

# RESTORATION OF IMMUNOGENICITY TO PASSENGER CELL-DEPLETED KIDNEY ALLOGRAFTS BY THE ADDITION OF DONOR STRAIN DENDRITIC CELLS

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The question as to why major histocompatibility complex (MHC)<sup>1</sup> antigens are uniquely powerful primary immunogens and in this respect differ from antigens of the minor systems has not yet been clearly answered. A number of suggestions have been made in the past (1-3), but they have not taken into account the remarkable variation in strength with which MHC antigens induce primary, T-dependent responses, depending upon how the antigens are presented. In some strains, for example, the donor tissue must contain viable, metabolically active cells, although these need not necessarily be capable of cell division; thus x-irradiated lymphoid cell suspensions can provoke strong primary alloimmune responses, but ultra-violet irradiated cells (4) or unfixed lymphocyte plasma membrane preparations (5) fail to do so, even though they carry MHC antigens structurally unaltered insofar as they specifically absorb alloantibodies. Furthermore, the donor tissue must contain cells bearing MHC alloantigens of class II type (i.e., homologues of Ia for mouse and HLA-D/DR for man); thus, incompatible rodent erythrocytes or platelets fail to induce strong primary alloimmune responses (6, 7), although they are quite sufficient for inducing secondary (memory) responses.

It has been previously shown (8) that long-surviving, immunologically enhanced MHC-incompatible rat kidney grafts, when retransplanted from a primary to a secondary recipient of the same genotype, do not elicit strong primary T-dependent alloimmunity in the secondary recipient. In contrast, normal primary kidney allografts in the relevant donor/recipient combination [(AS × AUG)F<sub>1</sub> donor, AS recipient] do arouse strong primary T-dependent immunity, and the grafts are regularly rejected within 12 d (8). The failure of long-surviving kidney grafts to activate T helper cells cannot be attributed to an absence of either class I or II MHC alloantigen because the grafts carry both (9, 10). We suggested that the crucial difference between the long-surviving and normal kidney allografts that accounted for the feeble immunogenicity of the former was that the long-surviving grafts did not contain incompatible passenger leukocytes. The experiments described in this report show that strong immunogenicity can be restored by injecting (AS × AUG)F<sub>1</sub> dendritic cells at the time of retransplantation. The immunogenic effect of purified, viable T or B lymphocyte suspension was found to be at least 2 log orders of magnitude less than that provided by dendritic cells. The results suggest that MHC-incompatible allografts are

<sup>1</sup> *Abbreviations used in this paper:* i.v., intravenous; MHC, major histocompatibility complex; MLC, mixed lymphocyte culture; PBS, phosphate-buffered 0.15 M saline.

uniquely powerful primary immunogens only if they also contain MHC-incompatible dendritic cells. The immunogenicity of conventional macrophage populations lacking dendritic cells or their precursors remains to be elucidated clearly, although our data points to their having only weak immunogenicity. With this qualification, it is suggested that MHC-incompatible tissues devoid of dendritic cells provoke responses comparable to those induced by minor system incompatibilities or the standard T-dependent protein antigens.

### Materials and Methods

*Animals.* We used the following inbred rat strains or hybrids derived from them: AS (RTIA<sup>b</sup>) and AUG (RTIA<sup>c</sup>) (11). The parental strains were bred at the National Institute for Medical Research, Mill Hill, England, and hybrids from them were bred in the experimental animal unit at this institution. All rats were maintained here.

*Kidney Transplants and Retransplants.* These were performed by conventional microsurgical technique, as previously described (8). Animals were inspected at regular intervals for survival and bled for serum urea estimations on days 7, 10, 14, 21, and 28. Enhancement of (AS × AUG)F<sub>1</sub> kidneys transplanted into AS recipients was induced by means of a combined active and passive regime (12, 13) by injecting transplant recipients intravenously (i.v.) with  $5 \times 10^7$  donor strain spleen cells 11 d before kidney transplantation, 1 ml of AS anti-AUG strain antiserum 1 d later, and a further 1 ml of the same anti-serum at the time of transplantation.

*Serum Ureas.* The glucose creatinine urea analyser 919 (Instrumentation Laboratories Ltd., Warrington, Cheshire, England) was used, using an area enzymatic rate reaction method on 40- $\mu$ l samples.

