THE SPECIFIC SELECTION OF RECIRCULATING LYMPHOCYTES BY ANTIGEN IN NORMAL AND PREIMMUNIZED RATS

By D. A. ROWLEY,* J. L. GOWANS,‡ R. C. ATKINS,§ W. L. FORD, AND MARILYN E. SMITH

(From the Medical Research Council, Cellular Immunology Unit, Sir William Dunn School of Pathology, Oxford University, Oxford, England)

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Small lymphocytes from the thoracic duct lymph of rats, after transfer to suitable recipients, will react to antigens and initiate various immunological responses (1). Since these cells are part of the pool of lymphocytes which recirculates through lymphoid tissue, it has been suggested that during the induction of immune responses in normal animals antigen may recruit lymphocytes of the appropriate specificity from the recirculating pool. In this way the efficiency of a regional response might be increased because a large number of lymphocytes could present themselves for selection by antigen within the confines, for example, of a single lymph node or of the spleen (2–4).

The notion of selection of recirculating lymphocytes by antigen was tested by assaying the immunological activity of the cells collected from the thoracic duct of rats 1–8 days after giving antigen; if recirculating cells are selected, then thoracic duct lymph should become deficient in cells reactive against the selecting antigen. In the first set of experiments, a specified depression of activity was observed in thoracic duct lymphocytes (TDL)[1] from nonimmunized rats given either sheep erythrocytes or allogeneic lymphoid cells similar to that reported in an independent study in mice by Sprent et al. (5). Further, it was shown that selection by sheep erythrocytes could be abolished by pretreating the lymphocyte donor with passive antibody. In the second set of experiments, selection by antigen in preimmunized rats led to a profound depression in the ability of TDL to transfer immunological memory. Since both marrow-derived (B) and thymus-derived (T) lymphocytes recirculate in the rat (6, 7), attempts were made to

* Permanent address: Department of Pathology, University of Chicago. Supported by a U.S. Public Health Service Special Fellowship: 1 F03 AI 50,622-01.

‡ Correspondence to J. L. G., Oxford.

§ Permanent address: Prince Henry's Hospital, Melbourne 3004, Australia.

[1] *Abbreviations used in this paper*: BGG, bovine gamma globulin, Cohn fraction II (Sigma Chemical Co., St. Louis, Mo.); B lymphocytes, marrow-derived lymphocytes; DNP, dinitrophenyl; GVH, graft-*versus*-host; HSA, human serum albumin, grade III (Sigma Chemical Co.); PBS, phosphate-buffered saline, Dulbecco's "A" (Oxoid Ltd., London); PFC, plaque-forming cells; SRBC, sheep erythrocytes; TDL, thoracic duct lymphocytes; T lymphocytes, thymus-derived lymphocytes.

select hapten- and carrier-specific cells separately in rats preimmunized with a hapten-protein conjugate. It will be suggested that the specific regional selection of lymphocytes by antigen of the kind demonstrated in the present experiments may explain a number of disparate immunological phenomena.

Materials and Methods

Rats.—Albino rats from a closed, noninbred colony were used in some experiments. In others, members of the highly inbred DA, HO, and AO strains and the F_1 hybrids between them were used. Donors of TDL weighed 160–220 g. In the experiments with sheep erythrocytes (SRBC) no donor had a serum hemolysin titer for SRBC $>1:2$.

Irradiation.—Rats received whole body radiation from a ^{60}Co source at a dose rate of about 50 rads/min. They were maintained on Terramycin (Pfizer Laboratories Division, New York), 9.1 g/liter of drinking water, starting several days before irradiation and continuing until the end of the experiment.

Antigens.—Erythrocytes from sheep's blood preserved in Alsever's solution (Wellcome Research Laboratories, Beckenham, Kent, England) were washed three times and resuspended in phosphate-buffered saline (PBS; Dulbecco's "A" [Oxoid Ltd., London]). All procedures in any given experiment employed erythrocytes from a single sheep except as noted below for the preparation of antisera for passive immunization. Washed burro erythrocytes were prepared from burro blood purchased from Tissue Culture Services Ltd., Slough, Bucks.

Alum-precipitated tetanus toxoid and fluid tetanus toxoid both at 40 Lf/ml were obtained from the Wellcome Research Laboratories.

Dinitrophenylated bovine gamma globulin, Cohn fraction II (Sigma Chemical Co., St. Louis, Mo.) ($DNP_{40}BGG$) and dinitrophenylated human serum albumin, grade III (Sigma Chemical Co.) ($DNP_{40}HSA$) were prepared by the method of Little and Eisen (8) and for some purposes were alum precipitated (9) so that 1 ml of the suspension contained 10 mg of the conjugate. For active immunization each dose of conjugate was combined with 2×10^9 killed pertussis organism (pertussis vaccine, Wellcome Research Laboratories).

