

THE ORIGIN OF CELL SURFACE IMMUNOGLOBULIN OF  
MARROW-DERIVED AND THYMUS-DERIVED  
LYMPHOCYTES OF THE RAT

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The simplest form of the hypothesis postulating immunoglobulin (Ig)<sup>1</sup> as the receptor for antigen on small lymphocytes predicts that Ig should be detectable on the lymphocyte surface and that it should be synthesized by the cell bearing it, not acquired from an external source. These predictions have been confirmed for the category of small lymphocytes which carry relatively large amounts of Ig on their surface (2-6), which at least in the case of the rabbit (2-4) are not acquired passively. These lymphocytes are thymus-independent (B) cells, and function as precursors for antibody synthesis (6-8). The other category of small lymphocytes are thought to have differentiated via the thymus (T cells) and to be involved in cell-mediated immune responses (9). T cells carry much less surface Ig than B cells as detected by anti-Ig binding studies (7, 10): the Ig can only be revealed by the most sensitive methods which employ autoradiography after binding of [<sup>125</sup>I]anti-Ig (10-12). The key question of the origin of this Ig has not been previously answered.

In an attempt to solve this problem we chose to examine rat thoracic duct lymphocytes (TDL) for two reasons. In the first place, analysis of the lymphocytes in rat TDL (12) has previously revealed a heavily labeling population which bound [<sup>125</sup>I]anti-Fab in the range 20,000-150,000 molecules per cell, and a lightly labeling one which bound 200-2,000 molecules. Secondly, the discovery of a light chain allotype in the rat (13-16) provides a genetic marker to identify the origin of the Ig irrespective of its class. In this paper we report the purification and radio-iodination of anti Ig-1a allotype antibody and its use in quantitative studies with rat TDL. The binding of [<sup>125</sup>I]antiallotype was

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<sup>1</sup> *Abbreviations used in this paper:* antiallotype, purified PVG/c F(ab')<sub>2</sub> anti-DA Ig-1a allotype antibody; anti-Fab, purified rabbit F(ab')<sub>2</sub> antirat Fab antibody; BSA, bovine serum albumin, isotonic and neutralized (1); BBSS, buffered balanced salt solution (1); DAB, Dulbecco's A + B solution; FBS, foetal bovine serum; DAB/FBS, DAB containing 2% FBS; GVH, graft-vs.-host; Ig, Immunoglobulin; MLC, mixed lymphocyte culture; SRBC, sheep red blood cells; TDL, thoracic duct lymphocytes.

compared with that of [<sup>125</sup>I]anti-Fab which recognizes rat L chain regardless of allotype.

Two types of experiments were carried out. First, we determined whether or not allelic exclusion occurred in animals heterozygous at the L-chain allotype locus, both in heavily and lightly labeled cells. Positive evidence for such a phenomenon would indicate a noncytophilic origin for the surface Ig (3, 4).

Secondly, we determined whether the surface Ig could exchange with serum Ig in vivo. Donor TDL from parental or F<sub>1</sub> animals were transferred to and recovered from irradiated host rats of the opposite allotype. The extent of binding to the recovered cells of [<sup>125</sup>I]antiallotype compared with [<sup>125</sup>I]anti-Fab antibody indicated whether the surface Ig was of donor or host origin.

Experiments to determine the class of Ig on the lightly labeled cells are also reported.

#### *Materials and Methods*

NaI<sup>125</sup> (14 Ci/mg) was from The Radiochemical Centre, Amersham, England (code IMS 30).

*Rats.*—Inbred male or female rats aged 8–12 wk of the following strains (17) were used: PVG/c (allotype W-1<sup>2</sup> [14] or Ig-1b [15]; Ag-B5): DA (SD-1 or Ig-1a; Ag-B4): AO (Ig-1b; Ag-B2): and the F<sub>1</sub> hybrids (PVG/c × DA), (AO × DA), and (AO × PVG/c).

*Cell Preparation and Transfer.*—TDL from normal rats were collected in heparinized (20 U/ml) DAB at 4–7°C between 12 and 22 h after cannulation for direct binding or up to 36 h for cell transfer experiments. For extended collections viability was >95% if cells were harvested each 12 h and resuspended in DAB/FBS.

In the transfer experiments, recipients were given 1,000 rads <sup>60</sup>Co irradiation at 100 rads/min, after which the thoracic duct was immediately cannulated. They were maintained with 0.9% terramycin in their drinking water. 16–20 h later, 1.5–1.9 × 10<sup>9</sup> donor TDL were infused into the tail vein over a 10-min period; the lymph was then collected immediately over three 10–11 hourly periods. Histocompatibility antigen and B-cell cytotoxicity typing<sup>3</sup> and binding studies were done on the third collection of TDL. GVH activity where appropriate was assayed (18) on the second collection.

After 30–33 h the spleen was removed and teased at 4°C into DAB/FBS with watchmaker's forceps. Cells were obtained for cytotoxicity typing and binding assays after filtering the suspension through a small plug of cotton wool. Before any assay all cells were washed 4 times through 5% BSA/BBSS as previously described (12). The viability of washed TDL was in all cases >95% and of spleen >70% as judged by trypan blue exclusions.

When required, TDL were depleted of cells with much surface Ig by the use of rabbit anti-rat IgG-coated Degalan columns as previously described (12).

*Binding Assays.*—5 × 10<sup>6</sup> washed lymphocytes were incubated with [<sup>125</sup>I]antibody (30–50 μCi/μg) at 10–20 μg/ml in 2% isotonic BSA in BBSS and 0.01 M Na<sub>3</sub>N for 60 min on ice. In all experiments controls with inhibitors added were included. Antiallotype binding was always checked in the presence of DA and PVG/c IgG, thus, providing a specificity control in every experiment. IgG inhibitors were at 100–300 μg/ml and IgM at 30 μg/ml (due to limited amounts of purified IgM). Incubations were done in 1.5-ml plastic tubes, and the cells were

<sup>2</sup> Typed with sera kindly provided by Dr. D. Armerding, Institute of Genetics, Köln, W. Germany.

<sup>3</sup> Howard, J. C., and D. W. Scott. 1974. The identification of sera distinguishing marrow-derived and thymus-derived lymphocytes in the rat thoracic duct. Manuscript in preparation.

washed twice in 3-ml tubes by suspending in 1 ml of 0.5% BSA/BBSS and centrifuging through 1 ml of 5% BSA/BBSS; all steps were at 4°C.

