

THE HOST CELL RESPONSE IN THE LOCAL GRAFT-*VERSUS*-HOST
REACTION INDUCED IN THE KIDNEYS OF F₁ RATS BY
PARENTAL THORACIC DUCT LYMPHOCYTES*

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Parental-type spleen cells or thoracic duct lymphocytes (TDL),¹ when injected under the renal capsules of genetically tolerant F₁ hybrid recipients, initiate an inflammatory reaction that results in the local destruction of tissue in the rat (1). Elkins has described many aspects of this local graft-*versus*-host (GVH) reaction, repeatedly stressing the requirement for hematogenous cells of the host to sustain an ongoing reaction (2-4). As described, the local GVH reaction appears to be an *in vivo* counterpart of a one-way mixed lymphocyte reaction in which the host cells serve primarily as the source of the histocompatibility (H) antigens that stimulate the donor cells to enlarge, divide, and destroy target tissues (5, 6). The situation *in vivo*, however, may well be more complex, particularly with respect to the role of the host cell. Host cell proliferation, for example, can be a conspicuous feature in both systemic (7-10) and local (11, 12) GVH reactions.

The present experiments were initiated to gain further information about the activities of the host cells. Attention was also directed to the need for free macrophages in the effector mechanism of the local GVH reaction in view of the requirement for these cells for the full expression of another form of cell-mediated immunity, the delayed hypersensitivity reaction (13, 14).

The results indicate that a high proportion of activated lymphoid cells within the reaction sites was host derived. Other observations suggest that all manifestations of the local GVH reaction could result from the interaction of parental-type TDL with F₁ cells of bone marrow origin. Tissue destruction could be effected in the apparent absence of macrophages. The possible effector activities of host lymphocytes are discussed.

Materials and Methods

Rats.—Highly inbred Lewis (Lew), Brown Norway (BN), and (Lewis × BN)F₁ hybrid (L/BN) rats were obtained from Microbiological Associates Inc., Bethesda, Md., and later

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¹ *Abbreviations used in this paper:* ALG, anti-lymphocyte globulin; BMC, bone marrow cells; GVH, graft-*versus*-host; H, histocompatibility; HBBS, Hanks' balanced salt solution; PAS, periodic acid-Schiff; PBS, phosphate-buffered saline; PE, peritoneal exudates; TC 199, tissue culture medium; TDL, thoracic duct lymphocytes; TdR³H, tritiated thymidine.

inbred at the Trudeau Institute, according to standard procedures. Both sexes, weighing between 150 and 250 g were used; cell grafts were always carried out between rats of the same sex. In experiments involving thymectomy or irradiation, Terramycin (oxytetracycline, Pfizer Laboratories Division, New York) was added to the drinking water, 125 mg/liter, from 48 hr before the procedure until the end of the experiment.

Thoracic Duct Lymphocytes.—Cannulation of the thoracic duct was performed according to the method of Gowans (15). Ringer's solution was mechanically pumped into the femoral veins of the restrained rats at a rate of 2 ml/hr. Lymph was collected overnight in either Ringer's solution or Dulbecco's phosphate-buffered saline (PBS) to which heparin, 20 units/ml, penicillin, 50 units/ml, and streptomycin 50 μ g/ml, were added. TDL were later washed in fresh PBS, centrifuged at 200 g, resuspended to appropriate volumes, and counted in a Coulter Counter Model B (Coulter Electronics, Inc., Hialeah, Fla.). Viability (eosin exclusion) was usually in excess of 98%.

Cell Suspensions.—TDL were prepared as above. Bone marrow cells (BMC) were flushed from the femurs and tibias with tissue culture medium 199 (TC 199) and strained through an 80 mesh stainless steel sieve. Peritoneal exudates (PE) were induced by injecting intraperitoneally 5 ml of a neutralized casein hydrolysate; exudate cells were collected 3 days later by washing the peritoneal cavities with Hanks' balanced salt solution (HBSS) containing 10 units of heparin/ml. Viabilities exceeded 80% (BMC) and 90% (PE). BMC and PE were counted in a hemacytometer, centrifuged at 200 g for 10 min, and resuspended in TC 199 (BMC) or HBSS (PE). Smears of the cell pellets of centrifuged aliquots were air-dried, fixed in absolute methanol, and later stained in May-Grünwald Giemsa.

Invasive cells were isolated from ongoing local GVH reactions by mincing kidneys in PBS and filtering through surgical gauze, or incubating minced kidney fragments in 0.2% pronase in PBS for 30 min at room temperature with the aid of a Teflon-covered magnetic stirring bar. In the second procedure, twice washed, filtered cell suspensions were further incubated for 60 min at 37°C in PBS or HBSS with 20% fetal calf serum to restore typical morphology.

Isotopic Labeling.—To estimate cell proliferation, TdR ³H (specific activity, 3Ci/mm, New England Nuclear Corp., Boston, Mass.) was rapidly injected intravenously at a dose of 1 μ c/g body weight; the kidneys were removed 30 min later. For extensive labeling of host cells, the same dose was divided into two to three equally spaced intravenous injections per day or continuously infused through a femoral vein cannula by means of a syringe-pump for 4 days.

