FAILURE OF LONG SURVIVING, PASSIVELY ENHANCED KIDNEY ALLOGRAFTS TO PROVOKE T-DEPENDENT ALLOIMMUNITY

II. Retransplantation of (AS × AUG)F_1 kidneys from AS primary recipients into (AS × WF)F_1 secondary hosts*

By K. I. WELSH,‡ J. R. BATCHELOR,‡ A. MAYNARD, and H. BURGOS

From the Blond—McIndoe Centre, Queen Victoria Hospital, East Grinstead, Sussex, England

(AS × AUG)F_1 rat kidneys show prolonged survival in AS recipients if AS anti-AUG-enhancing antiserum is given at the time of transplantation (1). After a few weeks residence in the AS recipient, such kidneys have lost the ability to stimulate T-dependent alloimmunity. In the preceding paper (2), (AS × AUG)F_1 kidneys, which had resided in AS recipients, were retransplanted into naive, secondary AS animals, these grafts failed to induce IgG cytotoxins, optimal killer T-lymphocyte responses, and acute graft rejection. Such kidneys, when retransplanted into specifically sensitized AS recipients, provoked acute graft rejection accompanied by IgG lymphocytotoxins and plentiful killer T-cell production. The explanation proposed was that rat major histocompatibility complex (MHC)[1] specificities are not themselves strong immunogens but require the concurrent action of an unknown second signal or function to induce strong primary T-dependent alloimmune responses. Although long-surviving kidney allografts carry MHC specificities, they are deficient in second signal. The strong T-dependent responses observed in control rats receiving normal kidney allografts was attributed to their capacity to supply the second signal as well as MHC specificity, the most likely source of second signal being passenger cells. In the accompanying paper, the recipients of retransplanted, long-surviving kidney allografts were AS rats. In this paper we describe experiments to determine whether long-surviving (AS × AUG)F_1 kidneys are acutely rejected when retransplanted from AS recipients into (AS × WF)F_1 recipients. Long-surviving kidneys taken from AS recipients should have had their (AS × AUG)F_1 passengers replaced by AS cells. If the AUG gene products present on the kidney parenchyma are unable to induce primary T-dependent alloimmunity, this should hold good in any secondary recipient. We have used an F_1 of which AS is one of the parental strains to avoid responses against the passenger cells, which by the time of retransplantation, should be of AS/AS genotype.

In addition, an experiment is described in which a 3 d residence of (AS × AUG)F_1

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‡ Present address: Department of Tissue Immunology, Hammersmith Hospital, Du-Cane-Road, London W.12. England.

[1] *Abbreviation used in this paper:* MHC, major histocompatibility complex.

TABLE I

Survival of Retransplanted (AS × AUG)F_1 ♂ Kidneys from AS Primary, ♂ Recipients into Secondary (AS × WF)F_1 ♂ Recipients

Days in 1° AS ♂ Recipient	Rat No: 2° Recipient	Survival	Comments
		d	
66	J.555	>50	
64	J.556	21	Histology: moderate rejection. ureteric kink.
63	J.557	18	Histology: mild rejection. ureteric kink.
91	J.562	27	No histology obtained.
84	J.564	43	
84	J.565	>50	

Control (AS × WF)F_1 recipients.
Allografted with normal (AS × AUG)F_1 kidneys.

Rat No:	survival	comment
J.558	10	histology: severe rejection
J.559	11	
J.560	8	
J.561	13	

kidney allografts was allowed before retransplantation of the grafts into secondary AS recipients. This period was insufficient to prevent acute graft rejection and indicates that the removal of the source of second signal may be a slow process.

Materials and Methods

These are described in the previous paper (2).

Results

Retransplantation of Long-Surviving (AS × AUG)F_1 Kidneys from AS Primary Recipients into Naive (AS × WF)F_1 Secondary Recipients. The survival times of six secondary recipients are shown in Table I. Two rats died within 3 wk and at autopsy were found to have hairpin kinks of the ureter at the site of anastomosis which caused partial outflow obstruction. The histological appearances of these two kidneys were those of mild and moderate rejection. Of the remaining four rats, two died at 27 and 43 d, and the other two continued to survive until 50 d, when they were killed.

Four control (AS × WF)F_1 recipients were allografted with normal (AS × AUG)F_1 kidneys, and as shown in Table I, survived for 8–13 d. The histological appearances of their grafts removed at autopsy were those of severe rejection.