Antisera to SRBC.—For each of the two pools of antiserum prepared for these experiments, the erythrocytes for immunization were derived from three different sheep. The sera were pooled from rats bled 10 days after the second of two intravenous injections of 10^9 SRBC given 1 month apart. One pool had a hemolysin titer of 1024, the other of 2048.

Assay of Splenic Plaque-Forming Cells (PFC).—Rats were anesthetized with ether, blood taken by heart puncture for assay of serum hemolysin, and the spleen removed. Direct splenic PFC were estimated using a modification of the technique described by Jerne and Nordin (10). The number of PFC per spleen was calculated from counts on duplicate plates and statistical calculations were performed on logarithmically transformed data.

Assay of Serum Antibody.—The titer of antibody against SRBC was taken as the reciprocal of the highest serum dilution producing 50% hemolysis of erythrocytes. Serum antibody to tetanus toxoid was measured by passive hemagglutination (11). Anti-DNP antibody in inactivated rat sera was titrated by passive hemagglutination of rat erythrocytes which had been coated with DNP_5 Fab prepared from a rabbit anti-rat erythrocyte serum.[2]

Thoracic Duct and Splenic Lymphocytes.—Lymphocytes were obtained from the thoracic duct as previously described (12). Individual collections were made for periods of up to 14 hr unless otherwise stated and the cells were washed once before injection. Spleens for transfer were cut into small fragments, sucked up into a wide-ended Pasteur pipette, and run into the peritoneal cavity of recipient rats through a small midline abdominal incision.

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[2] Hunt, S. V. 1972. Data to be published.

RESULTS

Selection by SRBC in Normal Rats.—The primary antibody response of rats to SRBC, which is abolished by a heavy dose of radiation, can be restored by an injection of syngeneic TDL (13, 14). To determine if antigen selects lymphocytes from the recirculating pool in normal rats, TDL were collected at various times after an injection of SRBC and assayed for their ability to restore the primary response to SRBC in irradiated recipients. Preliminary experiments confirmed that an intravenous injection of TDL from normal, noninbred albino rats restored the response of irradiated (750 rads) rats to 2×10^8 SRBC with a peak response at 6 days and established that giving increasing numbers of TDL, 0.11×10^8, 0.33×10^8, or 1.0×10^8 cells per recipient, resulted in progressively higher responses to SRBC with a well marked "premium effect," especially between the two lower cell doses (Table I). This effect is usually attributed to the fact that at least two different cell types in TDL are required for the response. For the purpose of detecting selection of specifically reactive cells by antigen, a dose of 1.0×10^8 TDL per recipient was selected as a suitable restorative inoculum; with this cell dose any procedure which reduced the number of reactive cells by more than threefold would be detectable. However, without dose/response data for each experiment it is not possible to assess the magnitude of the reduction in the number of reactive cells in any given experiment.

TABLE I

Response to SRBC of Irradiated Rats Given TDL from Normal Rats

Normal donors* Cell dose ($\times 10^8$)	Irradiated recipients‡			
	Log_{10} PFC per spleen		Log_{10} serum antibody titer	
0.11	3.217 ± 0.183	$P = <0.001$	0.778 ± 0.202	$P = <0.001$
0.33	4.228 ± 0.080	$P = <0.20$	2.021 ± 0.182	$P = <0.02$
1.00	4.355 ± 0.048		2.537 ± 0.061	

* Pooled TDL collected from three normal rats.

‡ Recipients injected intravenously with TDL and 2×10^8 SRBC 24 hr after 750 rads γ-irradiation; responses measured at 6 days. Each mean $\pm$ SE calculated on log transformed data from groups of seven rats.

In the first experiment selection was detected by cannulating the thoracic duct on the day after an intravenous injection of 2×10^8 SRBC and collecting TDL from 24 to 38 hr after antigen injection. Table II shows that TDL from donors receiving SRBC were much less effective in restoring the response of irradiated rats to SRBC than an equal number of TDL from control rats which had received saline only. The spleens of donors 4 days after the injection of SRBC contained >80,000 PFC, and the serum antibody titers were 264 or higher; clearly the donors were fully responsive although their TDL were rela-

tively ineffective in the adoptive response. In further experiments it was shown that either 10^8 or 10^9 SRBC produced an equivalent reduction in the immunological responsiveness of TDL and in the subsequent experiments a standard "selecting" dose of 2×10^8 SRBC was consequently employed.