Liquid Scintillation Counting.—The incorporation of TdR ³H was quantitated by extracting DNA from homogenized whole kidneys with 6 ml of hot trichloroacetic acid after repeated extractions with the cold acid. 1-ml aliquots in 10 ml of 2,5-diphenyloxazole in dioxane were counted in a Beckman liquid scintillation counter Model LS-100 (Beckman Instruments Inc., Fullerton, Calif.) at ambient temperature and recorded as counts per minute (cpm) per kidney after subtracting the cpm obtained in the contralateral noninjected kidney.

Radioautographs.—Slides of cell smears and tissue sections were dipped in Ilford liquid emulsion K-5, exposed in light-tight boxes for appropriate periods at 4°C, and developed in Kodak D-19 (Eastman Kodak Co., Rochester, N. Y.). Staining was carried out through the emulsion except for the periodic acid-Schiff (PAS) reaction that was performed before dipping.

Histology.—Tissues were fixed in 10% neutral buffered formalin and embedded in paraffin and plastic (16). Paraffin-embedded sections were cut at about 5 μ and stained with PAS-orange G-hematoxylin. Plastic-embedded material, 1–2 μ thick, was stained with toluidine blue-acid fuchsin.

Thymectomy.—The thymus was removed by suction through a sternum-splitting incision under ether anesthesia. After careful inspection for residual thymic tissue, the wounds were closed with skin clips. At the end of an experiment, the thoracic cavity was again inspected and suspicious looking tissue was studied histologically. Sections of lymph node and spleen were routinely examined for the absence of thymus-dependent zones. Evidence of incomplete

thymectomy was rarely found and data from such animals were discarded. 2-3 wk after thymectomy the rats received 800-900 R total body γ -irradiation and were reconstituted with 10^7 syngeneic BMC.

Irradiation.—A ^{137}Cs source provided total body γ -irradiation with a mean midphantom dose rate of 35.5 R/min and a maximum variation from the mean of $\pm 6\%$.

Mounting the Local GVH Reaction.—Under ether anesthesia, the left kidney was exteriorized through a flank incision and suspensions of parental-type TDL in 0.2 ml of TC 199 were injected under the renal capsule via a tuberculin syringe with a 30 gauge needle (1). The kidney was gently replaced and the incision closed in two layers with silk sutures and skin clips.

RESULTS

Morphology.—Gross and microscopic features of the renal GVH reaction, similar to those described by Elkins (1), were observed in the present studies. Three major characteristics were apparent by the 3rd day: the infiltration of renal parenchyma by mononuclear cells, proliferation of infiltrative cells, and renal tubular damage. Each roughly reflected the over-all intensity of the reaction, none being overly dominant. Histological scoring, however, proved unsatisfactory.

Fig. 1 shows a typical 7 day reaction. When stained with methyl green-pyronin, many of the larger cells had intensely pyroninophilic cytoplasm. Mitotic figures were readily found. Renal tubules were widely separated by infiltrative cells and showed indications of damage including cytoplasmic vacuolization and an apparent increase in the number of mitotic figures. Focal condensations of basement membranes containing only cellular remnants were considered indicative of more extensive injury; glomeruli were generally spared. Periglomerular and perivascular concentrations of infiltrative cells were best seen in early or mild reactions. Extension of the lesion into the renal medulla was rarely encountered.

When isolated from an ongoing reaction, a variety of lymphoid cells corresponding to those observed in the tissue sections was observed. By day 4 blast-like cells were conspicuous; about 18% of the cells were macrophages (Fig. 2). Granulocytes were generally infrequent.

The Contribution of Host Cells to the Local GVH Reaction.—After repeated or continuous isotopic labeling, presumptive hosts were continuously infused intravenously with nonradioactive thymidine (50 $\mu\text{g}/\text{ml}$, 2 ml/hr) to minimize possible salvage by donor cells of any TdR^3H made available by the death of labeled cells. Local GVH reactions were initiated 1-6 hr after beginning this infusion. Radioautographs prepared from tissue sections and smears of cells showed that by day 3, labeled (host) cells were present in the injection site under the renal capsule and also constituted a large part of the interstitial infiltrate. High proportions of the macrophages and lymphocytes isolated on day 4 were labeled (Table I, Fig. 3). Assessments at later intervals were unsatisfactory because of the decreasing grain density undoubtedly due to dilution by division. Some of this division was autochthonous as shown by the presence