*Preparation of T Cell- and B Cell-enriched Populations.* Lymphocytes were derived from either peripheral blood by density centrifugation on Ficoll Hypaque ( $P = 1.090$ ) or from thoracic duct lymph. Enrichment for T cells was achieved by incubation of the cells on nylon wool columns and collecting the nonadherent cell population (14). B cell-enriched populations were prepared by incubating neuraminidase (type VI; Sigma Chemical Co., St. Louis, Mo.)-treated cells on a Helix Pomatia coupled Sepharose 6MB column (AB; Pharmacia Fine Chemicals, Div. of Pharmacia Inc., Piscataway, N. J.) (15). To check that the enrichment procedures were satisfactory, some samples of the T-enriched and the B-enriched cell suspensions were incubated with fluorescein isothiocyanate-labeled anti-rat immunoglobulin, washed, and examined by fluorescence microscopy for the presence of lymphocytes with surface immunoglobulin. T cell-enriched suspensions contained <10%, and B cell suspensions contained >80% of surface immunoglobulin-positive lymphocytes.

*Preparation of Macrophages.* Macrophages were obtained from a starting population of spleen or peritoneal exudate cells. Single cell spleen suspensions were obtained by in vivo and in vitro spleen digestion; collagenase (Sigma Chemical Co., type I, 75 mg/kg in ~1 ml of phosphate-buffered saline [PBS]) was injected i.v., and the rat was killed 15 min later. The spleen was removed, diced into small pieces (~0.04 cm<sup>3</sup>) and rotated in RPMI 1640 medium containing protease from *Bacillus polymyxa* (Sigma Chemical Co., type 1X, 1 U/ml for 30 min at 37°C). After this, the cells were flushed through a metal sieve (pore size, 300  $\mu$ m) and washed three times. Macrophages were positively selected from these populations by adherence to Falcon plastic (Falcon Labware, Div. of Becton, Dickinson & Co., Oxnard, Calif.). The cells were plated out ( $\leq 4 \times 10^7$  cells/9-cm diam Falcon plastic petri dish; Falcon Labware) in RPMI 1640 medium supplemented with 5% fetal calf serum. After 1-h incubation at 37°C, the nonadherent cells were gently rinsed with warm medium, and fresh medium was added containing 0.6 mM EDTA. After a further 15-min incubation, the released adherent cells were vigorously pipetted off and washed. The majority of cells had the morphological appearance of macrophages, but they were not tested for the presence of relevant surface marker antigens.

*Preparation of Dendritic Cells.* These cells were derived from thoracic duct afferent lymph, as described by Mason et al. (16). In brief, abdominal lymph nodes—mesenteric, para-aortic, renal, and coeliac—were removed from donor strain rats aged ~2 mo. After an interval of 6 wk, these rats were x-irradiated (700 rad), and thoracic duct cannulation was performed within the following 24 h. Lymph was collected over a 48-h period. Further enrichment for dendritic cells

was achieved by a density centrifugation step (17). Cells were suspended in dense bovine albumin,  $P = 1.084$ , overlaid with less dense albumin,  $P = 1.050$ , and centrifuged at 10,000  $g$ ,  $4^{\circ}\text{C}$ , for 30 min. The interface contained a population of 50–75% dendritic cells, judged by the striking morphological appearance of these cells. No tests for surface marker antigens were made.

*Measurement of the Volume of Blood Contained in a Nephrectomized Rat Kidney.* Rat erythrocytes were washed and packed; to 1 ml packed cells was added 0.26 mC of  $^{51}\text{Cr}$  in 20  $\mu\text{l}$ . After 15 min at room temperature the cells were washed, and 100  $\mu\text{l}$  of packed cells in 1 ml PBS (emitting  $\sim 10^6$  CPM) was injected i.v. into each of three rats. After allowing 5 min equilibration time, the left renal vessels were clamped, and the left kidney was removed, blotted, and squeezed, as for transplantation. The animals were bled from the aorta. Gamma-emissions from 1 ml of blood and the left kidney of each rat were measured in an LKB 1280 ultra-gamma spectrometer (LKB Instruments LTD., Surrey, England). The results are set out in Table I. The amount of blood transferred in a kidney graft varies according to the size of the kidney and how vigorously the kidney is blotted and squeezed. However, the normal passenger blood volume of a rat kidney weighing 1 g is  $\sim 0.1$  ml when calculated from these results. In experiments to examine the immunogenicity of unfractionated blood cells, blood was withdrawn from heparin-treated (AS  $\times$  AUG) $F_1$  donors, and the plasma and excess heparin was removed after centrifugation. This procedure was found necessary to prevent persistent haemorrhage from the renal vascular anastomoses.