*Quantitation of Binding and Presentation of Results.*—Cells were suspended in 5% BSA/BBSS and smeared for autoradiography by methods previously described (12). In brief, smears were fixed in methanol, dipped in Ilford G5 Nuclear Emulsion (Ilford Ltd., Ilford, England), and stained with Harris' hematoxylin stain. Autoradiographic grains were measured photometrically (19) over 100 or 200 cells on each slide, and these readings can be converted to molecules bound per cell knowing the specific activity of antibody, exposure time, and yield of photometric units per disintegration (12). Results are presented as histograms, and the increments in molecules bound per cell vary since the initial assignment of cells was made in terms of photometric readings.

In the case of short-exposure autoradiographs (revealing heavily labeled cells) the distinction between labeled and unlabeled cells was so clear cut (Figs. 1, 2 *a* and *b*) that percentages of cells that were labeled could readily be determined (Table II). However, in the autoradiographs with long exposure it was difficult to distinguish by eye weak but significant labeling from background, and to avoid an arbitrary assignation of "significant" labeling, the complete distributions are presented for cells with and without inhibitor. On those smears with positively labeled cells, readings from background areas adjacent to cells were also made; but as the values obtained were the same as or less than those for cells incubated with antibody plus inhibitor the data are not shown. In some figures the anti-Fab binding data have been normalized by a factor of 0.55 to allow direct comparison with antiallotype binding. The rationale behind this is discussed under Results, section 2.

*Preparation of  $^{125}\text{I}$ -labeled Purified Antibodies.*—The methods of purification and  $^{125}\text{I}$  labeling of antibodies were as previously described (12, 20). Anti-Ig-1a allotype antiserum was raised in PVG/c rats against DA IgG according to the method of Armerding (14). The antibodies were purified by absorption to Sepharose 4B-DA IgG and elution with 1 M propionic acid; 5.1 mg of antibody was obtained from 68 ml of antiserum. Electrophoresis on SDS polyacrylamide gels showed the purified material to be largely IgG plus a smaller amount of IgM. 2.2 mg of the antibody were then digested with pepsin and the  $\text{F}(\text{ab}')_2$  fragment obtained after chromatography on a Sephadex G-200 superfine column (Pharmacia Fine Chemicals, Inc., Piscataway, N. J.). 0.54 mg of material was recovered and analysis by SDS polyacrylamide gel electrophoresis showed the major constituent to be  $\text{F}(\text{ab}')_2$  with a small amount of undegraded IgG also present; no IgM was detected.

The antibody was labeled with  $^{125}\text{I}$  at a specific activity of 30–50  $\mu\text{Ci}/\mu\text{g}$  (1.7–2.8 atoms  $^{125}\text{I}$  per molecule of antibody). After labeling, up to 40% of the reagent could bind to SRBC coated with DA anti-SRBC antibody, compared with <1% binding to cells coated with PVG/c anti-SRBC. 96% of this binding was inhibited by preincubation with a 10-fold excess of DA IgG, and PVG/c IgG had no effect. DA Fab in 10-fold excess inhibited the binding to coated SRBC by 96% while with purified DA light chain the figure was 63%. DA Fc and H chain did not inhibit binding provided contaminating Fab and L chain were removed with an anti-Fab-Sepharose 4B immunoabsorbent. The incomplete inhibition with L chain may be because aggregation was a problem in the preparation of rat L chain.

$^{125}\text{I}$ rabbit  $\text{F}(\text{ab}')_2$  anti-rat Fab,  $\text{F}(\text{ab}')_2$  antirat  $\text{IgG}_{2\text{a}+2\text{b}}$ , and  $\text{F}(\text{ab}')_2$  antirat IgM were the same reagents as used previously (12), as was the purified rat IgM (20) used to check the specificity of anti-IgM binding.

## RESULTS

### 1. Cell Populations Examined.—

a. *Normal TDL.* After binding with  $^{125}\text{I}$  antibody, heavily labeled cells in normal unfractionated TDL were detected by autoradiography on smears with  $\frac{1}{2}$ –2-day exposures. Lightly labeled cells were revealed by exposure for 3–5 wk.

In order to lower the density of background grains, cells with much surface Ig were removed before labeling by passing cells through a column of Degalan beads coated with rabbit antirat IgG antibodies. The frequency of heavily labeled cells was reduced from 30-45% (depending on the strain of rat) to 1-2%, while the recovery of lightly labeled cells was high (12). Cytotoxic testing with a heterologous anti-B cell serum kindly done by Dr. J. C. Howard showed a depletion of B cells from 40 to 4% by column extraction in 1 experiment.

b. *TDL passaged through irradiated recipients:* Normal TDL from DA, (PVG/c × DA)F<sub>1</sub>, or PVG/c rats were transferred intravenously into irradiated recipient DA or PVG/c rats which had been cannulated earlier. As can be deduced from Table I, recovered TDL numbered 13% on average (range 6-20%) of the total infused (B and non-B) and were practically all of donor origin as analyzed by cytotoxic alloantisera. The recovered cells were virtually all of non-B type on the basis of insensitivity to rabbit antirat B cell antiserum and heavy labeling with [<sup>125</sup>I]anti-Fab (Table I). In all cases except one, the frequency of B cells was estimated at less than 5% compared with 25-30% for DA, 40-45% for PVG/c and (PVG/c × DA)F<sub>1</sub> TDL in the original inoculum. This drop in frequency is consistent with the reported slower recirculation of B lymphocytes in the rat (21, 22). It may be calculated that the recovery of non-B cells over the first 30 h was about 21% of the non-B cells infused. Because of the low number of B cells in recovered TDL, binding assays with anti-Fab and antiallo type antibodies were carried out without depletion on Degalan anti-IgG columns.