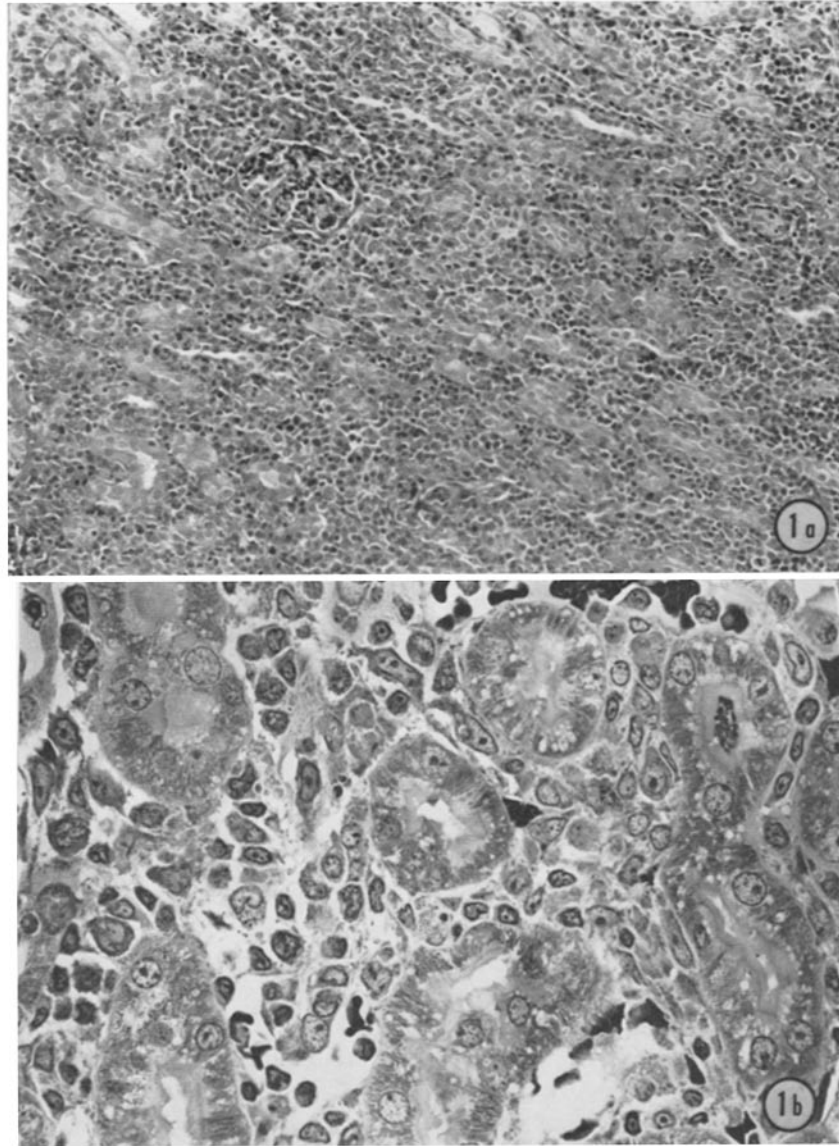


FIG. 1. 7 day local GVH reaction. (a) The renal capsule lies beyond the frame to the right. The accumulation of inflammatory cells extends well into the renal cortex and is associated with an apparent deletion of tubules. PAS-orange G-hematoxylin. $\times 150$. (b) Renal parenchyma has been infiltrated and in part replaced by a variety of mononuclear cells. Blast-like lymphoid elements with deeply staining cytoplasm and large nucleoli are conspicuous. Occasional cells resembling macrophages are also seen. Toluidine blue-acid fuchsin. Plastic embedded section. $\times 600$.

of labeled mitotic figures (Table I, Fig. 4). The proportion of mitotic figures in an otherwise replicate labeling experiment was increased by the intravenous injection of vinblastine sulfate, 2 mg/kg (Eli Lilly & Co., Indianapolis, Ind.)

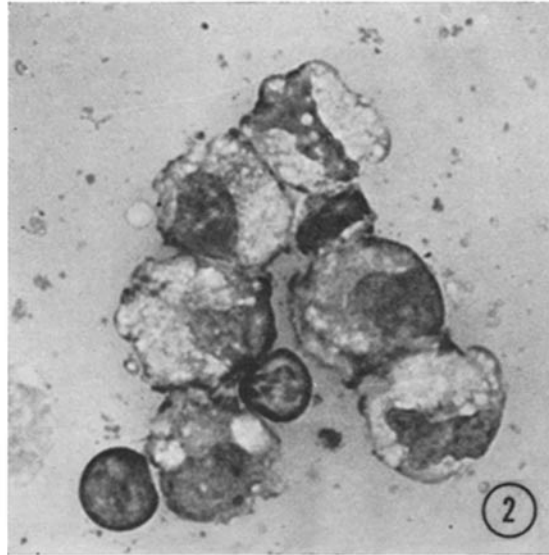


FIG. 2. A group of macrophages and lymphocytes isolated from a 6 day renal GVH reaction. Phagocytized polystyrene particles and cellular debris are seen in the macrophages. Toluidine blue-acid fuchsin. $\times 1500$.

TABLE I
Differential Counts and Radioautographic Labeling of Mononuclear Cells Isolated from Kidneys during Local GVH Reaction (Day 4)

Rat No.	Total counted	Small lymphocytes*	Large lymphocytes*	Macrophages	Mitotic figures			
1	614	46.6‡	41.6§	36.6	78.2	16.3	88.0	0.5
2	559	46.2	43.8	35.1	88.3	17.9	93.0	0.9
3	641	39.4	36.0	43.8	87.2	19.2	88.6	1.0

* Lymphocytes up to 8μ in diameter were classified as small, all others as large.

‡ Per cent of total.

§ Per cent labeled.

|| All of the mitotic figures seen were labeled. A cell with 5 or more grains overlying its nucleus was considered to be labeled; backgrounds in these preparations were almost nil.

6 hr before sacrifice. Of the 500 mitotic figures counted in radioautographs of plastic-embedded sections from three rats, 67% (range, 65–69%) were labeled and hence of host origin. The large and medium lymphocytes present must also be considered dividing cells because 26% of them were labeled in rats killed immediately after a single intravenous injection of TdR³H.