The specificity of the reduction in responsiveness was studied by comparing the performance of thoracic duct cells from donors injected with either 2×10^8 SRBC or burro erythrocytes. Table II shows that the response of recipients of TDL from donors injected with SRBC was again very low, while that of recipients given cells from donors injected with burro erythrocytes was equivalent to

TABLE II

Response to SRBC of Irradiated Rats given TDL from Donors Injected with SRBC

Donors* injected with	Irradiated recipients‡	
	Log_{10} PFC per spleen	Log_{10} serum antibody titer
Exp. 1		
SRBC i.v.	3.421 ± 0.139 $P = <0.01$	1.392 ± 0.160 $P = <0.001$
Saline i.v.	4.189 ± 0.126	3.176 ± 0.101
Exp. 2		
SRBC i.v.	3.350 ± 0.154 $P = <0.001$	1.763 ± 0.212 $P = <0.01$
BRBC i.v.	4.263 ± 0.118	2.520 ± 0.169
Exp. 3		
SRBC FCA i.d.	3.423 ± 0.230 $P = <0.05$	1.957 ± 0.273 $P = <0.05$
Not injected	4.254 ± 0.240	2.746 ± 0.144

* Pooled TDL collected from groups of two or three donors 24–36 hr after injection with 2×10^8 erythrocytes either intravenously (i.v.) or intradermally (i.d.) in Freund's complete adjuvant (FCA) as four depots in abdominal skin.

‡ Recipients injected intravenously with 10^8 TDL and 2×10^8 SRBC 24 hr after 750 rads γ-irradiation; responses measured at 6 days. Each mean $\pm$ SE calculated on log transformed data from groups of seven to nine rats.

those of the controls in other experiments. Again, the donors given SRBC had $>80{,}000$ splenic PFC and high antibody titers, while donors given burro erythrocytes had <1000 splenic PFC and antibody titers to SRBC of <4. In other experiments it was shown that the injection of donors with SRBC had no detectable effect on the response of their TDL to burro erythrocytes. Thus, in so far as it was examined, selection was specific for the antigen used. An additional experiment recorded in Table II showed that rats given an intradermal injection of SRBC in Freund's complete adjuvant also yielded, 24 hr later, TDL with a depressed restorative capacity.

The period of time for which intravenous injections of SRBC exerted their selective influence was studied by collecting TDL at various times after antigen injection. In one experiment donors were first cannulated and then injected with SRBC. The mean response of recipients of TDL collected for the first 12 hr after antigen injection was 4100 splenic PFC, while the mean response conferred by

cells collected during the second 12 hr was 1600. Thus, selection was demonstrable by 12–24 hr after antigen injection. The selective influence of a single intravenous dose of SRBC waned as the period between antigen injection and thoracic duct cannulation was extended. Table III shows that by 4 days after a single injection of SRBC, thoracic duct cannulation yielded cells which had a restorative capacity equivalent to that of TDL from normal donors. However, selection could be sustained by repeated injections of antigen. This was shown by collecting TDL from one group of donors given a single injection of SRBC 8 days before and from a second group which had received three injections given 8, 4, and 1 days before. Recipients of cells from the remotely injected rats gave normal responses to SRBC but, in contrast, the recipients of cells from the repeatedly injected donors gave low responses (Table IV).

TABLE III

Response to SRBC of Irradiated Rats Given TDL from Donors 1 or 4 days after Injection with SRBC

Donors* injected with	Irradiated recipients‡			
	Log_{10} PFC per spleen		Log_{10} serum antibody titer	
SRBC (−1 day)	3.170 ± 0.267	$P = < 0.01$	0.698 ± 0.285	$P = < 0.001$
SRBC (−4 days)	4.386 ± 0.068		2.104 ± 0.239	

* Pooled TDL from two groups of two donors injected intravenously with 2×10^8 SRBC either 1 or 4 days before TDL collected.

‡ Recipients injected with 10^8 TDL and 2×10^8 SRBC 24 hr after 750 rads γ-irradiation; responses measured at 6 days. Each mean ± SE calculated on log transformed data from groups of six rats.