## Results

Passenger cells in a kidney allograft can be loosely divided into intravascular and extravascular components. To determine which cells are responsible for the strong primary immunogenicity characteristic of conventional kidney allografts, the following experimental design was used throughout. Long-surviving, enhanced (AS  $\times$  AUG) $F_1$  kidneys residing in AS primary recipients for  $>4$  wk were retransplanted into secondary AS recipients. Animals receiving retransplants were also injected intravenously with cell subpopulations derived from (AS  $\times$  AUG) $F_1$  donors.

*The Effects of (AS  $\times$  AUG) $F_1$  Blood on the Survival of Retransplanted Kidney Allografts.* The rats in this experiment were injected with the cells from 0.1 or 0.5 ml of blood. Control rats received no blood. From Table II it can be seen that all animals given 0.1 ml of blood, i.e., the amount previously demonstrated to be contained within a kidney prepared for transplantation, showed extended survival. Three out of the four rats had negligible rejection episodes and were killed after 3–4 mo. The fourth (723) suffered a marked rejection episode but survived for over 4 wk. It appears that the amount of blood normally presented in a kidney allograft does not provide the necessary stimulus for graft rejection. When as much as 0.5 ml of blood was injected, two out of four rats suffered acute graft destruction and all had sharp early rejection episodes. This suggests that although the amount of blood known to remain

TABLE I  
Calculation of Renal Passenger Blood Volume

Rat number	1 ml Blood CPM	Kidney CPM	Kidney weight	Calculated blood volume	Blood volume corrected to kidney weight of 1 g
			<i>g</i>	<i>ml</i>	
1	216,505	12,157	0.872	0.056	0.064
2	164,564	19,540	1.078	0.119	0.110
3	203,304	17,599	0.753	0.087	0.113

TABLE II  
*Survival and Function of (AS × AUG)F<sub>1</sub> Kidneys Reimplanted into Secondary AS Recipients Treated with (AS × AUG)F<sub>1</sub> Blood Cells*

Rat number	Serum urea						Survival
	Day 0	Day 7	Day 10	Day 14	Day 21	Day 28	
	<i>mmol/liter</i>						<i>d</i>
716*	10.5	6.5	10.0	9.0	7.5	8.0	>100
717*	13.0	8.5	ND‡	13.8	15.0	14.0	>100
718*	11.5	ND	29.0	15.5	12.5	12.5	>100
723*	10.0	12.0	>50	ND	26	83	29
715§	9.0	7.0	50.0	22.5	12.0	13.5	>100
720§	17.0	8.3	>50.0	—	—	—	12
721§	10.6	8.1	>50.0	—	—	—	11
724§	12.0	7.4	44.0	22.0	22.0	18.0	>100
701	9.5	8.0	11.8	8.5	11.0	10.9	>100
703	14.2	8.8	12.0	8.0	8.0	ND	>100
713	14.5	7.5	11.8	9.5	10.8	10.0	>100
714	9.5	9.0	14.5	11.2	10.5	10.8	>100
719	8.5	6.0	8.0	ND	8.0	10.0	>100

\* 0.1 ml.

‡ Not done, in this and all other tables.

§ 0.05 ml.

|| Controls not given blood.

in a kidney prepared for transplantation is insufficient to provoke graft destruction if given to the secondary recipient, blood does contain a cell or cells in small numbers with this property. Their effect is demonstrated in the rats treated with 0.5 ml blood.

*The Effects of (AS × AUG)F<sub>1</sub> T and B Lymphocyte-enriched Suspensions on the Survival of Reimplanted Kidney Allografts.* These cell subpopulations were derived from either peripheral blood or thoracic duct lymph, and the relevance of the source will be referred to in the discussion section. In the T cell-treated animals (Table III), two of seven rats given 2 million cells derived from blood died from acute graft rejection; the other five rats showed extended or indefinite survival, although two appear to have undergone a transient rejection episode. Prolonged survival was observed in all four animals receiving 5 million thoracic duct T cells, and only one of these had a significantly elevated serum urea suggestive of a rejection episode. Furthermore, lower doses of T cells ( $10^5$ ) did not provoke acute graft destruction (data not shown).