Quantitation of Cell Proliferation.—Local GVH reactions were assayed on the basis of the incorporation of TdR³H by proliferating cells. The effect of increasing doses of Lew TDL is shown in Fig. 5. Theoretically, only the number of potentially reactive cells should have been varied and the total number of cells

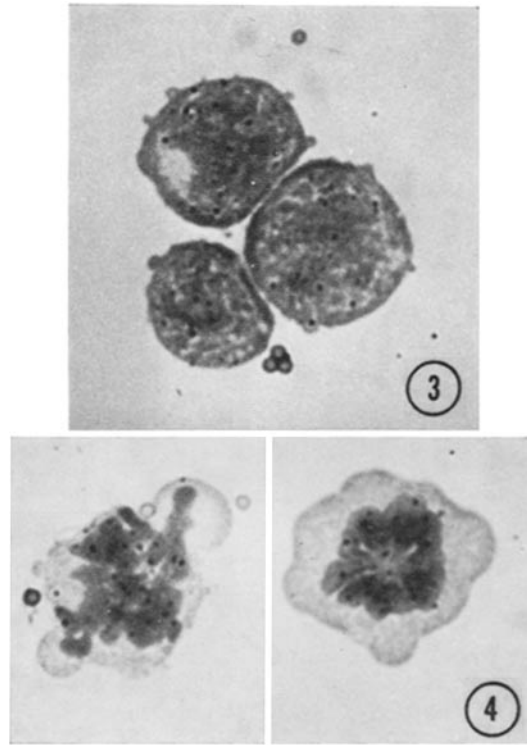


FIG. 3. Radioautograph showing labeled large lymphocytes isolated from a 4 day renal GVH reaction in a prelabeled host. The extracellular particles are spheres of polystyrene. $\times 2000$.

FIG. 4. Radioautograph showing labeled cells, presumably lymphocytes, in metaphase. $\times 2000$.

held constant. Even so, the response was essentially exponential. From these data 10^7 cells in 0.2 ml was selected as the most suitable concentration for later use. When recipients of this dose were sacrificed at increasing intervals the results shown in Fig. 6 were obtained. These correspond to data reported by Elkins (12).

The Effect of Ionizing Radiation.—Table II shows the influence of three levels of total body irradiation to presumptive hosts on the white blood cell (WBC) count and the local GVH reaction. Despite an apparent correlation,

some of the rats with very low WBC counts still showed a substantial renal uptake of TdR³H. This was further examined using one dose level (400 R) and varying the interval between irradiation and grafting. The WBC counts were about 20% of normal by the time of grafting. 3 days later, rats were sacrificed

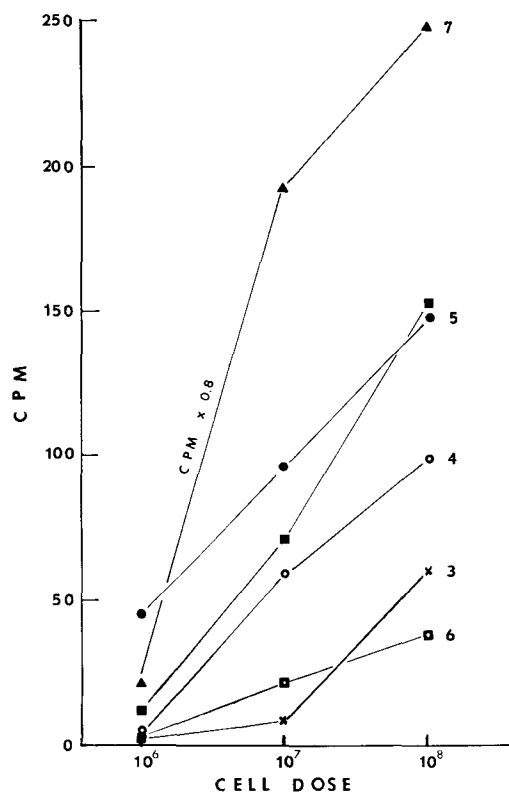


FIG. 5. The relationship of the intensity of the GVH reaction in F₁ kidneys to the number of donor TDL as measured by the incorporation of TdR³H (see text). CPM/kidney in thousands are shown on the ordinate and the number of parental cells on the abscissa. The day of sacrifice is indicated next to its respective result. The data for day 7 were reduced by a factor of 0.8 to accommodate the scale of the graph. Each point is the median of five rats.

after the usual injection of TdR³H. Some of the lowest activity occurred in the rats irradiated 8 days before grafting (Table III); however, the results were not significant at the 5% level of probability (χ^2). Taking the data overall, the statistical correlation between the WBC count and the magnitude of the GVH reaction was low.

The Thymectomized Host.—By 30 days after reconstitution with BMC, the mean WBC/mm³ (\pm SE) was 6200 (\pm 310) in 43 thymectomized rats and 14,100 (\pm 310) in an equal number of controls. In agreement with Elkins (3),

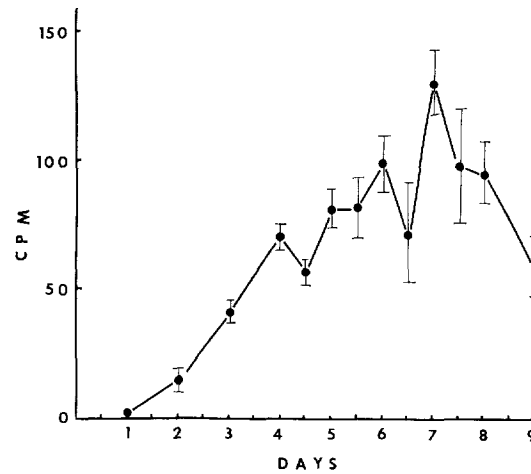


FIG. 6. The intensity of the renal GVH reaction as measured by the incorporation of TdR³H at various intervals after the subcapsular injection of 10^7 parental thoracic duct cells. Aggregate data from replicate experiments are shown. CPM/kidney in thousands are shown on the ordinate and the time in days on the abscissa. Each point is the mean of 8-12 rats \pm standard error.