Since the administration of passive antibody will severely depress the immune response to a subsequent injection of SRBC in rats (15), presumably by rendering antigen unavailable for induction, it was of interest to determine whether the prior injection of antibody would also prevent antigen from exerting its selective influence on recirculating lymphocytes. To study this point donors of TDL were injected with 1.5 ml of anti-SRBC serum or 1.5 ml of normal rat serum 3 hr before an intravenous injection of 2×10^8 SRBC. TDL collected 24–36 hr after giving antigen were injected into irradiated recipients together with sheep SRBC as in the previous experiments. In addition, the donors were released from restraint, their thoracic duct cannulae removed, and their splenic PFC and serum antibody response measured 4 days after the selecting dose of SRBC. Experiment 1 in Table V showed that the recipients of TDL from donors injected with normal serum and antigen had very low responses as had been observed in the previous experiments. In contrast, the response of rats given cells from donors passively immunized before injection with antigen were comparable with those observed in control animals. Similar results were obtained with a different pool of rat anti-SRBC serum as shown in experiment 2, Table V.

Donors given either normal rat serum or anti-SRBC serum alone without SRBC yielded TDL possessing the normal restorative capacity (experiment 3, Table V). All five donors of TDL in experiments 1 and 2 (Table V) which received normal serum and SRBC had >100,000 spleen PFC and antibody titers of 512

TABLE IV

Response to SRBC of Irradiated Rats Given TDL from Donors Given either a Single or Multiple Injections of SRBC

Donors* injected with	Irradiated recipients‡			
	Log_{10} PFC per spleen		Log_{10} serum antibody titer	
SRBC, × 1 (−8 days)	4.253 ± 0.141	P = <0.001	2.371 ± 0.259	P = <0.05
SRBC, × 3 (−8, 3, and 1 days)	3 350 ± 0 125		1.732 ± 0.168	

* Pooled TDL from two groups of three donors injected intravenously with doses of 2 × 10^8 SRBC either 8 days or 8, 3, and 1 days before TDL collected.

‡ Recipients injected with 10^8 TDL and 2 × 10^8 SRBC 24 hr after 750 rads γ-irradiation; responses measured at 6 days. Each mean ± SE calculated on log transformed data from groups of eight rats.

TABLE V

Response to SRBC of Irradiated Rats Given TDL from Passively Immunized Rats

Donors* injected with	Irradiated recipients‡			
	Log_{10} PFC per spleen		Log_{10} serum antibody titer	
Exp. 1				
SRBC + NS	2.415 ± 0.263	P = <0.001	0.301 ± 0.268	P = <0 001
SRBC + anti-SRBC	4.352 ± 0.177		1.954 ± 0.169	
Exp. 2				
SRBC + NS	2.819 ± 0.271	P = <0.001	0.778 ± 0.261	P = <0.001
SRBC + anti-SRBC	4.286 ± 0.174		2.021 ± 0.086	
Exp. 3				
NS	4.588 ± 0.119	P = <0.4	2.784 ± 0.186	P = <0.3
Anti-SRBC	4.267 ± 0.296		2.365 ± 0.356	

* Pooled TDL from groups of two or three donors; the donors were injected intravenously with either 2 × 10^8 SRBC and normal serum (NS), 2 × 10^8 SRBC and anti-SRBC serum, or with serum alone. TDL were collected 24–36 hr after injection.

‡ Recipients injected intravenously with 10^8 TDL and 2 × 10^8 SRBC 24 hr after 750 rads γ-irradiation; responses measured at 6 days. Each mean ± SE calculated on log transformed data from groups of six to eight rats.

or higher, while the five donors which were passively immunized before receiving SRBC had <10,000 PFC and serum antibody titers of 64 or less. These results show clearly that the prior administration of passive antibody severely depresses the ability of antigen to select lymphocytes from the recirculating pool;

TDL collected from such rats were able to restore responses to SRBC in irradiated recipients which were equivalent to those given by TDL from normal, untreated donors.

Selection with Strong Histocompatibility Antigens in Normal Rats.—Small lymphocytes from the thoracic duct of parental strain rats will react to histocompatibility antigens in F_1 hybrid rats and initiate a graft-*versus*-host (GVH) reaction (2). An attempt was made to deplete the recirculating pool of lymphocytes of such specifically reactive cells by injecting parental strain rats with antigen in the form of F_1 hybrid lymphoid cells teased out from the spleen and from cervical and mesenteric lymph nodes. Three inbred rat strains were used:

TABLE VI

Specific Selection of Lymphocytes from the Recirculating Pool of Parental Strain Rats by Injection of F_1 Hybrid Lymphoid Cells. Assay of Parental TDL by Popliteal Lymph Node Weight Assay (16)

Experiment No.	Source of lymphoid cells used as "selecting" antigen	Activity ratios* by GVH assay in	
		Specific F_1	3rd party F_1
1–3	(AO × DA)F_1	0.34	1.22
		0.49	1.13
		0.44	0.75
4–6	(AO × HO)F_1	0.44	1.36
		0.52	1.19
		0.35	1.10
	Geometric mean	0.42	1.11

TDL collected from AO strain rats 24–36 hr after an intravenous injection of 10^9 F_1 hybrid lymphoid cells. GVH activity of parental TDL assayed in the specific F_1 hybrid (donor of selecting antigen) and in a third-part F_1 hybrid.