Two groups of rats were treated with cell suspensions of enriched B lymphocytes (Table IV). For those receiving 2 million cells, the source before enrichment was peripheral blood, and for those receiving 5 million cells, it was thoracic duct effluent. All the animals in these groups have shown extended survival; one animal died at day 47 and the other five are likely to enjoy indefinite survival as judged by their serum urea values.

*The Effects of (AS × AUG)F<sub>1</sub> Adherent Cells on the Survival of Reimplanted Kidney Allografts.* The method used to prepare adherent cell suspensions was subject to some variability between experiments, but despite this, a consistent effect was observed after treatment with  $2 \times 10^6$  adherent cells, all animals suffering a rejection episode,

TABLE III  
*Survival and Function of (AS × AUG)F<sub>1</sub> Kidneys Retransplanted into Secondary AS Recipients Treated with (AS × AUG)F<sub>1</sub> T Cells*

Rat number	Serum urea						Survival
	Day 0	Day 7	Day 10	Day 14	Day 21	Day 28	
	<i>mmol/liter</i>						<i>d</i>
M30*	8.1	19.1	70.4	21.7	15.4	17.2	>100
M31*	10.1	ND‡	10.9	21.4	14.8	13.3	>100
M32*	11.1	11.1	21.8	13.5	21.3	12.8	>100
M37*	11.0	6.6	74.4	—	—	—	11
M40*	12.6	15.3	58.4	82.9	—	—	16
M41*	9.9	7.4	45.9	28.3	14.8	26.1	28§
M42*	9.8	9.6	21.2	14.3	9.1	8.6	>100
M48	17.2	7.3	55	27.5	42	24.0	47
M49	10.7	7.1	11.0	14.5	11.5	11.7	>60
M90	10.4	6.5	ND	15.4	12.6	13.6	>28
M91	9.3	7.2	ND	11.9	10.1	8.9	>28

\*  $2 \times 10^6$  cells.

‡ Not done.

§ M41 killed on day 28 because of large subcutaneous abscess. The cause of death was infection.

||  $5 \times 10^6$  cells.

TABLE IV  
*Survival and Function of (AS × AUG)F<sub>1</sub> Kidneys Retransplanted into Secondary AS Recipients Treated with (AS × AUG)F<sub>1</sub> B Cells*

Rat number	Serum urea						Survival
	Donor	Day 7	Day 10	Day 14	Day 21	Day 28	
	<i>mmol/liter</i>						<i>d</i>
M35*	8.0	6.9	32.7	12.2	ND‡	13.0 (3 mo)	>100
M38*	8.8	18.5	6.9	47.1	21.0	13.2	>100
M46*	8.2	8.1	20.4	15.4	18.3	27.5	>80
M47*	11.6	6.5	22.4	10.8	13.8	14.0	47
M94§	13.0	5.8	7.4	7.1	8.2	8.8	>42
M95§	11.8	8.1	8.1	8.5	8.6	11.2	>42

\*  $2 \times 10^6$  cells.

‡ Not done.

§  $5 \times 10^6$  cells.

and three of four rats dying within 12 d (Table V). Similarly, in the group given  $10^6$  adherent cells, six of seven rats had a very elevated serum urea at day 10, indicating rejection, although the survival times were extended in five rats and very prolonged in four. Only one early death occurred in the group of rats given  $5 \times 10^5$  cells, and graft function was good in the 1st mo for the other three recipients. These data show that preparations of adherent cells provide an immunogenic stimulus if  $1 \times 10^6$  to  $2 \times 10^6$  cells are given.

*Effects of (AS × AUG)F<sub>1</sub> Dendritic Cells on the Survival of Retransplanted Kidney Allografts.* As shown in Table VI, dendritic cells provide a very potent stimulus to the recipients' immune response, causing graft rejection in all the animals receiving

TABLE V  
*Survival and Function of (AS × AUG)F<sub>1</sub> Kidneys Reimplanted into Secondary AS Recipients Treated with (AS × AUG)F<sub>1</sub> Adherent Cells*

