2 ascites	0.69	—*	—
L-5178-Y	Tissue culture	<0.02	—	—
L-5178-Y	Tissue culture (+ heat-aggregated IgG2)	1.38	—	—

* —, not determined.

L chains, whereas thymus cell immune precipitates were distributed in a similar pattern to the control precipitates without any discernible H and L chain peaks.

Ig Associated with Theta-Positive Lymphomas.—Three theta-positive lymphomas were studied for membrane-bound Ig. Quantitation of κ-light chains and $\gamma 2$ and μ-heavy chains are shown in Table II. Tumors were studied as solid or ascites tumors, and in the case of L-5178-Y, also as a cultured cell line. Considerable Ig was associated with all tumors grown in mice. In particular large amounts of $\gamma 2$ were observed 5–10 times that seen in normal thymus, whereas usually less μ-chain determinants were found compared with normal thymus. These results contrasted sharply to the lack of any detectable Ig on the L-5178-Y cells grown in vitro. The presence of large amounts of IgG and the lack of any detectable Ig on in vitro grown cells suggested that Ig may be passively adsorbed to the surface of these cells. Proof of this came from studying the in vitro cultured L-5178-Y cells before and after incubation with normal mouse serum or heat aggregated IgG2 and then washed six times with Hanks'

balanced salt solution and the surface Ig quantitated. Whereas before incubation with mouse Ig no detectable surface Ig was present, after such incubation these cells contained 1.38 ng/10^6 cells when incubated with heat aggregates of IgG2. An even greater amount of passively adsorbed IgG was observed when cells were incubated in normal mouse serum. This amount of passively adsorbed immunoglobulin was sufficient to account for all the Ig detected on the in vivo grown cells. When these cells were examined by indirect immunofluorescence using a rabbit anti-mouse immunoglobulin conjugate, it was shown that mouse Ig could be detected on these cells by this procedure as well, although a considerable heterogeneity in the degree of fluorescence was observed in these preparations.

DISCUSSION

By three separate methods, it has been impossible to demonstrate large amounts of T cell Ig after cell disruption. The NP40 treatment of cells yielded the highest values of immunoglobulin, whereas urea-acetic acid treatment yielded somewhat lower values. The latter procedure was that used by Marchalonis et al. (6) in their studies which showed relatively large amounts of T cell immunoglobulin. The discrepancy between our data and theirs is at present unexplainable. However, in our hands no T cell Ig could be detected using the lactoperoxidase iodination procedure as well. These results are in accord with those of Vitteta et al. (5). The finding of some theta-positive lymphomas with passively adsorbed IgG on their surface further complicates the T cell Ig situation. It is not known to what extent this Ig receptor is present on normal T cell populations; however, it raises the possibility that such receptors may exist on some T cells, and therefore, any finding of T cell Ig must be evaluated as to whether it is a product of a T cell rather than a result of passive adsorption.

SUMMARY

Quantitation of surface and total cell Ig obtained after lysis by detergent, urea-acid treatment, and freeze-thawing were determined on spleen cells, thymus cells, and spleen cells specifically depleted of B cells. A two- to four-fold increase in measurable Ig was found after cell lysis. All cell populations showed a similar increase in measurable Ig indicating that no discordantly large amounts of buried Ig determinants were associated with the surface of T cells. The lack of appreciable amounts of T cell Ig was confirmed by immunoprecipitation of radioiodinated cells. A theta-positive lymphoma was described which, when grown in culture, lacked detectable surface Ig but contained a receptor site for IgG. This resulted in appreciable amounts of surface IgG being associated with the tumor line when isolated from ascitic fluid of tumor-bearing mice or after preincubation of cultured cells with either heat-aggregated IgG or normal mouse serum.

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