* Activity ratio = No. of TDL from uninjected AO rats/No. of TDL from injected rats giving equal increases in lymph node responses. Each ratio was derived from 24 lymph node weights.

AO (AgB-2), HO (AgB-5), and DA (AgB-4); the GVH activity of the parental TDL was assessed by the popliteal lymph node weight assay (16). In experiments 1–3 (Table VI), cells collected from the thoracic duct of AO rats given (AO × DA)F_1 lymphoid cells were assayed for their GVH activity in (AO × DA)F_1 (specific) and (AO × HO)F_1 (third-party) rats. In experiments 4–6 the reciprocal combinations were employed. The doses of cells used in each assay were based on the actual number of AO cells in the thoracic duct lymph as determined by a cytotoxic test with alloantisera. Between 10 and 20% of the cells in AO lymph were F_1 cells which had recirculated but this degree of contamination with either specific or third-party F_1 hybrid lymphocytes does not affect the assay. Table VI shows that the specific GVH activity of parental lymphocytes was reduced about 2.5-fold by an injection of F_1 hybrid cells (activity ratio 0.42), while the activity against the third-party antigen was un-

affected (activity ratio 1.11). The depression of GVH activity was again specific for the selecting antigen.

Selection with Tetanus Toxoid in Immunized Rats.—Small lymphocytes from the thoracic duct can carry the property of immunological memory. Thus, when

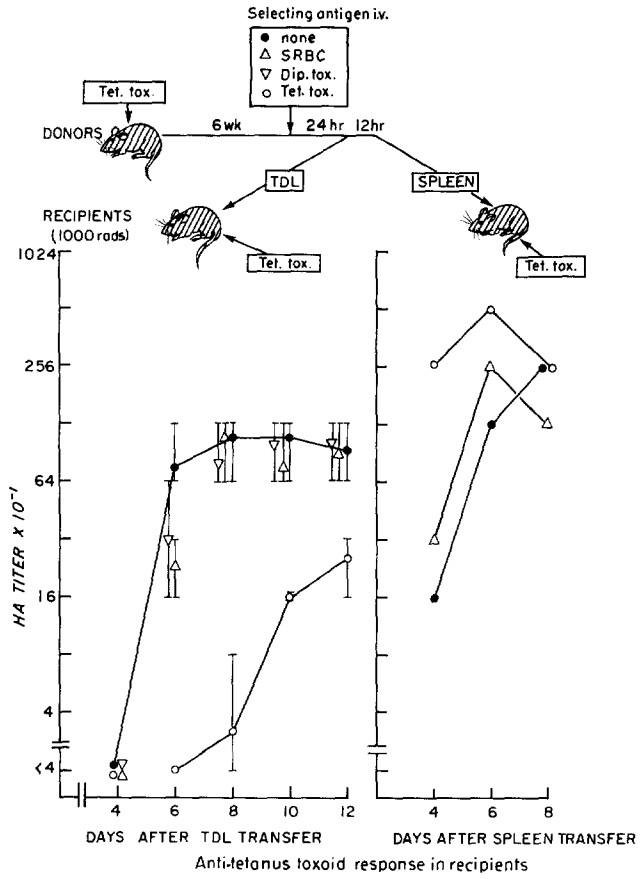

FIG. 1. Specific selection of lymphocytes from the recirculating pool of immunized rats by intravenous tetanus toxoid. Assay by transfer of TDL to irradiated recipients and challenge. *Donors*: Four inbred, HO strain ♀ rats (D 1–4) were immunized intraperitoneally with 20 Lf alum-precipitated tetanus toxoid 6 wk before cannulation of the thoracic duct. 24 hr before cannulation the donors were given intravenous injections of either 20 Lf fluid tetanus toxoid (D1), 20 Lf fluid diphtheria toxoid (D2), or 2×10^8 SRBC (D3). Control received no injection (D4). Lymphocytes were collected from thoracic duct for 12 hr, then donors killed and suspensions of cells prepared from their spleens. *Recipients:* Inbred HO strain ♀ rats were irradiated with 850 rads and injected intravenously 24 hr later with 20 Lf fluid tetanus toxoid together with either 2×10^7 TDL or cells from a half spleen. Each cell inoculum from a single donor. *Left*: Geometric mean and range of titers from groups of four rats; each group given TDL from one donor. Selecting antigen to donor: ○, tetanus toxoid (D1); ▽, diphtheria toxoid (D2); △, SRBC (D3); ●, none (D4). *Right*: Titers from single rats given spleen cells from D1 (○), D3 (△), or D4 (●).

