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

II. Retransplantation of (AS × AUG)F1 kidneys from AS primary recipients into (AS × WF)F1 secondary hosts*

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(AS × AUG)F1 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)F1 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)F1 kidneys are acutely rejected when retransplanted from AS recipients into (AS × WF)F1 recipients. Long-surviving kidneys taken from AS recipients should have had their (AS × AUG)F1 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 F1 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)F1

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1 Abbreviation used in this paper: MHC, major histocompatibility complex.
### Table I

Survival of Retransplanted (AS × AUG)F₁♂ kidneys from AS Primary, ♂ Recipients into Secondary (AS × WF)F₁♂ Recipients

| Days in 1° AS ♂ Recipient | Rat No: 2° AS ♂ Recipient | Survival | Comments |
|---------------------------|---------------------------|----------|----------|
| 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       |          |
| 84                        | J.564                     | 43       | No histology obtained. |
| 84                        | J.565                     | >50      |          |

Control (AS × WF)F₁ recipients. Allografted with normal (AS × AUG)F₁ kidneys.

| Rat No: 2° AS ♂ Recipient | Survival | Comment               |
|---------------------------|----------|-----------------------|
| J.558                     | 10       |                       |
| J.559                     | 11       |                       |
| J.560                     | 8        | histology: severe rejection |
| 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)F₁ Kidneys from AS Primary Recipients into Naive (AS × WF)F₁ 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)F₁ recipients were allografted with normal (AS × AUG)F₁ 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)F1 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 log2, 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)F1 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)F1 targets was observed at the 7–8th d with cells harvested from control rats, the net 51Cr release being 30%. In the case of cells from experimental rats, the mean net 51Cr release rose to 8% at the 12th d, fell, and then rose again during the 4th wk to 15% before falling again.
Retransplantation into Naive AS Secondary Recipients of (AS × AUG)F₁ 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)F₁ Kidneys Which Have Resided for 3 d in Primary AS Recipients. It has already been shown that if a passively enhanced (AS × AUG)F₁ 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)F1 kidneys, when retransplanted into naive (AS × WF)F1 recipients, showed prolonged survival. The immune response stimulated by these grafts had the following characteristics. All six (AS × WF)F1 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)F1 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)F1 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)F1 kidneys carried by AS recipients were retransplanted into (AS × WF)F1 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)F1 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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