TABLE I  
*Cells Recovered from Irradiated Recipient Animals*

Transfer	Expt.	TDL				Splenic lymphocytes			
		Recovered* × 10 <sup>-3</sup>	Labeled anti-Fab	B‡	Donor§	Recovered × 10 <sup>-3</sup>	Labeled anti-Fab	B	Donor
DA → PVG/c	1	1.85	0.8	ND	ND	ND	ND	ND	ND
	3	1.70	0.7	ND	98	1.1	33	ND	98
PVG/c → DA	2	1.05	0.5	ND	97	0.8	52	ND	100
F <sub>1</sub> → PVG/c	5	1.85	1.9	0	96	0.65	33	45	97
	6	3.23	2.3	2.9	93	ND	52	52	97
F <sub>1</sub> → DA	4	2.85	5.2	8.5	96	1.74	35	43	95
	5	1.83	4.7	0.4	91	0.85	32	40	100
	6	2.24	4.0	3.6	ND	ND	43	53	ND

Recipient rats were irradiated with 1,000 rads, cannulated, and infused with  $1.6 \times 10^9$  cells 20 h later. TDL were collected in three batches over the first 30 h postinfusion and spleens were taken at the end of this period.

\* Total for 30 h postinfusion.

‡ By cytotoxic testing with anti-B antiserum.

§ By cytotoxic testing with appropriate alloantisera.

ND: not done; F<sub>1</sub>:(PVG/c × DA).

Ford and Atkins (23) in a similar protocol to that described above have shown that TDL emerging from irradiated recipient animals 20–30 h after transfer are small lymphocytes fully competent in GVH reactions against third party Ag-B antigens. We confirmed their finding with PVG/c or DA TDL recovered from irradiated DA and PVG/c animals, respectively. The GVH assays were done in (PVG/c  $\times$  AO) or (DA  $\times$  AO) $F_1$  test animals by the popliteal lymph node assay (18) and passaged cells were as active as normal cells against the AO antigens.

It should be emphasized that the passaged TDL examined here were small lymphocytes emerging much earlier than the activated blasts (commonly referred to as T-TDL in the mouse [24]) derived from antigenic stimulation (23, 24). In the case of  $F_1$  TDL transferred to parental recipients any complications of a GVH reaction were avoided.

*c. Spleen lymphocytes obtained after transfer of TDL to irradiated recipients:* To obtain heavily labeled cells in the transfer experiments, spleens were taken from the same recipients as in part b above 30 h after transfusion. They contained 40–50% B lymphocytes, practically all of donor origin (Table I).

*2. Binding of [ $^{125}$ I]Antiallotype and Anti-Fab Antibodies to Normal TDL.—* In initial experiments the binding of [ $^{125}$ I]antiallotype and [ $^{125}$ I]anti-Fab antibodies to DA TDL was compared. When  $5 \times 10^6$  TDL were incubated with 50  $\mu$ l of each reagent at 10  $\mu$ g/ml, washed, and counted, binding was measured at  $1.18 \times 10^6$  dpm/ $10^7$  cells for [ $^{125}$ I]antiallotype compared with  $2.18 \times 10^6$  dpm/ $10^7$  cells for [ $^{125}$ I]anti-Fab, i.e., a ratio of 0.55:1. The lower labeling may be due to the fact that more sites are available to the multispecific anti-Fab than to the antiallotype antibodies. A concentration of 10  $\mu$ g/ml was routinely used in binding studies as both antibodies were found to saturate at this concentration. The favorable binding with antiallotype as well as anti-Fab antibody was confirmed by short-exposure autoradiography on the labeled cells. With both reagents the same percentage of DA cells was labeled (Table II *a*), the binding per cell being lower on average with antiallotype antibody (Fig. 1). The specificity of the binding of both anti-Fab and antiallotype antibody to heavily labeled cells is shown in Fig. 1. DA IgG completely inhibited the binding of both anti-Fab and antiallotype antibody, while PVG/c IgG could only inhibit the binding of the anti-Fab reagent. Photographs of heavily labeled cells are shown in Fig. 2 *a* and *b*; at the short exposure time the lightly labeled cells had almost no grains at all. The validity of counting heavily labeled cells as a separate category (Table II) is established by Fig. 2 *a* and *b*, and Fig. 1.

Fig. 2 *c* shows the result of extended autoradiographic exposure of the same population of cells as in Fig. 2 *b*. The background was too high to allow quantitation, but it is evident that a second category of cells carrying small amounts of antibody has been revealed. This was confirmed as previously reported (12) when heavily labeled cells were first removed. Both anti-Fab (Fig. 2 *d*) and antiallotype antibodies (Fig. 2 *e*) gave significant labeling of virtually all DA

TDL which had passed through the Degalan anti-IgG column.<sup>4</sup> Fig. 2 *f* shows the extent of labeling if binding of [<sup>125</sup>I]antiallotype was specifically blocked by the addition of DA IgG to the incubation. Inhibition of the binding of [<sup>125</sup>I]anti-Fab antibody gave similar results.

The quantitative aspects of the binding to lightly labeled cells are shown in Fig. 3. The extent of binding of [<sup>125</sup>I]antiallotype was less than for anti-Fab antibody. This difference was similar to that found for heavily labeled cells, for if the anti-Fab data are normalized by multiplying the values by 0.55 (see beginning of this section) the distributions of cells after binding with each

TABLE II  
*Percentage of Lymphocytes Heavily Labeled with Anti-Fab or Antiallotype Antibody*  
(a) Normal TDL

Expt.	DA		PVG/c	(PVG/c × DA)F <sub>1</sub>	
	A	B	C	D	E
Antiallotype	30	29	0	20	28
Anti-Fab	30	27	43	38	43
Ratio	1.0	1.07	0	0.53	0.65

(b) Spleen lymphocytes (transfer experiments)

Expt.	PVG/c → DA	DA → PVG/c	F <sub>1</sub> → DA			F <sub>1</sub> → PVG/c	
	2	3	4	5	6	5	6
Antiallotype	10	34	21	19	29	17	31
Anti-Fab	52	33	34	32	43	33	52
Ratio	0.19	1.03	0.62	0.59	0.67	0.51	0.60

All values were calculated after counting 500 cells on each smear.

reagent are virtually coincident (Fig. 4 A). This transformation was used on the results in subsequent sections where the comparison between antiallotype and anti-Fab binding is vital.

The specificity of binding to lightly labeled cells is also established in Fig. 3 B and C. There was no binding of antiallotype antibody to unfractionated PVG/c TDL (Fig. 3 B), and the binding to DA TDL was inhibited by DA but not PVG/c IgG (Fig. 3 C). As the PVG/c IgG had no effect on binding of antiallotype antibody, pooled data from labeling in the presence and absence of PVG/c IgG are presented in subsequent figures.