Rat number	Serum urea						Survival
	Donor	Day 7	Day 10	Day 14	Day 21	Day 28	
			<i>mmol/liter</i>				<i>d</i>
M12s*	12.9	8.6	73.0	15.3	ND‡	19.0	>100
M13s*	8.9	11.5	>80	—	—	—	11
M14s*	14.8	11.1	83.3	—	—	—	10
M16s*	9.7	11.9	—	—	—	—	10
M4p§	10.2	8.6	54	12.1	—	—	14
M7p§	9.0	8.5	37.6	9.5	ND	35.6	66
M9p§	13.2	8.0	69.8	18.7	46.9	28.6	>100
M10p§	11.6	14.9	10.5	17.6	19.6	ND	>100
M23s§	11.7	10.2	>83	—	—	—	10
M24s§	10.3	14.4	>83	21.3	20.4	21.4	>100
M25s§	8.0	17.1	54.5	20.7	28.3	31.6	30
M11p	12.8	ND	11.3	14.5	14.4	14.7	>100
M17s	17.0	6.8	9.4	ND	8.3	10.8	>100
M18s	10.4	7.4	11.9	9.0	7.8	ND	54
M20s	8.6	6.4	—	—	—	—	10

\*  $2 \times 10^6$  cells. Postfix: s, splenic.

‡ Not done.

§  $10^6$  cells. Postfix: s, splenic; p, peritoneal.

||  $5 \times 10^5$  cells. Postfix: s, splenic; p, peritoneal.

$5 \times 10^4$  or  $10^5$  cells. Eight of nine rats receiving the higher dose died within 2 wk, and at the lower dose, all animals died within 22 d. Even  $10^4$  dendritic cells exerted an effect; two of the three recipients receiving this dose died at 12 and 22 d.

The cell numbers in these groups refer to the morphologically identifiable dendritic cells removed from the albumin interface. The remaining 25–50% of cells have yet to be characterized but are probably a mixture of neutrophils and large lymphoblasts.

As a specificity control, three AS rats receiving long-surviving (AS × AUG)F<sub>1</sub> kidneys were injected intravenously with  $10^5$  AS strain dendritic cells. The retransplanted kidneys were not acutely rejected and all these rats have survived for more than 1 mo. However, all three rats have been persistently uraemic from the 2nd wk.

### Discussion

The main objective of our experiments was to identify the population(s) of passenger cells within rat kidney allografts which confer the property of activating a strong primary alloimmune response leading to acute graft destruction. Long-surviving, enhanced kidney grafts in the donor/recipient combination used here are not acutely rejected when they are retransplanted into a secondary recipient syngeneic with the primary one (8). As the retransplanted kidneys are assumed to be devoid of the original donor type [i.e., (AS × AUG)F<sub>1</sub>] passenger cells, various subpopulations of (AS × AUG)F<sub>1</sub> cells likely to be amongst the passengers can be injected into the

TABLE VI  
*Survival and Function of (AS × AUG)F<sub>1</sub> Kidneys Reimplanted into Secondary AS Recipients Treated with (AS × AUG)F<sub>1</sub> Dendritic Cells*

Rat number	Serum urea						Survival
	Donor	Day 7	Day 10	Day 14	Day 21	Day 28	
			<i>mmol/liter</i>				<i>d</i>
M61*	7.2	8.0	—	—	—	—	10
M62*	7.6	7.7	—	—	—	—	10
M63*	9.6	8.7	—	—	—	—	10
M65*	6.3	ND‡	82.9	—	—	—	10
M67*	8.4	6.6	—	—	—	—	10
M68*	11.6	5.8	83.0	—	—	—	11
M69*	8.2	5.7	>86	50.0	ND	51.0	42
M70*	9.0	10.8	72.1	—	—	—	13
M71*	8.4	15.4	73.5	—	—	—	12
M72§	11.3	8.6	55.8	29.6	69.0	—	22
M73§	8.8	6.9	69.5	29.8	—	—	20
M75§	14.7	5.9	47.9	—	—	—	12
M76	8.9	6.4	6.8	14.6	15.5	13.6	>56
M77	9.6	7.4	79.7	—	—	—	12
M78	10.7	6.2	15.9	42	59.6	—	22
Specificity controls, secondary recipients treated with 10 <sup>5</sup> AS dendritic cells							
M81	7.9	6.5	13.3	47.3	44.5	57.1	>28
M82	10.0	6.4	16.5	33.8	46.5	59.8	>28
M83	9.9	6.2	14.8	27.2	46.5	25.0	>28

\* 10<sup>5</sup> cells.

‡ Not done.

