REDISTRIBUTION OF RENAL ALLOGRAFT-RESPONDING LEUKOCYTES DURING REJECTION

II. Kinetics and Specificity*

By ARTO NEMLANDER, ANU SOOTS, EEVA VON WILLEBRAND, BO HUSBERG, and PEKKA HÄYRY‡

From the Transplantation Laboratory, Fourth Department of Surgery, University of Helsinki, Helsinki, Finland, and Department of Surgery, Malmö Allmänna Sjukhuset, Malmö, Sweden

Based on adoptive transfer of allograft immunity (1, 2), Gowans (3) suggested that "activated" lymphoid cells are released from the regional lymph nodes, recirculate in the blood, enter the graft and destroy it by some still unknown mechanisms.

Several studies (4, 5) have failed to demonstrate any preferential homing of immune spleen cells to a relevant allograft. Later studies using extensive purification of allograft-responding lymphoid cells, e.g., passage through thoracic duct (6) or selective labeling of only those cells proliferating in response to the allograft (7), have made it possible to demonstrate a small, though significant, incremental localization of allograft-responding leukocytes in a relevant, compared with an irrelevant, allograft or an autograft. A common finding both in these and further investigations (8–11) has, however, been that the actual amount of indicator label reaching the allograft has been, on most occasions, very small (<1%), whereas most of the injected label has been recovered from the recipient liver, lung, and spleen (6, 12).

The small rate of specific homing observed in these experiments is clearly discrepant with the observation that large numbers of inflammatory cells leave the allograft during rejection (13, 14). Although it has been documented that the inflammatory response of rejection is accompanied by a massive proliferation of lymphoid and even nonlymphoid cells *in situ* (15, 16), this is not enough to explain this discrepancy.

Recirculation and homing of leukocytes is largely dependent on interactions between the surface of the migrating cell and that of the vascular endothelium (17). Even a slight injury of the leukocyte surface, e.g., one produced by a mild treatment with *N*-acetyl-neuraminidase (18) or alloantibody (19), drastically alters the recirculation pattern of normal and immune cells. In all homing experiments referred to above, the test cells were isolated, washed, or otherwise treated in vitro and reinfused to the recipient. It was therefore possible that the in vitro procedures had caused artefactual relocalization of the labeled cells and thus given artefactual data on the localization of the cells in the grafts.

We recently described a model that makes it possible to investigate the traffic of

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‡ To whom correspondence should be addressed at the Transplantation Laboratory, University of Helsinki, Haartmaninkatu 3, SF 00290 Helsinki 29, Finland.

allograft-responding leukocytes between the graft and the host without handling of these cells in vitro (20). By disconnecting the vascular and lymphatic connections between the graft and the host, the proliferating cells are selectively labeled either in the graft or in the host with [^{3}H]thymidine. The label is neutralized 15 min later by systemic infusion of a 1,000-fold excess of cold thymidine, and the circulation is reestablished. The traffic of labeled cells is quantitated by autoradiography from single cell dispersates of the graft and the various lymphoid organs of the host. The type of proliferating cells travelling to each direction can be identified by combining cytological morphology to autoradiography.

With this model we reinvestigated the recirculation pattern of allograft-responding inflammatory leukocytes between the graft and the host and the types of inflammatory (white) cells participating in the response. The rate of traffic to the graft appeared far higher than in any one of the previous estimates. An entirely new observation is that after an initial exponential increase, the traffic in both directions slows down and tapers off 4–5 d after the transplantation, i.e., 3 d before the blood circulation to the graft is compromised.

Materials and Methods

Experimental Animals. The nuclei for the AO (AgB2;RT1^v), DA (AgB4;RT1^a), and BN (AgB3;RT1^n) rat strains were obtained from Professor J. L. Gowans, Dunn School of Pathology, Oxford, England; the nucleus for the Lewis strain (AgB1;RT1^l) was obtained from Dr. J. Howard, Agricultural Research Council, Institute of Animal Physiology, Babraham, Cambridge, England; and the WF (AgB2; RT1^v) strain was obtained from Professor O. Sjögren, Department of Tumor Biology, University of Lund, Lund, Sweden. All rats were bred in our colony. Rats weighing 200–250 g were used for the experiments.

Operative Procedures. The operative setup has been illustrated in detail (20). In short, the donor kidney was removed, perfused from blood with heparinized phosphate-buffered saline, and transplanted to recipient abdominal aorta and inferior vena cava by end-to-side anastomosis. On most occasions, recipient right kidney was removed during the same operation. Alternatively, the kidney vessels were exposed, the kidney was perfused intravenously (to avoid arterial thrombosis), and after appropriate ischemia time, the circulation was reestablished. On these occasions the right kidney served as an "autograft."