The specificity of antibody binding was further established by the observa-

<sup>4</sup> In early experiments the binding of antiallotype antibody to lightly labeled cells from TDL was examined by incubating cells with reagent either before or after separation on Degalan antirat IgG columns. The results were the same in either case, confirming the validity of removing cells before assaying binding.

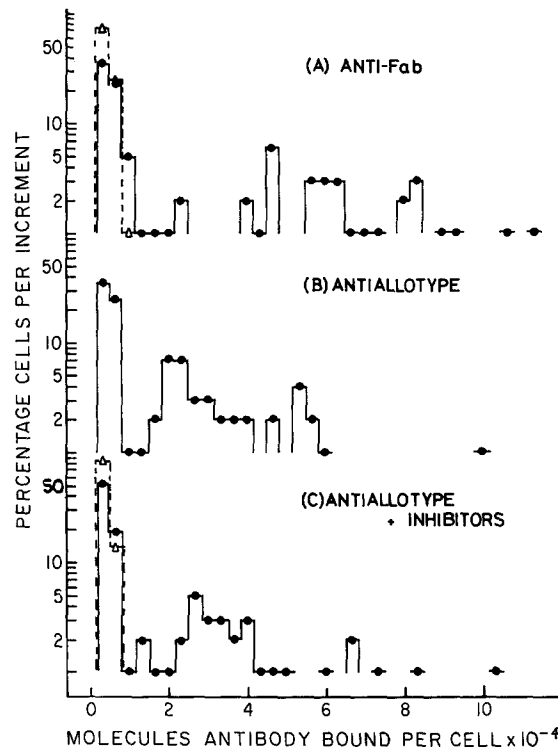


Fig. 1. Heavily-labeled DA TDL. DA TDL were labeled with [ $^{125}$ I]anti-Fab or [ $^{125}$ I]anti-allotype antibody and processed for autoradiography, with an exposure time of 6 h. The distribution of cells in terms of molecules of antibody bound are shown for labeling with: (A)  $\bullet$ , [ $^{125}$ I]anti-Fab;  $\Delta$ , [ $^{125}$ I]anti-Fab + PVGn (or DA) IgG. (B)  $\bullet$ , [ $^{125}$ I]antiallotype. (C)  $\bullet$ , [ $^{125}$ I]antiallotype + PVG/c IgG;  $\Delta$ , [ $^{125}$ I]antiallotype + DA IgG. For each distribution 100 grains over 100 cells were measured photometrically. Note (i) weaker binding of antiallotype (compare A and B) (ii) specificity of antiallotype (C).

tion that the small number of red blood cells present were not labeled above background under any conditions.

3. *Binding to (PVG/c  $\times$  DA) $F_1$  TDL.*—In order to look for evidence of allelic exclusion, the binding of [ $^{125}$ I]antiallotype antibody to  $F_1$  TDL heterozygous at the Ig-1 locus was next determined and compared with labeling by [ $^{125}$ I]anti-Fab. The results for heavily labeled cells are shown in Table II *a*; 20–28% of unfractionated TDL were so labeled with antiallotype compared with 38–43% with anti-Fab reagent. This clearly shows that the Ig-1a Ig was not expressed at the surface of about 40% of the heavily labeled cells.

The results for lightly labeled cells are shown in Fig. 4 B which compares transformed anti-Fab data with antiallotype binding. If allelic exclusion occurred there should be a bimodal distribution of antiallotype binding with