TABLE II

*Intensity of Local Graft-vs.-Host Reactions on Day 5 in Animals Exposed to Varying Doses of Total Body Irradiation 24 hr before Initiating the Reactions**

Dose	WBC count‡	TdR ³ H uptake‡
<i>R</i>		<i>cpm</i>
0	18.4	74.6
	15.5	139.5
	11.3	147.6
	16.4	121.0
200	3.4	43.9
	4.6	68.2
	2.6	77.3
	2.9	72.7
400	1.5	49.0
	2.5	12.8
	2.3	61.3
	3.0	43.4
800	11.5	43.1
	9.5	12.2
	6.5	26.1

* Kidneys of F₁ hosts injected with 10^7 parental thoracic duct cells; rats sacrificed 30 min after i.v. TdR³H.

‡ WBC and cpm expressed in thousands.

the local GVH reactions in such animals did not differ quantitatively (Fig. 7) or histologically (Fig. 8) from those in intact rats. Again it is apparent that

TABLE III
*Local Graft-vs.-Host Reaction 3 Days after 400 R Total Body ^{137}Cs Irradiation at Varying Intervals before Receiving Grafts**

No. of rats	Time of irradiation†	WBC counts‡	TdR ³ H uptake§
	<i>day</i>		<i>cpm</i>
8	-8	4.0 (1.0-8.9)	40.8 (8.7-57.9)
4	-4	9.8 (5.7-18.3)	38.3 (33.1-49.9)
3	-2	4.3 (2.5-8.5)	33.9 (31.1-44.6)
4	-1	3.5 (2.5-5.6)	48.5 (24.8-67.9)
4	0	3.3 (1.3-4.1)	47.9 (22.6-62.5)
20	Unirradiated controls	16.2 (6.4-24.4)	60.4 (15.8-75.2)

* F₁ kidneys harvested 3 days after the injection of 10⁷ parental thoracic duct cells; rats sacrificed 30 min after i.v. TdR³H.

† Number of days preceding grafting; rats irradiated at 0 time received their grafts about 1 hr after irradiation.

‡ WBC and cpm, median and range in thousands. The coefficient of correlation (r) between all cell counts and their respective cpm = +0.44.

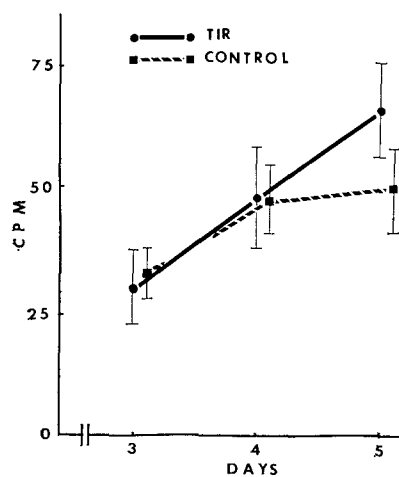


FIG. 7. The intensity of the renal GVH reaction as measured by the incorporation of TdR³H in normal and thymectomized, irradiated and bone marrow reconstituted (TIR), F₁ hosts on various days after the subcapsular injection of 10⁷ parental thoracic duct cells. CPM/kidney in thousands are shown on the ordinate and the time in days on the abscissa. Each point represents the mean of eight rats \pm 95% confidence interval.

the WBC count can be considerably reduced without influence on the intensity of the reaction. From this experiment it also appears probable that cells of bone marrow origin can provide whatever host functions are necessary for a

full-scale local GVH reaction. Additional data concerning this point were obtained by employing anti-lymphocyte globulin (ALG) which is said to act preferentially upon lymphocytes that receive thymic processing rather than those derived solely from bone marrow (17). The immunosuppressive potency of this batch of ALG was proven earlier by the ability of 1 ml injected intravenously to totally suppress the tuberculin reaction in highly sensitive rats. Hence, 1 ml of rabbit anti-rat ALG was given intravenously to L/BN hosts daily from 1 hr before grafting to the end of the experiment. This treatment did not diminish the ensuing reactions. In another experiment, parental-type

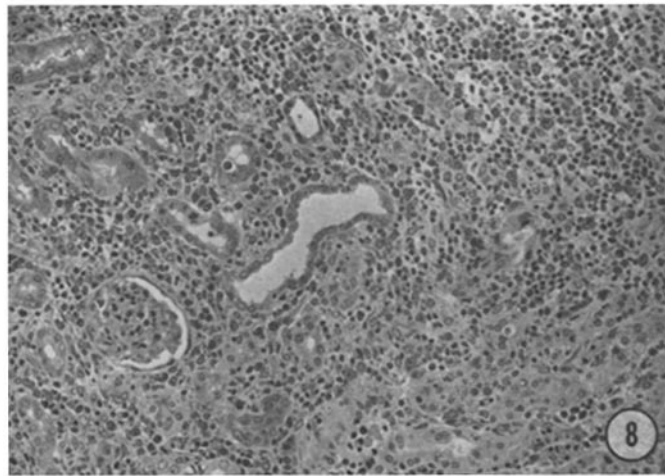


FIG. 8. Invasive-destructive reaction in renal cortex of thymectomized host 7 days after the subcapsular injection of parental TDL. PAS-orange G-hematoxylin, $\times 150$.