Labeling Procedures. At different times after transplantation, a relaparotomy was performed, and the vessels of the kidney allograft (and autograft) were reexposed. The kidney artery, vein, and draining lymphatics were clamped at the kidney pedicle. Either the recipient or the graft was selectively labeled with [^{3}H]thymidine (New England Nuclear, Boston, MA; sp act, 6.7 Ci/mmol). When labeling the graft, the intra-arterial route was used; the recipient was labeled intravenously. The dose for the recipient was 1 μCi/g body weight. One tenth of the systemic label was used for the graft. After 15 min, the label was neutralized by injecting 3 mg (an ~1,000-fold excess) of cold thymidine into the systemic circulation. The clamp was opened and the circulation to the graft reestablished. We previously demonstrated (20) that an ischemia time of up to 30 min does not influence the function of the rat kidney graft, nor induce any nonspecific inflammation in the transplant. As the redistribution of labeled cells reaches a balance at 18 h (20), this was taken as the end point for the redistribution. The recipient was killed 18 h after labeling, and appropriate organs were removed for analysis.

Dispersion of the Allografts. As described in detail before (20), the transplant was perfused with heparinized phosphate-buffered saline, removed, minced with a scalpel, and immersed in Hepes-buffered RPMI 1640 medium (Gibco Bio-Cult, Glasgow, Scotland) containing collagenase (0.2 mg/ml; Worthington Biochemical Co., Freehold, NJ) and DNAse (0.2 mg/ml; Sigma Chemical Co., St. Louis, MO). The material was incubated at 37°C for 30 min with a magnetic stirrer until most of the tissue was dissolved. The remaining clumps were removed via sedimentation, the total recovery of nucleated cells was determined with a hemocytometer, and cell smears were made for differential counts.

Preparation of Leukocytes from the Recipient Lymphoid Organs. Heparinized blood was collected from the recipient, and the erythrocytes were hemolyzed with aqueous solution of 0.83% ammonium chloride. Spleen leukocytes were obtained by teasing apart the organ, removing the clumps via sedimentation, and hemolyzing the residual erythrocytes as above. The total recovery of nucleated leukocytes was determined with a hemocytometer, and cell smears were made for differential counts.

Differential Counts. Differential counts were performed from May-Grünwald-Giemsa (MGG)-stained cytocentrifuged (Shandon Scientific Co., London) cell smears. Blast cells (lymphoblasts, plasmablasts, and plasma cells), lymphocytes, monocytes, macrophages, and granulocytes were recognized by commonly accepted morphological criteria (21). The recovery of inflammatory cells of a given type was calculated from the total recovery of nucleated cells and from the percentual distribution of inflammatory (white) cells in the differential counts.

Autoradiograms. The autoradiograms were performed on the cytocentrifuged cells smears with Kodak AR-10 stripping film (Eastman Kodak Co., Rochester, NY), exposed for 2 wk, and stained with Giemsa stain. The different types of labeled cells, i.e., blast cells, lymphocytes, mononuclear phagocytes, granulocytes, and parenchymal (predominantly endothelial) cells were recognized again by morphological criteria. The total recovery of labeled cells from each particular organ was calculated on the basis of total leukocyte recovery and the fraction of labeled inflammatory cells.

Quantitation of Renal Blood Flow. Allogenic kidneys were transplanted across an AgB barrier, as described by Bergman and Husberg (22) from WF to BN strain and from DA to WF strain. The technique involves the placement of a catheter in such a way that permanent access to the renal arterial system is obtained.

The blood flow through the kidney was recorded daily by the Xenon infusion (Byk-Mallinckrodt Holland, 17–35 mCi/ml xenon, diluted 1/10 in saline). T ½ washout time was recorded with a Selektronic Analyser 45-22 (Nuclear Data Electronic AB, Horsholm, Denmark).

Results

Total Traffic of Labeled Leukocytes between the Graft and the Host. The net traffic of allograft-responding leukocytes between the graft and the host was investigated with 19 AO recipients of DA kidney allografts. The proliferating cells were selectively labeled in the host or in the graft. The rats were killed 18 h thereafter, on days 0, 1, 2, 3, 4, 5, and 6 after the transplantation.

When the label was injected into the recipient, excluding the graft (Fig. 1), only a background recovery of labeled cells was observed in the spleen and blood for the first 3 d after the transplantation. Thereafter, the frequency of labeled cells increased exponentially in both organs. The first host-derived labeled cells were seen in the graft already on the day of transplantation. The recovery of labeled cells increased exponentially up to day 4, whereafter the recovery of host-derived labeled cells in the graft rapidly declined. This decline was not due to the exhaustion of supply, as the frequency of labeled cells in the spleen and blood still kept increasing.