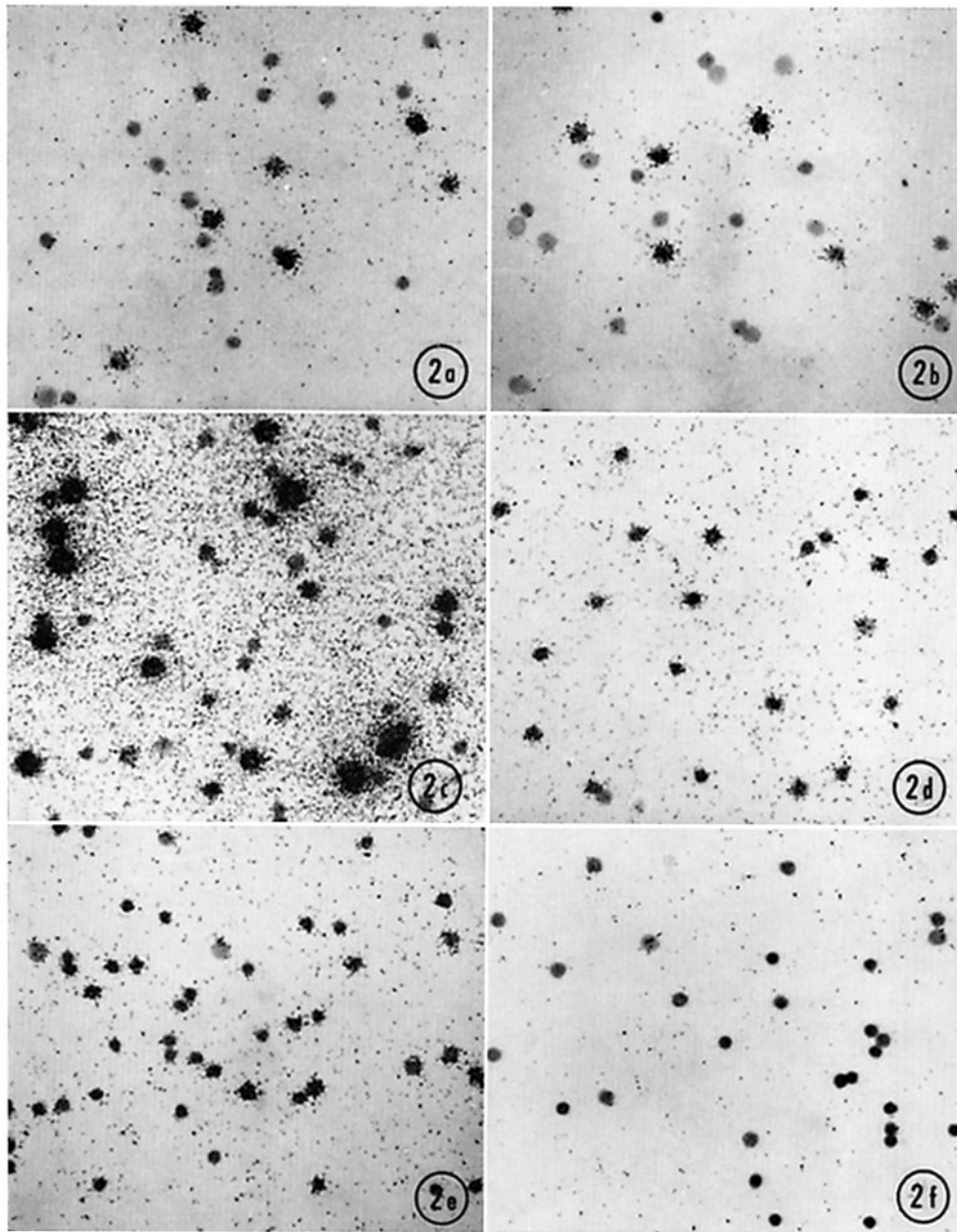


Fig. 2. Photographs of heavily- and lightly-labeled DA TDL. Unfractionated TDL labeled with: (a) [ $^{125}\text{I}$ ] anti-Fab and (b) [ $^{125}\text{I}$ ] antiallotype with 18 h exposure; (c)  $^{125}\text{I}$  antiallotype, 22 days exposure. TDL passed through Degalan antirat IgG columns and then labeled with: (d) [ $^{125}\text{I}$ ]anti-Fab, (e) [ $^{125}\text{I}$ ]antiallotype and (f) [ $^{125}\text{I}$ ]antiallotype + DA IgG; all with 22 days exposure. Note (i) Heavy labeling cells with each reagent (2a and b) (ii) Abolition of background grains at long exposures by removal of heavy labeling cells (compare 2c and e). (iii) Low labeling in presence of inhibitor (2f). (iv) Lightly labeling cells (2d and e). Magnification:  $\times 230$ .



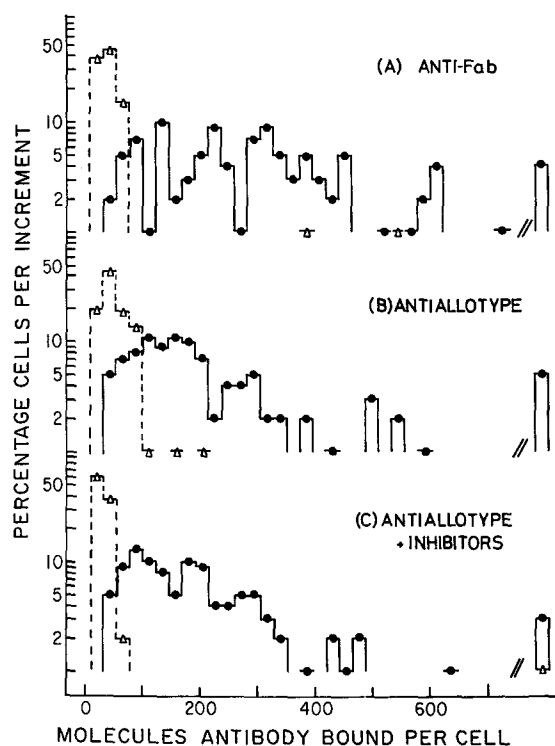


Fig. 3. Lightly-labeled DA and PVG/c TDL. In all cases except for labeling of PVG/c TDL with [ $^{125}$ I]antiallotype, normal TDL were passed through a Degalan anti-rat IgG column and then labeled as follows: (A) DA cells: ●, [ $^{125}$ I]anti-Fab; △, [ $^{125}$ I]anti-Fab + PVG/c (or DA) IgG. (B) [ $^{125}$ I]antiallotype: △, PVG/c cells (whole TDL); ●, DA cells. (C) DA cells: ●, [ $^{125}$ I]antiallotype + PVG/c IgG; △, [ $^{125}$ I]antiallotype + DA IgG. Autoradiographs were exposed for 35 days and 100 cells were measured for each smear. Note demonstration of specificity of antiallotype reagent, binding only to DA TDL (Ig-1a) and inhibited only by DA IgG.

some cells being unlabeled and others binding to the same extent as anti-Fab  $\times$  0.55. The results show, however, that most cells were labeled above background with [ $^{125}$ I]antiallotype within one mode of distribution, and that fewer molecules were bound per cell than expected if the Ig was all of the Ig-1a type. This finding is consistent with the adsorption of a mixture of Ig-1a and Ig-1b to all cells. Similar results were obtained in another experiment.

#### 4. Cell Transfer Experiments.—

a. *Heavily labeled cells:* Cells with large amounts of surface Ig were examined in the spleens of irradiated, transfused recipients (see section 1 for details of transfers). When DA (Ig-1a) cells were given to PVG/c (Ig-1b) recipients 33% of spleen cells were labeled with [ $^{125}$ I]anti-Fab ( $>20,000$  molecules per cell), and as many with [ $^{125}$ I]antiallotype antibody (Table II *b*). In the reciprocal

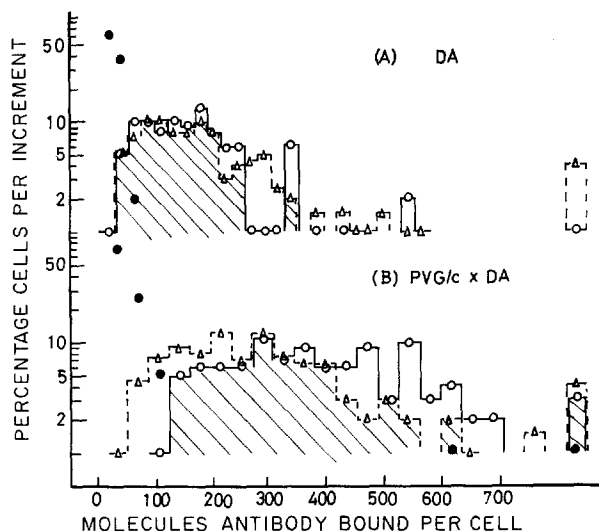


Fig. 4. Lightly-labeled DA and (PVG/c  $\times$  DA) $F_1$  TDL. TDL were passed through a Degalan antirat IgG column and labeled as indicated below. The anti Fab data have been normalized by multiplying by 0.55 and thus the abscissa values are this fraction of the true anti-Fab binding. O, [ $^{125}$ I]anti-Fab;  $\Delta$ , [ $^{125}$ I]antiallotype  $\pm$  PVG/c IgG (pooled);  $\bullet$ , [ $^{125}$ I]antiallotype + DA IgG. (A) DA cells, data from Fig. 3; (B) (PVG/c  $\times$  DA) $F_1$  cells. Exposure was 35 days for A and 20 days for B. 100 cells were measured on each smear. (Thus 200 cells were used for pooled [ $^{125}$ I]antiallotype distribution.) Cross hatching indicates the regions where the distributions overlap. Note superposition of distributions with DA cells and displacement with  $F_1$  cells. All  $F_1$  cells were labeled with antiallotype within a single mode.

experiment (PVG/c to DA) although more cells were labeled heavily with [ $^{125}$ I]anti-Fab (52%), only 10% bound [ $^{125}$ I]antiallotype antibody.