TDL (10^7 /ml of TC 199) were incubated for 10 min at room temperature in ALG (final concentration, 2 mg protein/ml). The cells were washed three times in fresh medium and inoculated as usual. In this instance, the local GVH reaction was manifestly depressed when examined histologically.

The Effect of Mixing Defined Populations of Parental and F_1 Cells.—TDL was collected overnight from Lew and L/BN rats. During processing, cells adhering to the bottoms of the flasks were not disturbed. Specimens contaminated by blood were discarded. BMC were obtained from F_1 rats after 3 days of thoracic duct drainage to achieve a degree of lymphocyte depletion. Peritoneal exudates raised in F_1 rats were used as a macrophage-rich source.

F_1 TDL, BMC, or PE cells were respectively combined with parental-type TDL in a 2:1 ratio. The cell mixtures were then injected under the renal capsules of F_1 recipients that had been exposed to 1200 R total body γ -irradiation 24 hr earlier. Table IV shows that at 5 and 7 days after grafting the

highest counts were achieved with the mixture of parental and F₁ TDL. The median values obtained with BMC were roughly half of the foregoing but the overlap on day 7 suggests that the differences are probably not significant. The PE cells did not yield values appreciably higher than the control of parental-type TDL alone.

Histologically, the reactions due to combined parental and F₁ TDL were brisk with unequivocal destruction of renal parenchyma (Fig. 9). Macrophages were not detected either in the inocula used in this experiment or among cells isolated from the kidneys in replicate experiments.

The Specificity of Tissue Destruction.—2 wk after thymectomy young adult

TABLE IV
Intensity of Graft-vs.-Host Reactions in Kidneys of Irradiated (1200 R) F₁ Rats Injected with Mixtures of Parental Lymphocytes and F₁ Cells from Different Sources

Time of graft*	Source of F ₁ Cells											
	Control‡			Thoracic duct lymph			Peritoneal exudate			Bone marrow		
	Sample size	Median§	Range	Sample size	Median§	Range	Sample size	Median§	Range	Sample size	Median§	Range
5				4	56.0	80.3				4	22.9	28.6
						15.9						11.9
7	10	6.9	15.9	3	100.1	104.0	4	9.2	17.8	5	55.5	83.5
			2.6			76.3						54.1

* 10^7 parental and 2×10^7 F₁ thoracic duct cells were mixed in vitro as specified immediately before injection.

‡ 10^7 parental cells; no F₁ cells.

§ Median and range are expressed in $\text{cpm} \times 10^{-3}$ (see text).

Lew rats were exposed to 800–900 R total body γ -irradiation and reconstituted with 10^7 L/BN BMC. Although the quantitative degree of chimerism was not determined, the data in Table V showing reactions of comparable intensity in the chimeric and conventional hosts can only be explained by the interaction of the parental-type inocula with F₁-type leukocytes. Tissue destruction (Fig. 10) was equally conspicuous in both host types. These results agree with recently reported data (3).

In another experiment, 1 day after the exposure of Lew and L/BN rats to 1200 R total body γ -irradiation, a mixture of 10^7 parental and 10^7 F₁-type TDL was injected under the renal capsule. 7 days later, reactions of comparable intensity were found in both host genotypes.

DISCUSSION

A proliferative response constituted predominantly of host-type cells was seen as early as the 4th day of the intrarenal GVH reaction in F₁ hosts that had been labeled with TdR³H before receiving their grafts of parental-type TDL.

This conclusion is supported by (a) the preponderance of labeled mitotic figures and (b) the finding that about 87% of the large and medium lymphocytes in the lesions were labeled in the radioautographs. These cells, taken as a class

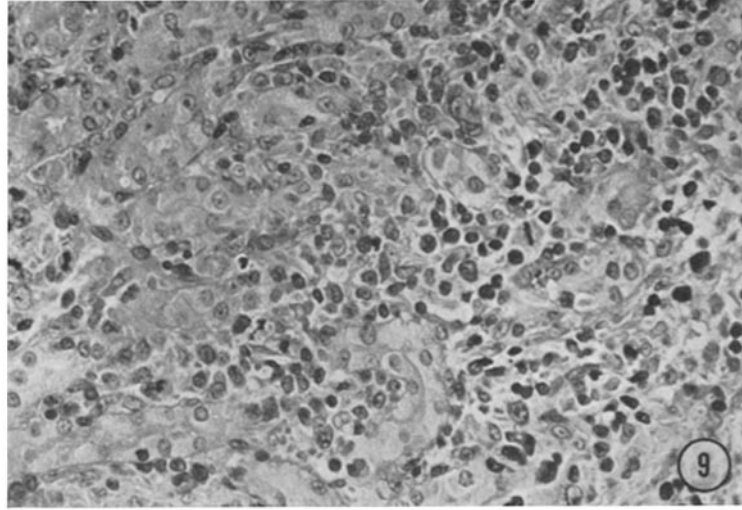


FIG. 9. Invasive-destructive reaction in renal cortex of heavily irradiated F_1 host 7 days after the subcapsular injection of a combined inoculum of parental and F_1 TDL. Toluidine blue-acid fuchsin. $\times 375$.