When the label was injected into the graft, excluding the recipient (Fig. 2), an exponential increase in the number of labeled cells was observed in the graft up to days 5 or 6 after the transplantation, whereafter it leveled off. The first graft-derived labeled cells appeared in the recipient blood and spleen already on the 1st d. There was an initial exponential increase in the number of graft-derived labeled cells in the host, and, 2–4 d after the transplantation, nearly as many labeled cells left the graft for blood and spleen as remained *in situ*, provided that ~30% of the inflammatory cells were recovered from the allograft with the present method of disaggregation (23). Thereafter, the recovery of graft-derived labeled cells in the host spleen and blood rapidly declined.

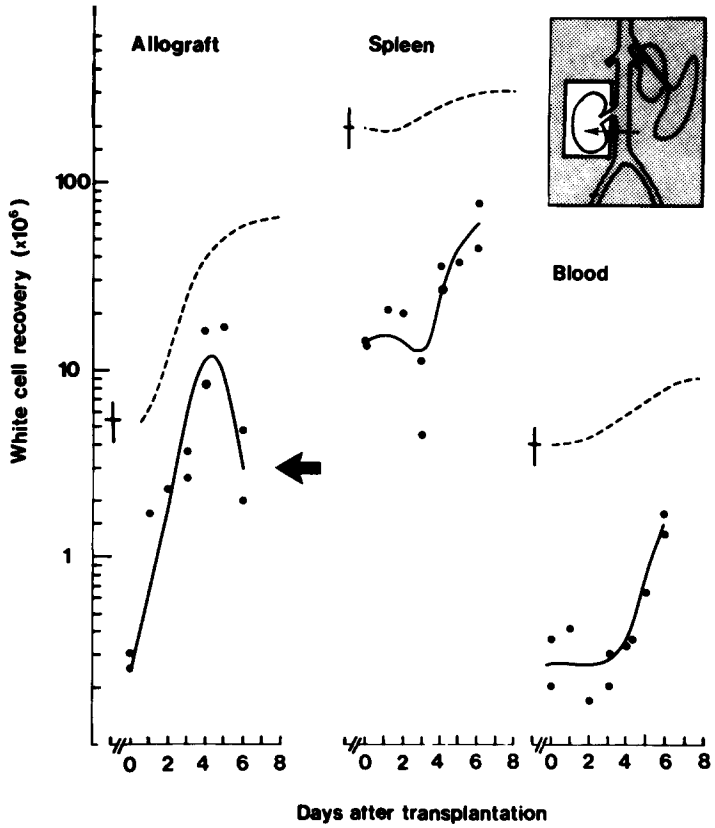

FIG. 1. Recovery of white cells (-----) and labeled white cells (—, ●) from DA renal allograft in AO rat from the recipient spleen and from 1 ml of recipient blood after selective labeling of the recipient and excluding the graft. Note that, while the recovery of the host-derived labeled cells in the allograft declined 4 d after the transplantation, the frequence of labeled cells in the recipient spleen and blood still kept increasing. The normal passenger cell background level in a normal DA kidney and normal white cell content in the DA spleen and blood (± SD) is indicated in the left of each graph. Arrows indicate the direction of net traffic of labeled cells.

Structure of Inflammation and Composition of Labeled Cells Entering the Graft. Differential distribution of graft-infiltrating inflammatory cells and labeled inflammatory cells was done in 11 cases after transplantation of a DA renal allograft to AO recipient.

The first inflammatory cells detected over the passenger background level in the allograft (Fig. 3) were monocytes and lymphocytes. The first blast cells appeared in the graft between days 1 and 2 after the transplantation. The peak of the blastogenic response was observed on day 6, whereafter the recovery of blast cells in the graft declined. The recovery of *in situ* lymphocytes leveled off between days 5 and 6, while the frequency of mononuclear phagocytes steadily increased until the graft was lost. Granulocytes were relatively few at the beginning and increased in number only later, when necrotic changes began to appear in the transplant.

The first host-derived labeled inflammatory cells (Fig. 3) were mononuclear phagocytes and small lymphocytes. Significant numbers of labeled monocytes appeared in the graft during the immediate posttransplantation period; from day 3 onwards,