§ 5 × 10<sup>4</sup> cells.|| 10<sup>4</sup> cells.

secondary recipients at the time of retransplantation and the effect on allograft survival observed. Our results show that cell suspensions enriched for dendritic cells are remarkably effective in provoking acute graft destruction. The minimum number of cells required was  $\sim 1 \times 10^4$  to  $5 \times 10^4$ , and, at a dose of  $10^5$  dendritic cells, all but one of a group of nine rats destroyed their grafts within 13 d. This acute rate of rejection is similar to that observed in conventional allografts between (AS × AUG)F<sub>1</sub> donors and normal AS strain recipients. The degree of purity of the dendritic cells in the suspensions used was 50–75%, and the reservation has to be made that it is possible but unlikely that another cell that has been copurified is responsible for the results observed.

In contrast to the effectiveness of dendritic cell suspensions, those enriched for T or B cells were approximately 2 log orders of magnitude less potent. Because of the very powerful immunogenicity of dendritic cells, we cannot be certain that the two examples of acute graft rejection seen in the group of 11 rats treated with  $2 \times 10^6$  to  $5 \times 10^6$  T cells were not due to incomplete purification of the T cell suspension. Contamination with 1–2% dendritic cells or their precursors could be sufficient to initiate acute rejection. It is interesting that both examples of acute rejection occurred

in a group of rats given T cells purified from peripheral blood rather than thoracic duct lymph; possibly, this reflects differing numbers of dendritic cells or their precursors carried in blood and lymph.

The inability of  $5 \times 10^6$  B lymphocytes to trigger acute graft rejection is important further evidence that the capacity of a cell to act as a strong primary alloimmunogen is not conferred by the mere presence of class II MHC molecules. Rat B cells carry  $\sim 10^5$  of these molecules per cell (18) and dendritic cells carry  $\sim 10^6$  molecules per cell (16), so that additional factors other than the quantity of class II MHC molecules are implicated.

Suspensions of plastic adherent spleen or peritoneal cells were clearly less active than dendritic cell suspensions. For example, only two of seven rats given  $10^6$  adherent cells died with acute graft destruction during the first 2 wk after retransplantation. Although dendritic cells did not adhere strongly to plastic in our own experience and that of others (19), there is no data on their precursors, and possible contamination of the adherent cell suspensions with these cannot be assessed.

The amount of intrarenal blood remaining within a graft at transplantation was calculated to be 0.1 ml. Cells from this volume of blood failed to induce acute graft destruction, and the results are consistent with the conclusion that extravascular dendritic cells, known to be present in rat kidneys (20), are the major stimulus.

The criteria for MHC-incompatible cells behaving as uniquely powerful primary immunogens *in vivo* have been mentioned earlier. These criteria are strikingly similar to the requirements necessary for a positive response in mixed lymphocyte cultures and have led to the view that the MLC reaction is the *in vitro* equivalent of the recognition stage of primary immunization by alloantigen *in vivo* (21). A primary MLC *in essence* provides a direct measurement of cellular proliferation by the responder  $T_H$  cells. Steinmann and colleagues (reviewed in 22) and Mason et al. (16) have demonstrated that dendritic cells are extremely potent stimulators of primary MLC reactions, and the lineage may be unique in this regard. Furthermore, Mason et al. (16), using purified  $T_H$  cells as the responder population, have found that MHC-incompatible dendritic cells stimulate maximum responses. Our results indicate that allogeneic dendritic cells provide the major primary *in vivo* immunogenic stimulus of kidney allografts. It seems reasonable to conclude that the very reason for the powerful immunogenicity of these cells *in vivo* is because they directly activate the recipients'  $T_H$  cells. Although the three control AS rats that received passenger cell-depleted (AS  $\times$  AUG) $F_1$  kidneys and  $10^5$  AS strain dendritic cells did not reject their grafts acutely within 2 wk (c.f. those that were given  $10^5$  (AS  $\times$  AUG) $F_1$  dendritic cells), nevertheless, their serum urea levels were obviously raised from the second week onwards. Because even syngeneic dendritic cells can stimulate a proliferative response *in vitro*, an interesting possibility is that syngeneic AS dendritic cells *in vivo* also can have a weak activating effect upon the recipient's helper T cells.