When (PVG/c  $\times$  DA) $F_1$  cells were transferred to DA or PVG/c recipients and recovered from the spleen, the ratio of cells labeled with antiallotype compared to anti-Fab was virtually the same regardless of the allotype of the recipient animal. The figure of about 0.6 obtained in transfer experiments was very similar to that found in normal (PVG/c  $\times$  DA) $F_1$  TDL (Table II a).

These results all suggest that host Ig did not bind in large amounts or replace surface Ig of heavily labeled TDL during their 30 h of residence in the recipient animal.

b. *Lightly labeled cells:* The allotype of Ig on lightly labeled cells after transfer was determined by binding studies on the recovered TDL (see section 1 b). In these experiments the crucial point was the extent to which the Ig could be accounted for as Ig-1a allotype and thus the transformed anti-Fab data were compared with antiallotype binding. The results from all the experiments done are shown in Fig. 5 for cells recovered from DA animals and Fig. 6 for cells from PVG/c recipients. It can be seen that in the case of the DA recipients of either PVG/c or  $F_1$  cells the antiallotype and anti-Fab binding distributions

coincide well, i.e., all the surface Ig on all the recovered TDL types as Ig-1a. In contrast, however, with PVG/c recipients there was always a striking deficit of antiallotype binding: indeed the labeling with antiallotype was so weak that it practically merged into the background (represented by incubation with DA IgG inhibitor).

These results showed that virtually all the detectable Ig on lightly labeled cells had been replaced by host Ig during the 20–30 h allowed for exchange.

5. *The Class of Immunoglobulin on Lightly Labeled Cells.*—Normal PVG/c TDL were depleted of B cells with the anti-IgG Degalan column and incubated with either [<sup>125</sup>I]anti-Fab, anti-IgM, or anti-IgG<sub>2a+2b</sub>. Fig. 7 shows the results of extended autoradiographic exposure. Anti-IgM labeled virtually all cells as did anti-Fab, and quantitatively the grain density distribution also approached that of anti-Fab. The binding of anti-IgM was inhibited by IgM but not by IgG. [<sup>125</sup>I]anti-IgG<sub>2a+2b</sub> bound to a lesser extent and about 80% of the cells bound less than 150 molecules of this antibody. The low binding of [<sup>125</sup>I]anti-IgG<sub>2a+2b</sub> was not due to an ineffective reagent (12) nor to the Degalan columns since unfractionated TDL also gave very low binding of this antibody (Fig. 7 D). This confirmed previous results showing that a very low percentage of rat TDL bound anti-IgG<sub>2a+2b</sub> reagent (12).

#### DISCUSSION

The specificity of the antiallotype antibodies is of paramount importance in these experiments, and the evidence clearly suggests that the antibodies recognize the Ig-1a allotype of rat L chain and no other Ig or cell surface determinant. This was assured by the immunization and purification procedures, by the binding of [<sup>125</sup>I]antiallotype to the appropriate Ig on SRBC and its inhibition by L chains, and also by the binding pattern of antibodies to TDL from DA and PVG/c animals (see Materials and Methods and Results).

Both antiallotype and anti-Fab antibodies detected two kinds of small lymphocyte: i.e., a heavily and lightly labeled category. With regard to heavily labeled cells, the experiments clearly showed that the cell surface Ig was not acquired but synthesized by the cells themselves. Only 60% of the cells which bound anti-Fab in heterozygous TDL could bind antiallotype antibodies, although both reagents labeled the same percentage of cells from homozygous animals of the DA strain. On the basis of equal allotype expression one would expect the antiallotype:anti-Fab ratio to be 1:2 and the figure of 0.6 obtained may indicate a small percentage of cells carrying cytophilic Ig, or that the Ig-1a allotype is favored in F<sub>1</sub> animals. The latter explanation is supported by the reported preference for Ig-1a allotype in serum Ig of heterozygotes (16). In any case, the allelic restriction is marked, and consistent with the results obtained for surface Ig of rabbit lymphocytes binding large amounts of antibody (2–4).