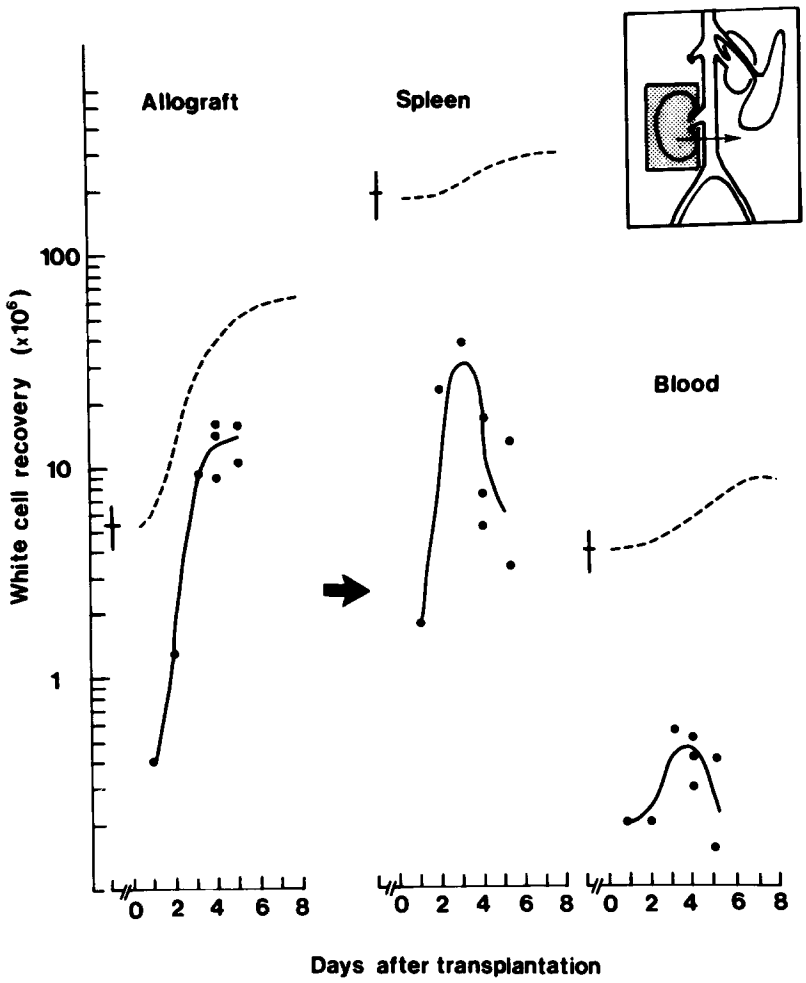

FIG. 2. Recovery of white cells (- - -) and labeled white cells (—, ●) from DA to AO renal allograft, the recipient spleen and 1 ml of recipient blood after selectively labeling the transplant and excluding the recipient. The normal passenger cell background level in a normal DA kidney and normal white cell content in the DA spleen and blood (± SD) is indicated in the left of each graph. Arrows indicate the direction of net traffic of labeled cells.

labeled lymphoid cells, consisting of approximately equal numbers of blast cells and lymphocytes, dominated. The influx of all types of labeled cells to the graft declined 5 d after the transplantation, when the total number of inflammatory cells in the graft was still rapidly increasing. No labeled granulocytes were seen to enter the graft within the observation period of 6 d.

Composition of Labeled Inflammatory Cells Leaving the Graft for Host Spleen. A similar analysis made from spleen demonstrated that the size of the recipient spleen increased by 50% during the early posttransplantation period (Fig. 4). This increase was mainly due to lymphocytes, although, especially during later stages of rejection, blast cells and cells of the mononuclear phagocyte series also contributed to the increase.