Titers of lymphocytotoxic antibodies formed by the two groups of rats are shown in Table II. The notable features are (*a*) both experimental and control group rats produced IgM antibody, although experimental rats were slightly slower to respond; and (*b*) control rats developed brisk IgG antibody responses, whereas those of the experimental rats were undetectable in three cases (J.557,562,565), doubtful in two others (J.564,555), and only in one case did a definite but slow IgG develop (J.556).

The blood urea levels shown by the two groups of rats are given in Table III. On day 7, at the time of removal of their own remaining kidney, all rats had virtually

TABLE II

Lymphocytotoxic Antibody Titers (vs. AUG Targets) of (AS × WF)F_1 Recipients‡*

	Group rat no	IgM + IgG antibody§				IgG antibody only§			
		Day 7	Day 10	Day 14	Day 21	Day 7	Day 10	Day 14	Day 21
Experimental	J.555	0	7	5	5	0	0	2	2
	J.556	0	>6	>6	>6	0	0	2	5
	J.557	0	6	4	—	0	0	0	—
	J.562	0	5	4	4	0	0	0	0
	J.564	0	>6	>6	4	0	1	0	0
	J.565	5	>6	4	5	0	0	0	0
Controls	J.558	>6	>6	—	—	5	4	—	—
	J.559	>6	>6	—	—	4	0	—	—
	J.560	>6	—	—	—	4	—	—	—
	J.561	>6	—	—	—	4	—	—	—

* Titers = highest dilution expressed as reciprocal of $\log_2$, end point = 30% kill.
‡ Details of rats shown in Table I. —, indicates rat dead.
§ Titers of IgM + IgG obtained with guinea pig complement; titers of IgG only obtained with rat complement.

TABLE III

*Blood Urea Levels (mg/100 ml) of (AS × WF)F_1 Recipients**

	Group rat no	Day 7	Day 10	Day 14	Day 21	Day 28
Experimental	J.555	42	74	59	57	60
	J.556	48	49	86	191	—
	J.557	45	45	82	—	—
	J.562	44	116	245	76	—
	J.564	54	96	90	75	74
	J.565	49	95	172	87	72
Control	J.558	38	428	—	—	—
	J.559	38	449	—	—	—
	J.560	39	—	—	—	—
	J.561	45	ND	—	—	—

ND, not done, because rat clinically, severely uraemic and likely to die if anaesthetized for bleeding.
—, rat dead.
* Details of rats shown in Tables I and II.

normal blood ureas. Thereafter, blood ureas reflected allograft function. In the control group, only two of the three rats which survived to the 10th d were judged clinically to be likely to survive anesthesia for blood sampling, and both had blood ureas over 400 mg/100 ml. Rats in the experimental group showed much less striking rises in blood ureas.

The cell-mediated immune responses of the two groups showed a similar contrasting pattern (Fig. 1). High T-cell mediated cytotoxicity against (AS × AUG)F_1 targets was observed at the 7–8th d with cells harvested from control rats, the net ^{51}Cr release being 30%. In the case of cells from experimental rats, the mean net ^{51}Cr release rose to 8% at the 12th d, fell, and then rose again during the 4th wk to 15% before falling again.

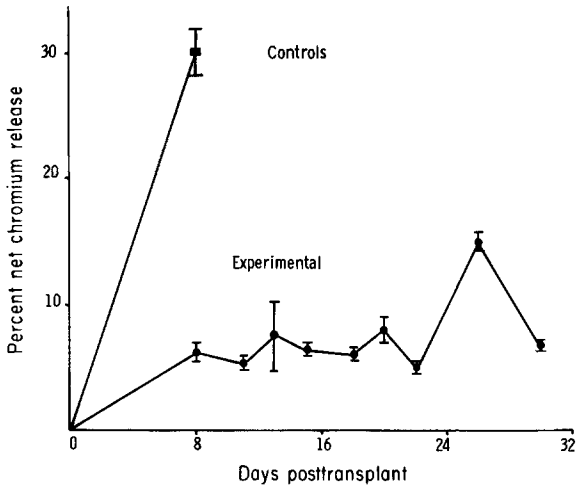

FIG. 1. Cell-mediated immunity of (AS × WF)F_1 rats transplanted with long-term (AS × AUG)F_1 kidneys. ■, control AS rats transplanted with normal (AS × WF)F_1 kidneys. ●, experimental AS rats transplanted with (AS × WF)F_1 kidneys which have resided 60 d in primary AS recipients. Results are the means of net chromium release produced by peritoneal lymphocytes from 3–5 rats ± SE. As previously, effector:target ratio = 100:1.