heavily irradiated rats are injected with TDL from donors immunized with tetanus toxoid they will respond in a secondary manner if, but only if, they are challenged with antigen. (13). The experiment illustrated in Fig. 1 shows that the capacity of TDL to transfer specific responsiveness to tetanus toxoid was severely depressed when the immune donors were given an intravenous injection of fluid toxoid 24 hr before thoracic duct cannulation. However, the TDL from such donors were fully reactive to an unrelated antigen, SRBC, as shown by their ability to restore the primary hemolysin response in irradiated recipients (Table VII). Similar evidence for the specificity of the unresponsiveness induced by the selecting antigen was the normal reactivity to tetanus toxoid of

TABLE VII

Specificity of Immune Deficiency of Rat TDL after Selection by Tetanus Toxoid In Vivo. Assay by Transfer of TDL to Irradiated Recipients and Challenge.

Donors*	Recipients‡				
Selecting antigen i.v.	Response to challenge with	Antibody responses§ Days after cell transfer and challenge			
		6	8	10	
Tetanus toxoid	Tetanus toxoid	<40	40	130	1/HA titer
—	Tetanus toxoid	280	1760	1120	
Tetanus toxoid	SRBC	5.0	5.75	6.0	$\log_2$ hemolysin titer
—	SRBC	4.7	6.75	6.5	

* Donors immunized intraperitoneally with 20 Lf alum-precipitated tetanus toxoid 8 wk before cannulation of thoracic duct.

‡ Recipients given 2×10^7 TDL from immunized donors and either 20 Lf fluid tetanus toxoid or 10^8 SRBC intravenously, 24 hr after 850 rads γ-irradiation.

§ Each value: geometric mean response of four rats.

TDL from immune donors injected with either SRBC or diphtheria toxoid 24 hr before cannulation of the thoracic duct (Fig. 1). It can be seen in Fig. 1 that the donors of the lymphocytes were not immunologically tolerant since their spleens were able to confer a substantial adoptive immunity to tetanus toxoid after transfer to irradiated recipients. The experiment suggests that recirculating lymphocytes competent to react to tetanus toxoid had been selected and immobilized in lymphoid tissue during the interval of 24 hr between the injection of the selecting antigen and cannulation of the thoracic duct.

Selection with Antigen in Rats Preimmunized with DNP BGG.—Anti-hapten responses in mice depend upon collaboration between B and T lymphocytes with the latter responding specifically to the carrier protein (17). If such collaboration also occurs in rats it might be possible to select B and T cells separately since both these classes of lymphocytes recirculate in the rat (6, 7), and it is known that TDL from specifically immunized rats will normally generate an anti-hapten response after cell transfer and challenge (18).

Fig. 2 shows that irradiated rats given 4×10^7 TDL from donors immunized

about 3 months previously with DNP BGG developed substantial titers of anti-DNP antibody after challenge with the homologous conjugate but that the response was completely abolished by injecting the donors with DNP BGG 24