For heavily labeled cells, the results of the transfer experiments (in which

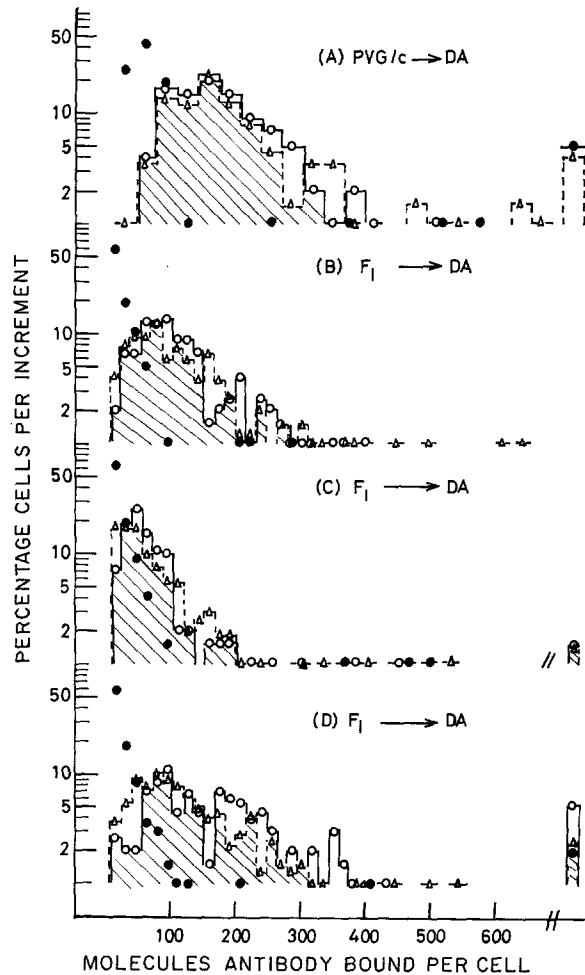


Fig. 5. Transfer of TDL into DA recipients. PVG/c or (PVG/c  $\times$  DA)F<sub>1</sub> TDL were passed through DA host rats, recovered from the thoracic duct and labeled as follows. The anti-Fab data are normalized as in Fig. 4. O, [<sup>125</sup>I]anti-Fab;  $\Delta$ , [<sup>125</sup>I]antiallotype  $\pm$  PVG/c IgG (pooled);  $\bullet$ , [<sup>125</sup>I]antiallotype + DA IgG. Results in (A), (B), (C), and (D) are from experiments 2, 4, 5, and 6 in Table I and exposure was for 22, 33, 26, and 28 days, respectively. For A, 100 cells were measured per smear, while for (B), (C), and (D) the number was 200. The crosshatching (as in Fig. 4) emphasizes that the distributions coincide closely. Practically all the anti-Fab binding can be accounted for by the antiallotype.

TDL were passed through irradiated recipients of the opposite allotype) supported those from the binding to F<sub>1</sub> cells. Transferred cells retained their surface Ig which was characterized as of donor not host type.

The opposite results were obtained with the binding of antiallotype antibodies to lightly labeled cells. First, no hint of allelic restriction in heterozygous

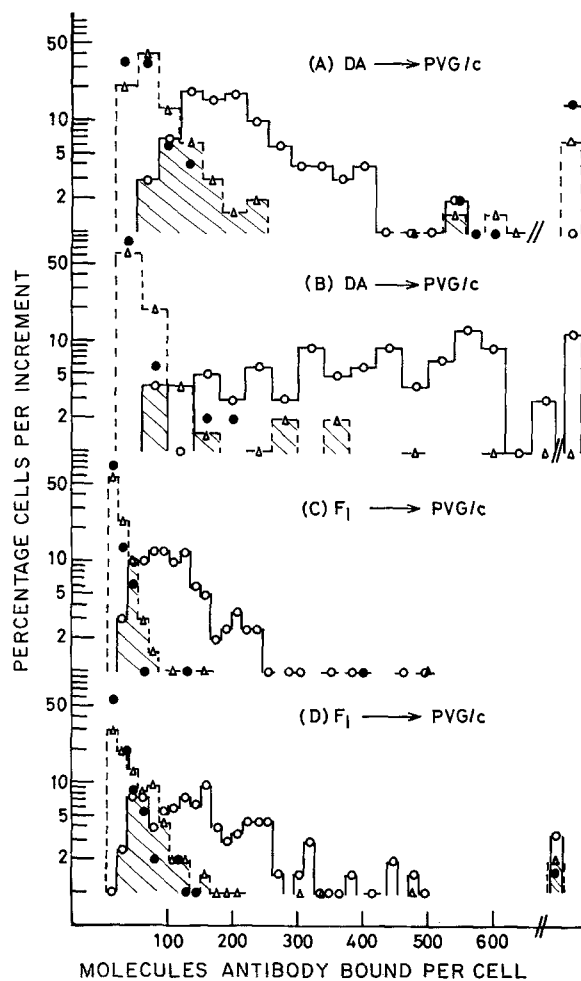


Fig. 6. Transfer of TDL into PVG/c recipients. DA or (PVG/c  $\times$  DA)F<sub>1</sub> TDL were passaged through PVG/c host rats, recovered from the thoracic duct and labeled as in Fig. 5. Data in (A), (B), (C), and (D) are from experiments 3, 1, 5, and 6 in Table I and exposures were for 22, 15, 26, and 28 days, respectively. Cross hatching as in Fig. 4. In these experiments very little of the anti-Fab binding can be accounted for by antiallotype.

animals could be found. Therefore either these cells do not show the phenomenon, or they had acquired the Ig passively. Second, in the transfer experiments, passaged cells which labeled lightly could be detected but the great majority of the Ig found was always of host origin. The acquisition of the host Ig might be attributed to the binding of pre-existing natural host antidonor alloantibodies, but this was unlikely since quantitatively binding was of the same order as or slightly lower than that of lightly labeled cells in normal TDL. In