Autoradiographic analysis demonstrated that all major types of labeled inflam-

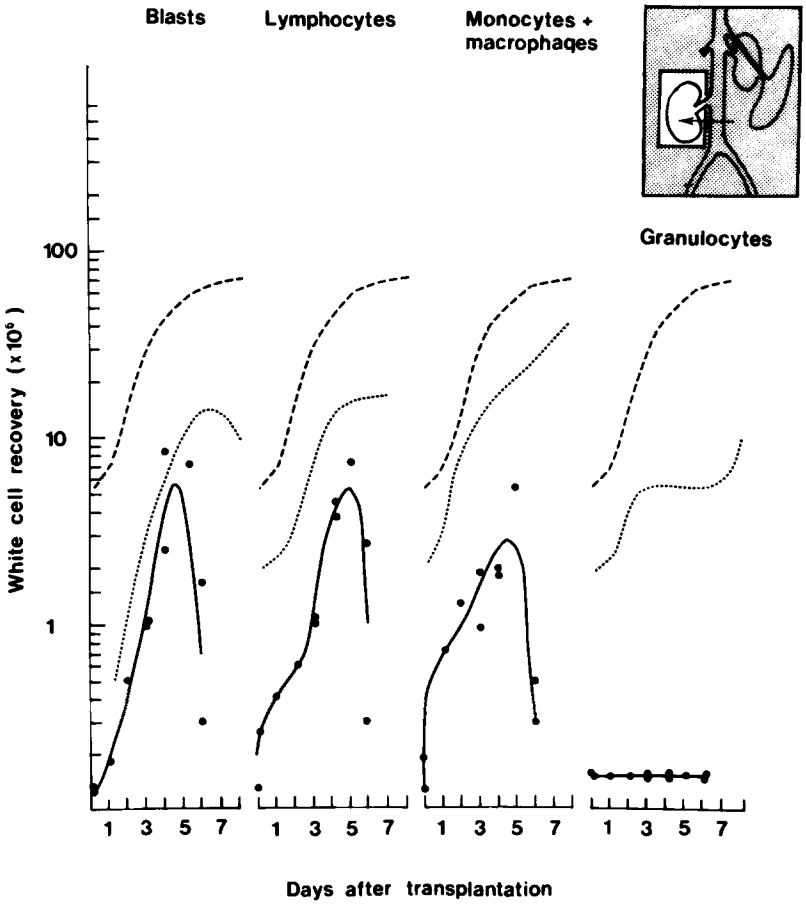

FIG. 3. Recovery of white (inflammatory) cells (- - -), different types of inflammatory cells (• • •), and different types of labeled inflammatory cells (—, ●) from DA to AO renal allografts after selective labeling of the recipient and excluding the graft.

matory cells left the graft to the recipient spleen (Fig. 4). The first graft-derived cells were blast cells and lymphocytes. These were seen in the spleen already on the 2nd d with a peak on the 3rd d. Thereafter, the outflux declined and leveled off. The outflux of labeled mononuclear phagocytes was smaller in size and shorter in duration: graft-derived labeled mononuclear phagocytes were observed in the recipient spleen only between days 2 and 4 after the transplantation. No labeled granulocytes were seen to enter the spleen.

Specificity of Labeled Cell Traffic. The specificity of allograft-responding leukocyte traffic was investigated in two types of experiments. In both experiments, WF recipients were transplanted with a DA allograft and a WF autograft. On the 3rd d after the transplantation (technically, it was not possible to perform this experiment later) either the host (excluding the autograft and allograft) or the allograft (excluding the autograft and the host) were selectively labeled with [^{3}H]thymidine, and the accumulation of labeled cells in the allograft, autograft, recipient's own kidney, spleen and blood was followed 18 h after the labeling.

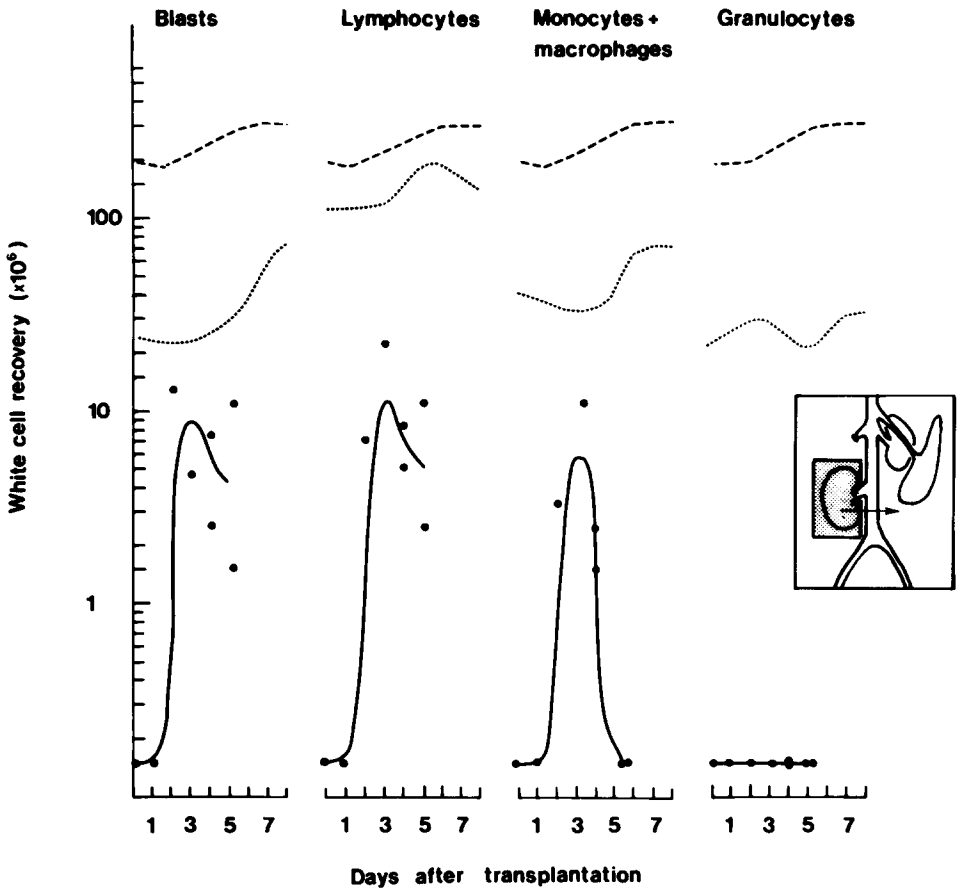

FIG. 4. Recovery of white cells (- - -), different types of white cells (• • •), and different types of labeled white cells (—, ●) from the spleen of DA to AO renal allograft recipient after selective labeling of the graft and excluding the recipient.