TABLE IV

*Retransplantation into Naive AS Secondary Recipients of (AS × AUG)F_1 Kidneys Which Have Resided for 3 d In Primary AS Recipients**

Rat No.	Blood urea (mg/100 ml) of Secondary recipient on day:					Survival of secondary recipient
	7	10/11	14	21	28	
						d
J.522	37	743	245	212	139	69
J.537	43	317	157	330	167	49
J.548	33	—	—	—	—	11
J.549	52	232	152	223	96	68
J.550	41	—	—	—	—	11

—, rat dead.

* Primary AS recipients received enhancing AS anti-AUG antibody (see previous experiments).

Retransplantation into Naive AS Secondary Recipients of (AS × AUG)F_1 Kidneys Which Have Resided for 3 d in Primary AS Recipients. It has already been shown that if a passively enhanced (AS × AUG)F_1 kidney has resided in a naive AS recipient for 27 d or longer, it does not sustain acute rejection nor induce normal T-dependent alloimmunity when retransplanted into a naive, secondary AS recipient (2). The present experiment was performed to determine whether a 3-d residence in the first AS recipient was sufficient to produce the same effect. Except for the duration of time spent by the allograft in the primary recipient, the experiment was conducted as described previously. Table IV contains the survival times and blood urea levels of five naive secondary AS recipients, grafted with hybrid kidneys which had spent 3 d in their primary AS hosts. All the secondary recipients developed acute rejection

responses, two of them dying at 11 d, and the remaining three surviving for 7–10 wk with chronic uremia. It is evident that 3-d residence by allografts in primary AS recipients is insufficient to produce those changes which protect long-surviving grafts from acute rejection after they have been retransplanted.

Discussion

In the experiments described here, passively enhanced, long-surviving (AS × AUG)F_1 kidneys, when retransplanted into naive (AS × WF)F_1 recipients, showed prolonged survival. The immune response stimulated by these grafts had the following characteristics. All six (AS × WF)F_1 recipients formed high titers of IgM lymphocytotoxins, whereas in only one was there a substantial level of IgG lymphocytotoxin produced. The generation of cytotoxic T lymphocytes was delayed and very markedly diminished. These results are indicative of a T-independent immune response and are compatible with the hypothesis put forward in the previous paper.

The (AS × WF)F_1 recipients developed higher titers of IgM lymphocytotoxins than the AS secondary recipients and the survival times of the retransplanted kidneys were shorter than in the AS secondary recipients. Strain differences in the strengths of the T-independent response are likely to explain these variations.

As indicated earlier, the strong T-dependent alloimmunity provoked by normal kidney allografts is attributed to passenger cells which carry MHC specificities and can provide the necessary second signal. Obviously, if passenger cells are solely responsible for inciting T-dependent immunity, it would be valuable to find out which populations of cells are concerned and their route through the kidney. The second experiment described in this paper shows that a 3-d residence in a primary AS recipient is not long enough to eliminate all the T-dependent immunogenicity of an (AS × AUG)F_1 kidney. This suggests that at least some of the cells responsible for inciting T-dependent alloimmunity are extravascular and unlikely to be removed by simple perfusion methods. The result also shows that pretreatment of kidney donors with cytotoxic drugs to eliminate passenger cells (3) must ensure adequate extravascular levels of drug. In the experiments of Lafferty and Woolnough (4) and Sollinger and Bach (5) in vitro culture of thyroid tissue for >1 wk was needed to eliminate lymphocytes from the cultured tissue and render it nonimmunogenic. Our results show that similarly the loss of immunogenic passenger cells in vivo is also time dependent.

Summary

Long surviving, passively enhanced (AS × AUG)F_1 kidneys carried by AS recipients were retransplanted into (AS × WF)F_1 second hosts. Acute graft rejection did not occur. Only one of six secondary recipients mounted a significant T-dependent IgG lymphocytotoxic antibody response. In all six, generation of cytotoxic T cells was markedly slower and depressed. These results are compatible with the hypothesis that kidney parenchyma, although carrying major histocompatibility complex specificity is able to induce T-independent but not T-dependent alloimmunity. A corollary is that passenger cells are responsible for exciting the T-dependent alloimmune response normally observed after grafting. The practical difficulty of eliminating all T-dependent immunogenicity from (AS × AUG)F_1 kidneys was emphasized by the observation

that a 3-d residence in an intermediate AS recipient was insufficient time to prevent acute graft rejection after retransplantation.

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