The donor/recipient combination used in these experiments provides a system in which the passenger cell-depleted kidney has a greatly reduced immunogenicity and does not provoke acute graft rejection. In some other combinations, passenger cell-depleted kidneys can be rapidly rejected, albeit without the generation of cytotoxic antibodies (10). Our interpretation of this strain-dependent variability is that primary alloimmunization can occur by two routes (Fig. 1). By route 1, allogeneic dendritic cells present in MHC-incompatible tissues can bypass the need for further antigen



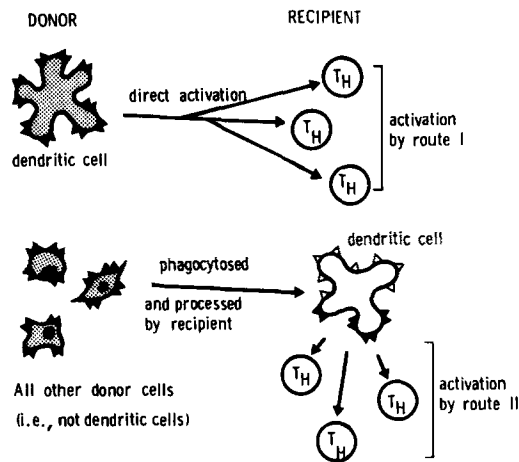


FIG. 1. Dual route of sensitization by alloantigen. Direct stimulation of T<sub>H</sub> (helper T cells) by allogeneic dendritic cells leads to sensitization by route 1. Route 2, operative in the absence of donor strain dendritic cells, involves the processing and presentation of alloantigen by recipient strain accessory cells.

processing and presentation, directly activating relevant T<sub>H</sub> clones of the recipient. Arguing from the results of MLC reactions, it would be predicted that this route of immunization only occurs where the incompatibility of the allogeneic dendritic cells involves class II MHC antigens. By route 2, MHC alloantigen of the graft other than that expressed on the donor's dendritic cells is processed and presented by the recipients' own accessory cells. The handling and presentation of MHC antigen to T<sub>H</sub> clones by this route does not differ significantly from that involved for minor system incompatibilities or the commonly used protein antigens.

According to our hypothesis, the crucial distinction between MHC-incompatible grafts and all other types of antigen is that by virtue of their content of viable dendritic cells, primary alloimmunization can proceed by route 1 as well as by route 2. All other antigens activate T<sub>H</sub> clones only by route 2. Route 1 is likely to be an optimally efficient method of activation. Although the detailed molecular mechanisms of the process remain uncertain, triggering of T<sub>H</sub> cells involves their recognition of an alteration to self in association with self class II MHC substances (23, 24). Class II MHC molecules expressed by the allogeneic dendritic cells appear to provide the functional equivalent. Alloimmunization by route 1 would be optimally efficient not only because every viable allogeneic dendritic cell would express the functional trigger, but also because of the high density of the triggering molecules on each cell. Alloimmunization by route 2, in which antigen is handled by the recipients' own accessory cells, is most unlikely to lead to such a high density of altered self molecular triggers on the recipients' dendritic cells. Furthermore, in extreme antigen excess, which would be necessary for this situation to develop, there would also be the opportunity for activation of suppressor circuits.

Returning to the problem of the strain-dependent variation in survival time of passenger cell-depleted kidney allografts transplanted into secondary recipients, we would point out that acute graft destruction is not a precise measure of a recipients' response. In those strain combinations where acute graft destruction does follow, no

quantitative comparison has been made to determine the strength of the responses induced by ordinary allografts and those depleted of passenger cells. Our prediction is that MHC-incompatible, passenger cell-depleted kidney allografts immunize only by the relatively inefficient route II, and that in those combinations where such grafts are acutely rejected, minimal immunosuppression at levels ineffective for ordinary grafts would maintain passenger cell depleted allografts in good function.

### Summary

The immunogenicity of long-surviving, enhanced (AS  $\times$  AUG) $F_1$  renal allografts retransplanted into secondary AS recipients was restored by the injection of small numbers of donor strain dendritic cells derived from afferent lymph. Whereas  $1 \times 10^4$  to  $5 \times 10^4$  dendritic cells were able to trigger an acute rejection response, neither the passenger volume of donor strain blood nor  $5 \times 10^6$  T or B lymphocytes were able to do so, thereby demonstrating more than a 100-fold difference in immunogenic potency.

It is concluded that intrarenal dendritic cells provide the major immunogenic stimulus of a kidney allograft. These results suggest that the antigenic strength of major histocompatibility complex-incompatible tissue correlates with the content of donor strain dendritic cells. They also provide further evidence that antigens of the major histocompatibility complex behave like conventional antigens unless they are on the surface of allogeneic dendritic cells.

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