TABLE V

Local Graft-vs.-Host Reactions Induced by Parental-Type Lymphocytes in Chimeric† Hosts*

Time of graft <i>day</i>	Host type	No. of hosts	cpm/kidney $\times 10^{-3}$	
			<i>mean</i>	<i>SE</i>
5	Chimeric‡	5	50.6 \pm 8.2	
	Control§	5	35.7 \pm 6	(0.3 < <i>P</i> < 0.2)
7	Chimeric	4	91.2 \pm 17.5	
	Control	5	54.9 \pm 10.9	(0.2 < <i>P</i> < 0.1)

* 10^7 Lewis TDL injected under renal capsule.

† Lewis rats, thymectomized, lethally irradiated and reconstituted with L/BN bone marrow (see text).

§ Conventional (L/BN) F_1 hosts.

|| Student's *t* test; observed differences between the means of the chimeric and conventional hosts are not significant.

have been shown to be actively dividing by others (18, 19) and in the present experiments by their ability to incorporate TdR^3H . A precise sequence of donor and host cell proliferation has not yet been established with the present techniques, but the early dominance of host cell activity is in accord with observa-

tions in systemic GVH reactions where donor cell proliferation is known to occur early (10, 18) before host cell proliferation becomes dominant (7-10). It is thus not surprising to find a corresponding sequence in the renal GVH reaction.

Results temporally different from the above were reported by Elkins (12) who used sex chromosomes to identify the source of cells in mitosis in the kidney reaction sites and found that donor cell proliferation was predominant until about the 9th day. An explanation for these differing results is not apparent, particularly since the same strains of rats were used in both studies. A possible

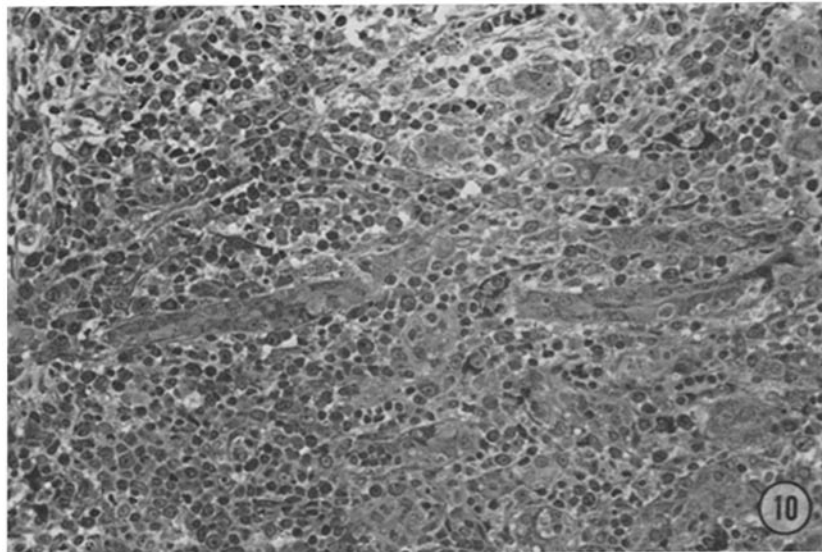


FIG. 10. Invasive-destructive reaction (7 days) in renal cortex of chimeric parental-type host reconstituted with F₁ bone marrow (see text). Toluidine blue-acid fuchsin. Plastic embedded section. $\times 150$.

source of inaccuracy in the present study could be the adventitious isotopic labeling of dividing donor cells due to the reutilization of TdR³H released from dead host cells. This contingency is believed to have been minimized by maintaining the hosts on a continuous intravenous infusion of nonradioactive thymidine on a schedule that has proved to be highly effective for this purpose in this laboratory. The discussion is accordingly based on the present observations. An explanation for the existing discrepancy is, however, being sought experimentally.

Taken with the isotopic labeling data, the abundance of blast-like cells in the renal GVH reactions probably represents activation of host lymphocytes. Lymphocyte activation can result in destructive activity directed against target cells even when achieved nonspecifically by the exposure of lymphocytes to phytohemagglutinin (20), for example. But the exposure of lymphocytes to

allogeneic antigens is said to result in a specific (6, 21) cytotoxic activity. Other evidence, however, indicates that such specifically stimulated lymphocytes can also launch a nonspecific attack on target tissues (22-26). Tissue destruction in the renal GVH reaction is clearly nonspecific, as reported by others (4, 12) and confirmed in the present studies by inducing renal damage with Lew TDL in immunologically unresponsive Lew hosts whose nucleated white blood cells were derived from L/BN bone marrow. These observations raise the intriguing possibility that the activation of the host's own lymphocytes can result in the creation of a population of effector cells which contributes to the destruction of tissue that is characteristic of GVH reactions in general.

The self-limiting course of the local GVH reaction may be attributable to a low level of proliferation among donor cells coupled with the loss of such cells from the kidney. An observation bearing on this point is that a high proportion of the donor cells emigrate from the host's kidney soon after injection (Volkman, unpublished experiments). Possibly this dissipation and dissemination of the grafted cells continues until they no longer form a critical mass in the kidney or elsewhere. More specifically, the eventual ratios of donor-to-host cells may favor the induction of tolerance on the part of donor cells with respect to H antigens of the host. The possibility that the progress of the reaction is inhibited by the formation of a circulating agent does not seem likely in view of the finding that a brisk second GVH reaction can be induced in the contralateral kidney while the first is in decline (Volkman, unpublished observations).