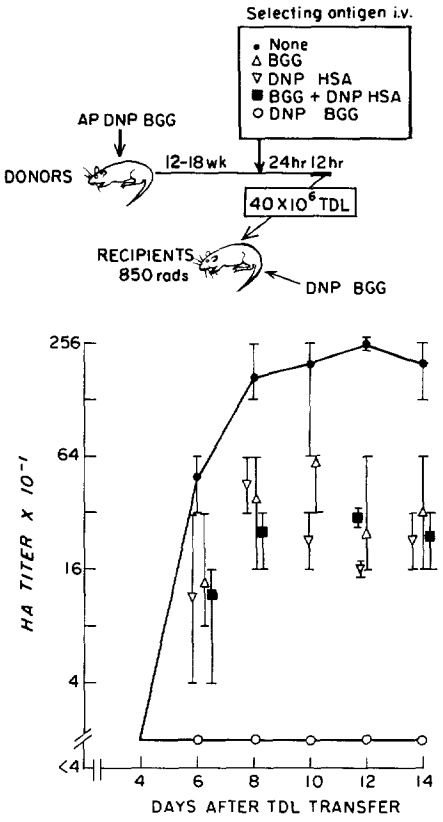

FIG. 2. Selection of lymphocytes from the recirculating pool by antigen in rats immunized with DNP BGG. Assay by transfer of TDL to irradiated recipients and challenge. *Donors*: HO or DA strain ♀ rats were immunized intraperitoneally with 1 mg of alum-precipitated (AP) $DNP_{40}BGG$ and 2×10^9 killed pertussis organisms 12–18 wk before cannulation of the thoracic duct. 24 hr before cannulation the donors were given intravenous injections of either (*a*) 1 mg of $DNP_{40}BGG$ in PBS, (*b*) 1 mg of BGG in PBS, (*c*) 1 mg of alum-precipitated $DNP_{40}HSA$, or (*b*) + (*c*). Lymph was collected for 12 hr. *Recipients*: Syngeneic rats were irradiated with 850 rads and injected intravenously 24 hr later with 4×10^7 TDL together with 1 mg of $DNP_{40}BGG$ in PBS. Geometric mean and range of hemagglutination titers from groups of six rats.

hr before cannulation of the thoracic duct. Selecting injections of the carrier protein alone (BGG), the hapten on a noncross-reacting carrier (DNP HSA), or both (BGG + DNP HSA) significantly depressed the response but did not abolish it.

DISCUSSION

Lymphocytes from the thoracic duct (TDL) of normal rats will restore a primary antibody response to SRBC in heavily irradiated syngeneic recipients (13, 14) and cause a GVH reaction in F_1 hybrids (2); TDL from rats immunized with either tetanus toxoid (13) or DNP BGG (18) will generate substantial quantities of specific antibody after cell transfer to irradiated recipients and challenge. In the present experiments it has been shown that an intravenous injection of the specific antigen shortly before cannulation of the thoracic duct will severely depress the ability of TDL to mediate each of these responses. Two peculiarities of this antigen-induced unresponsiveness deserve emphasis. First, it was not attended, in any of the experiments, by a measurable fall in the output of cells from the thoracic duct. Presumably, small numbers of specific lymphocytes are selected from the recirculating pool into the regions of lymphoid tissue containing antigen. Second, it was not a reflection of immunological tolerance in the lymphocyte donors. Thus, rats injected with either SRBC or tetanus toxoid 24 hr before cannulation of the thoracic duct both developed normal antibody responses although they yielded lymphocytes showing a marked depression of reactivity; and the powerful initial selection exerted by SRBC waned to zero when the interval between injection of antigen and cannulation of the thoracic duct was extended to 4 days. The normal responsiveness of rats subjected to selection by an intravenous injection of allogeneic cells was not established in the present experiments. However, Ford and Atkins (19) have recently shown that F_1 hybrid rats with an established thoracic duct fistula die from a GVH reaction after an injection of parental lymphocytes. The selected lymphocytes were fully active in the F_1 recipient but the remainder, which were sampled in the thoracic duct lymph, were specifically depressed by a factor of 50 in GVH assays.

An intravenous injection of antigen could depress the ability of TDL to generate antibody responses by selecting either marrow-derived (B) or thymus-derived (T) lymphocytes, since both B and T cells recirculate in the rat (6, 7). No information on the class of lymphocyte selected was obtained in the experiments with SRBC or tetanus toxoid although the strong selection exerted by SRBC emulsified in Freund's complete adjuvant which, by itself induces delayed hypersensitivity but little early antibody, suggests that a common cell type, possibly a T lymphocyte, may be required for the induction of both delayed hypersensitivity and antibody formation to SRBC.

Studies on rats immunized with DNP BGG were undertaken to determine whether both B and T lymphocytes could be selected. It was first shown that a single intravenous dose of the immunizing conjugate, injected 24 hr before cannulation of the thoracic duct, completely abolished the ability of TDL to generate an anti-DNP response after cell transfer and challenge with DNP BGG. Additional experiments established that an injection of the carrier protein alone (BGG) or of the hapten on a heterologous carrier (DNP HSA) signifi-

cantly reduced but did not abolish the response. No further reduction of the response was observed when a combined injection of BGG and DNP HSA was given. The interpretation of these results depends on the assumption that in rats, as in mice (17), anti-hapten responses depend upon collaboration between B lymphocytes carrying specificity for the hapten and T lymphocytes with specificity for the carrier. The fact that the adoptive anti-DNP response in rats is highly carrier specific[3] is some justification for this assumption. The failure of the combined injection of BGG and DNP HSA (designed to select carrier- and hapten-specific cells, respectively) to rival the suppressive effect of the homologous conjugate may possibly be explained by the presence of determinants on DNP BGG not represented on either of the other antigens. The selection achieved in vivo with the carrier alone is of some interest since antigen-coated columns, which will retain B lymphocytes, are very inefficient at removing carrier-specific lymphocytes (20).