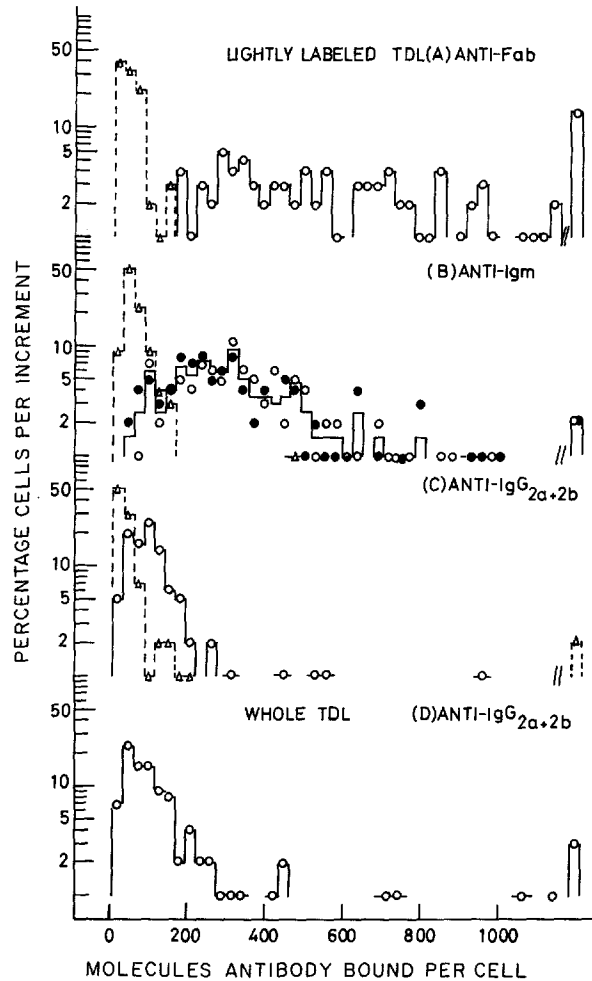


Fig. 7. Class of Ig on lightly-labeled cells. Cells from PVG/c TDL were passed through a Degalan anti-rat IgG column and labeled as follows. (A)  $\circ$ , [ $^{125}$ I]anti-Fab;  $\Delta$ , [ $^{125}$ I]anti-Fab + PVG/c IgG. (B)  $\circ$ , [ $^{125}$ I]anti-IgM;  $\bullet$ , [ $^{125}$ I]anti-IgM + PVG/c IgG;  $\Delta$ , [ $^{125}$ I]anti-IgM + PVG/c IgM. Note (i) binding of anti-IgM comparable with (A). (ii) specificity of anti-IgM. (C)  $\circ$ , [ $^{125}$ I]anti-IgG<sub>2a+2b</sub>;  $\Delta$ , [ $^{125}$ I]anti-IgG<sub>2a+2b</sub> + PVG/c IgG. Note very weak binding. (D) shows unfractionated PVG/c TDL labeled as in (C). Binding not improved over (C), therefore anti-IgG column extraction did not influence the result. Exposure was for 29 days and 100 cells were measured for each histogram.

any case the donor Ig was lost within 20 h down to a level of detection where less than 100 molecules of anti-allotype antibodies were bound. The most likely explanation is that serum and surface Ig can exchange on these cells.

Most of the cell surface Ig of lightly labeled lymphocytes from normal

TDL was IgM with a smaller amount of IgG<sub>2a+2b</sub>. This is in marked contrast to the concentration of these classes in lymph where there is 40 times more IgG<sub>2a+2b</sub> than IgM (20). The amount of IgM on lightly labeled cells was such that this class must be considered to constitute much of the acquired immunoglobulin. Hammerling and Rajewsky (25) also found that most of the Ig on T cells from mouse lymph nodes was IgM.

In discussing these results heavily labeled and lightly labeled lymphocytes will be equated with B and T cells, respectively. This identity is justified by analogy with experiments in mice (6-8, 10, 11) and also by the distribution of B-cell markers in rat TDL which correlate with cells carrying large amounts of surface Ig. This applies to the heterologous anti-B cell antiserum marker (see Results) and also to the distribution of C3 and Fc receptors which are considered to be B-cell markers (J. A. Hayward and C. R. Parish, manuscript in preparation). Thus we would conclude that Ig found on rat B cells is synthesized by each cell itself, while that on most T cells has been acquired from the serum, and therefore is unlikely to function as receptor.

This assertion must be reconciled with several studies claiming that Ig is the antigen receptor on T cells. Experiments positively demonstrating such Ig include some examining the nature of receptor on antigen-binding T cells (26-28) and others reporting the discovery of antigen-specific Ig on T-TDL (29, 30).

The experiments on antigen-binding T cells involve the use of "classical" (nonhistocompatibility) antigens such as sheep erythrocytes (26, 27) and hemocyanin (28), and deal therefore with only one type of T-cell function. However, a large part of the T-cell pool may be devoted to cells committed to function in the immune response to strong histocompatibility antigens. Ford and Atkins (31) have estimated that as many as 7-12% of peripheral T cells may recognize and respond to one single set of Ag-B allelic differences in initiating GVH reactions, so that most T cells would have to be committed to these strong antigens to cope with the 9 Ag-B alleles already known (32). The immune response in GVH or MLC reactions appears sufficiently different from other responses that it may have a unique non-Ig recognition system (33). Thus it could be argued that our studies and those on antigen-binding cells do not conflict, but that different subpopulations are being studied; antigen-binding T cells could constitute less than  $\frac{1}{10}$  th, perhaps, of the total T-cell pool, which would pass unnoticed in our experiments.

Alternatively even the surface Ig identified on antigen-binding T cells may have been acquired, not synthesized by the cell itself. The main argument against this is the specificity and low frequency of antigen-binding cells. However, the possibility of a "sandwich" of non-Ig-receptor:antigen:Ig at the cell surface able to bind further antigen when incubated in vitro remains a tenable explanation (33). No experiment on antigen-binding T cells has been reported in which genetic markers were used to identify the origin of the Ig.

The other positive experiments which apparently contradict the findings in

this paper report the identification of alloantigen-specific, surface Ig on T-TDL obtained from the thoracic duct of mice undergoing a GVH reaction (29, 30). However, in these experiments no evidence for the origin of the surface Ig was reported and it now seems likely that this material was antibody adsorbed to the T-TDL. This antibody has recently been shown to derive from B cells contaminating the thymocyte inoculum initiating the GVH reaction (Hudson and Sprent, personal communication).

#### SUMMARY

The origin of immunoglobulin on the surface of TDL in the rat has been studied by comparing the binding of purified alloantibodies recognizing the Ig-1a allotype of rat light chain, with that of rabbit antirat Fab antibodies. Both reagents labeled all TDL from rats of the DA strain (Ig-1a) with two categories of cells being detected; one binding 100–2,000 molecules of antibody, the other 10,000–100,000 molecules. These categories were likely to be synonymous with T and B cells, respectively. The [<sup>125</sup>I]antiallotype antibodies did not bind to TDL from rats of the PVG/c strain (Ig-1b).

When the binding to TDL from (PVG/c × DA)F<sub>1</sub> animals was studied it was found that allelic exclusion occurred in the heavily labeled cells, but not in the lightly labeled ones. Furthermore, when lymphocytes of one allotype were transferred to irradiated recipients of the opposite allotype and recovered from the TDL or spleen of the recipient 20–30 h later, the immunoglobulin on heavily labeled cells was of the donor type, while that of lightly labeled ones bore the recipient marker. Thus heavily labeled cells (B lymphocytes) had synthesized their own immunoglobulin while lightly labeled cells (T lymphocytes) had acquired theirs passively by adsorption. The class of immunoglobulin on lightly labeled cells was also studied and it was found that [<sup>125</sup>I]anti-IgM antibodies bound to an extent approaching the [<sup>125</sup>I]anti-Fab binding, while [<sup>125</sup>I]anti-IgG<sub>2a+2b</sub> antibodies gave much less binding.

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