Substantial local GVH reactions could still be induced after 400 R total body irradiation of host rats indicating that host cell functions are not dependent upon cells with a high degree of radiosensitivity. Some of these reactions were obtained despite an 80% reduction in the WBC count. One explanation for this may be that migratory leukocytes are concentrated within the inflammatory focus and thus compensate for their reduced numbers in the blood.

On the basis of certain physiological and immunological properties, lymphocytes have been divided into two major classes: thymus-derived (T) cells and bone marrow-derived (B) cells (27-31). In this connection, it is of interest that in B hosts, (presumptive F₁ hosts that were thymectomized, lethally irradiated, and reconstituted with bone marrow) local GVH reactions were quantitatively comparable to those in intact controls. In addition, a mixed inoculum of host-type BMC and parental-type TDL when injected under the renal capsules of heavily irradiated hosts yielded results that by the 7th day were again comparable with those obtained by mixtures of parental and F₁ TDL. Thus B cells appear capable of appropriate host functions in the renal GVH reaction. Although the data do not indicate whether this capacity resides exclusively with these cells, it nevertheless seems plausible to infer that B cells are an important component of the activated cells in a local GVH reaction in the intact host. It is interesting that the association of B cells with the host proliferative

response has been demonstrated in the spleen during a systemic GVH reaction (32). Synergism between T and B cells has been reported in this situation (32–34) although opposing views have been expressed (35–37).

Despite the predominance of lymphoid cells in the reaction sites, a relatively high proportion of macrophages (16–19%) was found. A typical reaction was, nevertheless, obtained in the apparent absence of such cells. By contrast, mononuclear phagocytes are said to be necessary for optimal activity in a mixed lymphocyte reaction *in vitro* (38–40). If events *in vivo* do indeed correspond to those *in vitro*, it follows that whatever function the mononuclear phagocyte performs *in vitro* can be effected by another cell or tissue in the intact animal. Taking another *in vivo* model of cell-mediated immunity, cutaneous delayed hypersensitivity, recent evidence indicates that the reaction cannot be fully expressed in the absence of macrophages (13, 14). One possible explanation for this seeming paradox is that the parameters for assay of the local GVH reaction, cell proliferation and tissue destruction, are related solely to lymphocytic functions, whereas the “bump,” largely due to the local hyperemia and interstitial edema, that is measured in the delayed hypersensitivity reaction, depends upon the liberation of pharmacological mediators for which macrophages may be responsible. A second is that the effector cell functions under consideration may not be performed exclusively by a particular class of cells. Thus, the lymphoid cells involved in GVH reactions after appropriate stimulation *in vivo*, may develop functional characteristics in common with macrophages.

The belief that migratory white blood cells provide the critical source of stimulatory H antigens in the local GVH reaction rests in part on the observed correlation between the WBC count and the intensity of the reaction in irradiated hosts (2, 3). In the present experiments, however, this correlation proved to be very weak. If the renal GVH reaction is largely manifested as a host-proliferative response, one can argue that the impairment of the capacity of surviving cells to divide, rather than a reduction in their numbers, is the more important effect of irradiation. Thus, the stimulation of donor cells by allogeneic antigens in kidney tissue could escape detection by procedures that apparently measure the consequences of host cell proliferation. In this connection, Strober and Gowans (41) reported that immunocompetent cells can become sensitized by a relatively brief transit through allogeneic kidney. On the other hand, the potent immunogenicity of the white blood cell is emphatically underscored by the positive GVH reactions in parental-type kidneys that could have resulted only from the interaction of immunocompetent parental and F₁-type leukocytes, as observed in the present study and by others (3, 4). Taken with the data indicating an early host-proliferative response, however, this experiment still would not exclude the possibility that kidney tissue, too, is immunogenic in the intact host and the question clearly requires further appraisal.

SUMMARY

Radioautographs of infiltrative cells in the kidneys of (Lewis \times BN) F_1 rats labeled with tritiated thymidine (TdR 3 H) before the subcapsular injection of parental (Lewis) thoracic duct lymphocytes (TDL) showed a predominantly host-proliferative response by 4 days after grafting. The immediate renal incorporation of TdR 3 H was used to measure the local graft-vs.-host (GVH) reactions. Substantial reactions could still be induced in the face of the considerable degree of leukopenia after 400 R whole body γ -irradiation. These results suggest that radioresistant cells are capable of carrying on the appropriate host activities and that the weakness of GVH reactions induced after higher doses of irradiation may be due to impairment of the mitotic mechanism of host cells. The importance of circulating leukocytes as a source of immunogenic stimulation was nevertheless substantiated by inducing local GVH reactions with Lew TDL in chimeric parental-type rats that had been repopulated with F_1 bone marrow. This result also emphasizes the nonspecific nature of tissue destruction in the renal GVH reaction in confirmation of Elkins. In this and other situations in which B cells were the predominant F_1 type elements available for interaction with parental-type TDL the reactions were nearly equivalent or equivalent to those in the appropriate controls.

Typical local GVH reactions could be induced in heavily irradiated hosts by an inoculum of combined parental and F_1 -type TDL in the apparent absence of mononuclear phagocytes.

The possible relationship between the activation of host lymphocytes, the involvement of B cells, and the nonspecific nature of tissue damage in the renal GVH is discussed.

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