When the host was labeled (Table I), excluding the autograft and allograft, on the average, 26.5 ± 2.7 (SD) × 10^6 labeled leukocytes were recovered from the recipient spleen. There was a large and specific influx of labeled cells to the allograft. On the average, 4.6 ± 4.8 × 10^6 labeled cells were recovered in the allograft, compared with only 0.1 ± 0.08 × 10^6 in the autograft. The recovery of labeled cells in the autograft was of the magnitude found in the recipient's own kidney (0.06 ± 0.05 × 10^6).

When the allograft was labeled excluding the autograft and the recipient, on the average, 9.6 ± 7.3 × 10^6 labeled leukocytes were recovered from the allograft. Many of the labeled cells were found to emigrate into the recipient's spleen, with a mean recovery of 14.0 ± 4.5 × 10^6 cells. The recovery of labeled cells in the autograft was low (0.04 ± 0.05 × 10^6), of the magnitude that was recovered from the recipient's own kidney (0.03 ± 0.05 × 10^6).

Migration of Labeled Cells to Irrelevant Locations. The extent of localization of labeled cells in "irrelevant" positions, such as in the lung and liver, was investigated on the 3rd d after transplantation of a DA allograft to WF recipient (Table II). When the graft was labeled, excluding the recipient, only 2.4 × 10^6 labeled cells were recovered

TABLE I

Recovery of Labeled White Cells from Different Sites after Selective Labeling of the Allograft of the Recipient

Recipient	Graft donor	Labeled compartment	Recovery of labeled white cells									
			Allograft		Autograft		Own kidney		Spleen		Blood	
			Labeled WBC × 10^6*		Labeled WBC × 10^6		Labeled WBC × 10^6		Labeled WBC × 10^6		Labeled WBC × 10^6‡	
				%		%		%		%		%
WF	DA WF	DA allograft	8.3	(20.0)	ND§	ND	0.006	(0.5)	9.8	(2.0)	0.0	(0.0)
WF	DA WF	DA allograft	20.3	(15.0)	0.1	(0.5)	0.1	(0.5)	13.6	(5.0)	0.0	(0.0)
WF	DA WF	DA allograft	3.7	(8.0)	0.02	(1.0)	0.0	(0.0)	ND	ND	0.0	(0.0)
WF	DA WF	DA allograft	6.1	(10.0)	0.0	(0.0)	0.0	(0.0)	18.8	(5.0)	0.0	(0.0)
Mean ± SD			9.6 ± 7.3		0.04 ± 0.05		0.03 ± 0.05		14.0 ± 4.5		0.0 ± 0.0	0.0
WF	DA WF	Host	1.5	(6.0)	0.04	(2.0)	0.0	(0.0)	24.9	(6.0)	0.3	(5.0)
WF	DA WF	Host	10.2	(20.5)	0.1	(4.0)	0.1	(6.0)	25.0	(10.0)	0.1	(1.0)
WF	DA WF	Host	2.2	(18.0)	0.2	(5.0)	0.08	(3.0)	29.7	(9.0)	0.0	(0.0)
Mean ± SD			4.6 ± 4.8		0.1 ± 0.08		0.06 ± 0.05		26.5 ± 2.7		0.13 ± 0.15	

* WBC = white cells.
‡ Per 1 ml.
§ Not done.

TABLE II
Recovery of Labeled Cells in the Blood, Lung, and Liver

Labeled compartment	Recovery of labeled white cells					
	Blood*		Lung‡		Liver‡	
	Labeled WBC§ × 10^6		Labeled WBC × 10^6		Labeled WBC × 10^6	
		%		%		%
Allograft	0.6	0	2.4	2	0.9	5
Recipient	1.0	3	1.2	1	1.8	10

* Per 1 ml of blood.
‡ Per 1 g of tissue weight.
§ WBC, white blood cells.

per 1 g of tissue in the recipient lung, and 0.9 × 10^6 cells in the recipient liver. After labeling the recipient, the entrapment of labeled cells in the lung and liver was of the same order of magnitude, i.e., 1.2 × 10^6 and 1.8 × 10^6 labeled leukocytes per g of tissue, respectively.

Completion of Rejection: Cessation of Renal Blood Flow. Finally, we found it necessary to relate these kinetic changes to the actual phenomenon of rejection. To titrate the end point of rejection, we quantitated the renal plasma flow by the xenon washout method. The time-course of xenon flow in nine DA kidneys transplanted into WF recipients was followed. The T ½ recordings of the transplant blood flow are seen in Fig. 5. The results of another MHC-incompatible strain combination (WF to BN) are shown for comparatory purposes.