The anatomical site into which lymphocytes are selected is presumably that in which the major part of the injected antigen localizes. After intravenous antigen this site is most likely the spleen; indeed, in rats receiving selecting injections of either SRBC or tetanus toxoid the spleens of the selected animals were the site of vigorous specific antibody formation. Although the high flux of recirculating lymphocytes through the spleen (21) probably makes it a particularly efficient organ for mediating antigen-induced selection, antigen deposited in subcutaneous depots in complete Freund's adjuvant was also effective. In the latter case the recirculating cells must have been engaged by antigen either in the skin itself or in the draining lymph nodes. The precise mechanism by which antigen traps lymphocytes and the anatomical zone within lymphoid tissue where this occurs are not known; nor is it clear whether the temporary, large scale hold up in lymphocyte recirculation which has been observed in antigen-stimulated lymph nodes (22) and spleen (21) is related to the specific selection observed in the present experiments.

Passively administered antibody was shown to prevent specific selection of recirculating cells by SRBC and may suppress immune responses by this mechanism. For example, passive antibody given 24 hr or more after antigen, that is, after selection has occurred, causes much less suppression of the immune response than when given with the antigen (23). In addition, the kinetics of responses suppressed by passive antibody indicate that a smaller number of cells respond initially (24).

A number of authors have observed the temporary inhibition of immune responses by prior antigen injection in which regional selection of recirculating lymphocytes may provide the common explanation. Thus, Schlossman et al. (25) showed that a single injection of DNP-lysine rendered previously sensitized guinea pigs specifically unreactive to a subsequent intradermal injection of

[3] Smith, M. E., and J. L. Gowans. 1972. Data to be published.

antigen, although the lymph node lymphocytes in such animals responded normally in the antigen-induced thymidine incorporation assay. Similarly, O'Toole and Davies (26) found that the response of the draining lymph nodes of mice to a subcutaneous injection of SRBC was reduced by a previous injection of the same antigen into the peritoneal cavity, a phenomenon they termed "preemption." The phenomenon of "immune deviation" (27) in which pretreatment with antigen prevents induction of delayed hypersensitivity probably also has a similar basis. Indeed, the present findings are particularly relevant to two examples where deviation was achieved in rats using SRBC or allogeneic lymphocytes as antigen. In the first, delayed hypersensitivity to SRBC was suppressed by giving antigen intravenously 24 hr before the intradermal injection of SRBC in Freund's complete adjuvant (28). In the second, the survival of renal allografts was considerably prolonged by injecting recipients with donor strain lymphocytes 24 hr before grafting (29). The development of cell-mediated immunity apparently requires the interaction of recirculating lymphocytes with antigen either at the site of sensitization or in the regional nodes (30) and intravenous antigen may suppress by reducing the number of recirculating cells available to interact with the sensitizing antigen.

SUMMARY

Thoracic duct lymphocytes (TDL) from normal rats will restore a primary antibody response to sheep erythrocytes (SRBC) in irradiated recipients and cause a graft-*versus*-host reaction in F_1 hybrid rats; lymphocytes from rats immunized with either tetanus toxoid or dinitrophenylated bovine gamma globulin (DNP BGG) will generate specific antibody after cell transfer and challenge. The ability of TDL to mediate each of these responses is severely depressed by giving a single intravenous dose of the specific antigen shortly before cannulation of the thoracic duct, although the lymphocyte donors themselves respond normally. The injection of antigen does not decrease the output of lymphocytes in the thoracic duct and the effect is specific for the antigen injected. The findings are most readily accounted for by assuming that small subpopulations of specific lymphocytes are selected from the recirculating pool by antigen which has localized in lymphoid tissue. The observation that passive antibody abolishes selection by SRBC supports this interpretation.

The strong selection exerted by a subcutaneous injection of SRBC in Freund's complete adjuvant, which induces delayed hypersensitivity but little early antibody, suggests that a common cell type may be involved in the induction of both delayed hypersensitivity and antibody formation. The anti-DNP antibody response generated by TDL from rats immunized with DNP BGG was abolished by a selecting injection of the homologous conjugate. The response was depressed to a smaller degree by injections of either BGG or dinitrophenylated human serum albumin, suggesting that carrier-specific (T) and hapten-specific (B) lymphocytes could be separately selected from the recirculating pool.

The regional selection of recirculating lymphocytes by antigen may explain a number of phenomena in which the prior injection of antigen has been found to inhibit a subsequent immune response.

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