In both strain combinations, the renal blood flow stayed within a normal level till the 6th d after transplantation. On the 7th d, a sudden decline in the plasma flow was recorded.

Discussion

As in several previous investigations (9, 11, 22), we used [^{3}H]thymidine to mark the "allograft-responding" leukocytes. It is, however, obvious that all labeled leukocytes do not represent cells responding to the allograft. This is especially true during the immediate posttransplantation period, when only the background proliferation of leukocytes was detected, e.g., in the recipient spleen. The more than 10-fold proliferative response observed during a later stage of rejection is obviously linked to the rejection and, thus, represents the host response to the allograft. However, the effector specificity of the proliferating cells and/or their receptor idiotype was not formally established in this study.

A significant entrapment of host-derived proliferating cells was observed in the allograft already on the 1st d after the transplantation. Because the allograft immune response at this stage is only beginning, it seems that many of the early labeled cells seen in the graft are proliferating either spontaneously or in response to unrelated antigens. This entrapment was followed by an exponential increase in the labeled cell traffic, and nearly one-third of the graft-infiltrating inflammatory cells were replaced as a consequence of relocalization during each 18-h-period. As dry weight measurements have previously documented (23) that only 30% of the inflammatory cell

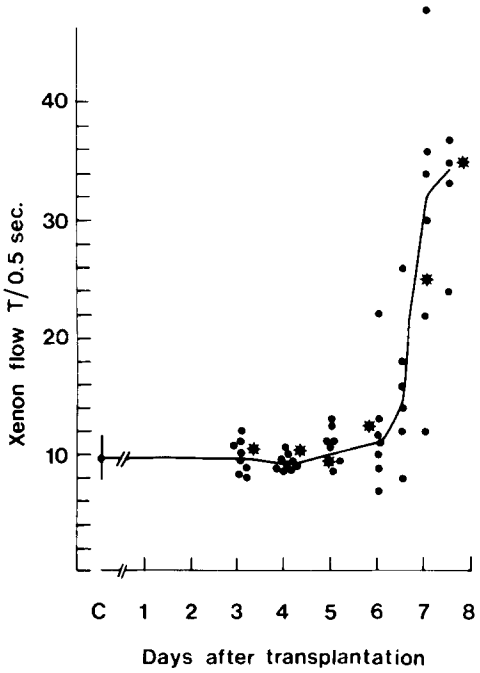

FIG. 5. Blood flow in WF to BN (●, $RT1^v \rightarrow RT1^n$) (★, $RT1^a \rightarrow RT1^v$) renal transplants at different times after the transplantation.

contents are recovered from the allograft with the present method of disaggregation, a correction factor of 3 may be given when estimating the actual inflammatory cell contents in the graft. Taking this factor into account, one may conclude that the actual size of labeled cell traffic is about the same in both directions. This very high turnover rate is a new and unexpected finding, but is compatible with the high turnover rate of inflammatory (white) cells in other sites of immune activation, e.g., complete Freund's adjuvant-induced granulomas (24) and lymph nodes responding to allogeneic lymphocytes or tuberculin (25).

Our estimate on the inbound traffic to the allograft is far higher than any one of the previous estimates (6, 9–11) based on reinfusion of in vitro or in vivo labeled spleen or thoracic duct leukocytes into the graft-carrying host. It should be emphasized that, in contrast to the previous analysis, no significant sequestration of labeled cells (over the blood background level) was observed in our study into irrelevant positions, such as the recipient liver or lung. On the other hand, our estimates on the outbound traffic from the graft to the host is of the same order as previously demonstrated in the lymphatic cannulation experiments in sheep (13) and man (14).

All morphological components of the host mononuclear leukocytes seem to participate in the anti-allograft response. Although the first host cells arriving in the graft are mononuclear phagocytes, the dominating labeled cell population emigrating into the graft consists of lymphoid cells, i.e., blast cells and small lymphocytes. It is also apparent that most lymphoid cells emigrating from the spleen to the graft at early stages of the anti-allograft response are morphologically blast cells, whereas at later stages of the rejection they are morphologically small lymphocytes (Figs. 3 and 4).

Most of the blast cells in the graft and spleen and most of the lymphocytes in the graft are apparently newly synthesized cells. In contrast, only a small fraction of the lymphocytes in the recipient spleen seem to derive from a recently divided cell population. Thus, most lymphoid cells participating in intra-graft immune events are results of recent cell divisions. This is in contrast to the mononuclear phagocytes, the largest inflammatory population infiltrating the graft. Although significant numbers of labeled mononuclear phagocytes were observed *in situ* and in the recipient spleen after selective labeling the host or the graft, the labeled mononuclear phagocytes in the graft were distinctly smaller than the labeled lymphocytes. This indicates that most of the graft-infiltrating mononuclear phagocytes derive from a pre-existing pool.

Criss-cross experiments with two allografts were not technically possible with the present model. However, upon a simultaneous transplantation of an allograft and an autograft, the traffic of labeled inflammatory cells was predominantly directed to the allograft. This observation confirms the previous results using the dual antigen to dual isotype assay by Emeson (10), Tilney and Ford (9), and Chang and Sugarbaker (11). However, in these studies only an incremental entrapment of leukocytes in a relevant graft was observed: in most experiments the ratio of entrapment in a relevant vs. an irrelevant allograft or autograft was only 1.5 to 2. In our case, a nearly 50-fold entrapment into a relevant graft over an autograft consistently took place.

Only direct conclusions can be drawn in regard to the mechanics of allograft-responding leukocyte traffic. The size of the traffic is far too high to be explained on the basis of allograft-responding lymphocytes only, i.e., lymphocytes carrying the relevant idiotypic receptor(s) to the graft antigens (26). Moreover, also mononuclear phagocytes participated in the traffic.

Previous investigations (27, 28) using sponge matrix allografts and ordinary transplants in rats and mice have demonstrated a specific enrichment of allograft-directed killer cells *in situ*. Moreover, both lymphocytes and phagocytic cells carrying an idiotype directed to the major locus antigens of the graft donor are specifically enriched inside the allograft (29). Because these cells represent obviously only a minority of cells passing through the graft, the idiotype specificity seems not to be the driving force for the increased traffic. Instead, we would like to agree with R. Simmons (personal communication) on an alternate hypothesis: it would be more conceivable to suggest that the increased traffic is due to some mechanism, immune or nonimmune, altering the permeability of a graft vascular endothelium. This would result in an increased leukocyte influx. The entrapment of the "relevant" cells *in situ* takes place via binding with a preformed cell receptor (lymphoid cells), alloantibody (K cells), and/or T cell-derived macrophage arming factors.

Although the traffic of labeled cells increased exponentially to both directions between days 2 and 4 after transplantation, the recovery of host-derived labeled cells in the graft and graft-derived host-labeled cells in the spleen and blood rapidly declined from the 5th d onwards. This decline was not due to the exhaustion of supply as the exponential proliferation continued in both compartments. Quantitation of blood flow by the xenon flush method demonstrated that this decline was not due to a slowdown of blood circulation, but, in fact, took place 2 d before the blood circulation to the graft was compromised. This observation is probably of biological importance. One likely explanation is that, at this time, graft rejection has become independent of the lymphoid system of the host and indicates irreversible rejection.

This interpretation is also supported by the finding of Ascher et al. (30) with sponge matrix allografts. If a recipient mouse is irradiated, with the sponge matrix allograft shielded before day 5 after transplantation, the maturation of cytotoxic cells within the sponge is inhibited. However, if a similar irradiation is performed after the 5th d, maturation of cytotoxic cells *in situ* takes place as in an unirradiated mouse.

Summary

We investigated the traffic of allograft-responding leukocytes between the host and graft without handling of these cells in vitro. The blood flow between the host and graft was disconnected, the proliferating cells were labeled with [^{3}H]thymidine selectively in the graft or in the host, the label was chased with cold thymidine, and the circulation was reestablished. The localization of labeled cells was quantitated by autoradiography.

The first host-derived labeled cells appeared in the graft and graft-derived labeled cells in the host, already on the 1st d after transplantation. This was followed by an exponential increase in the labeled cell traffic in both directions. The peak of traffic was observed on day 4 after transplantation, whereafter the traffic rapidly declined and tapered off. This decline was not due to exhaustion of supply, as the labeled cells continued to proliferate in their original compartments, nor to a slowdown of blood circulation, which took place 2–3 d later. We consider the decline to indicate that the rejection has proceeded to a (irreversible) stage autonomous of the host lymphatic and hematopoietic system.

During the exponential increase, nearly one-third of the graft-infiltrating inflammatory cells were replaced as a consequence of relocalization during each 18-h-period. All mononuclear white cell types, with the exception of granulocytes, participated in the traffic. Most lymphoid cells entrapped in the graft were descendents of recent cell divisions; most of the mononuclear phagocytes derived from a preexisting phagocyte pool. The entrapment of labeled leukocytes in a relevant graft was specific: when an allograft and an autograft were simultaneously transplanted, a more than 50-fold entrapment was observed in the allograft, compared with the autograft. Very few of the cells localized in irrelevant positions, such as the liver and lung, of the recipient.

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