isolated which exhibits a circadian rhythm and can be phase shifted by light pulses (*11*).

The persistence of an unaltered circadian period in reduced eyes from *Bulla* is significant in that it is not in accord with one proposal that a population of higher frequency noncircadian pacemakers located throughout the retina in *Aplysia* interact to produce a circadian periodicity (*12*). Although our retinal fragments varied in the number of cells remaining, we did not observe any changes in period length. Either the circadian period in *Bulla* is the property of individual retinal cells or is the product of a subpopulation of cells which escaped fractionation in our experiments. Whether or not individual neurons are circadian pacemakers is an important issue. The relatively large size of many of the cells at the base of the *Bulla* retina should be an aid in investigating this question.

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### References and Notes

1. J. W. Jacklet, *Science* **164**, 562 (1969).
2. A. Eskin, *Z. Vgl. Physiol.* **74**, 353 (1971).
3. J. C. Woolum and F. Strumwasser, *Proc. Natl. Acad. Sci. U.S.A.* **77**, 5542 (1980); A. Eskin, *J. Neurobiol.* **8**, 273 (1977).
4. F. Strumwasser, *Physiologist* **16**, 9 (1973). Strumwasser and his co-workers [*Soc. Neurosci. Abstr.* **5**, 809 (1979)] reported that isolated *Aplysia* photoreceptors in cell culture do not exhibit a circadian rhythm in evoked response to periodic light pulses. This result suggests that the photoreceptors are not involved in generating the circadian rhythm in the *Aplysia* eye.
5. G. D. Block and W. O. Friesen, *Soc. Neurosci. Abstr.* **7**, 45 (1981).
6. G. D. Block, W. O. Friesen, S. F. Wallace, in preparation.
7. The mean correlation coefficient for four preparations in which receptor membrane potential and optic nerve impulse activity was measured was −.20.
8. The free-running period of reduced eyes ($N = 5$) measured between the midrise points on the first two cycles in darkness (16°C) was 23.9 hours with a standard deviation of ±0.4 hour, whereas for intact eyes ($N = 5$) the period was 23.8 ± 0.3 hours. There was a difference in peak impulse frequency, with intact eyes ($N = 23$) exhibiting a mean peak rate of 64.1 ± 15.6 impulses per 1/2 hour compared to 34.9 ± 16.4 impulses per 1/2 hour for reduced retinas ($N = 12$). This difference in impulse peak frequency is statistically significant [$t(33) = 5.1$, $P < .005$].
9. Light pulses were delivered to the reduced eyes by means of a plastic light guide placed near the dish with the isolated eye. Pulses were delivered between 2000 and 0200 hours (circadian time). Light intensity varied between 5,000 and 10,000 lux. The phase shift per eye pair was determined during the second in vitro cycle by comparing the midrise time of an eye pulsed with light and one that was not pulsed. The mean phase difference between reduced retinas receiving a light pulse and intact retinas that did not receive a light pulse was compared to the mean phase difference of a control group of reduced and intact eye pairs, neither of which were pulsed with light.
10. The mean phase difference (± standard deviation) between reduced eyes that were pulsed with light and intact eyes that were not pulsed was 1.1 ± 0.8 hours ($N = 7$), whereas the difference between reduced and intact retinas in a control group that did not receive a light pulse was −0.4 ± 0.8 hour ($N = 7$). The mean phase difference between the treatment and control groups was statistically significant [$t(12) = 3.5$, $P < .005$]. In order to compare the phase shift of reduced eyes to phase shifts of intact retinas, one eye of six intact eye pairs was pulsed for 6 hours. The mean phase advance for the pulsed intact eyes was +1.5 ± 0.9 hours, which is similar to the light-induced shift observed in reduced eyes.
11. Cell R-15 in the abdominal ganglion of *Aplysia* expresses one circadian cycle of membrane potential fluctuations after synaptic transmission is blocked with tetrodotoxin and calcium-free seawater (*4*). There is no evidence, however, that the isolated cell rhythm is sustained or can be phase shifted.
12. J. Jacklet and J. Geronimo, *Science* **174**, 299 (1971).
13. We thank L. Shoda and A. Lusska for technical assistance. This work was supported by grant NS15264 from the National Institutes of Health.

19 November 1981; revised 10 May 1982

## Prevention of Allograft Rejection by Immunization with Donor Blood Depleted of Ia-Bearing Cells

Abstract. *Strain-specific unresponsiveness was induced in adult mice by immunizing them with donor blood treated with antiserum to Ia (I region–associated antigens) prior to the transplantation of islets of Langerhans. This regimen alone produced greater than 100-day survival of islet allografts transplanted across a major histocompatibility barrier.*

Studies in our laboratory have demonstrated that allografts of islets of Langerhans in mice survive indefinitely in nonimmunosuppressed recipients if the islets are treated in vitro with donor-specific antiserum to I region–associated antigens (Ia) and with complement before transplantation (*1*). Because islet cells lack Ia determinants (*2*), the treatment with Ia antiserum and complement apparently removes Ia-bearing donor lymphoid cells (passenger cells) that initiate the rejection reaction. Islet allograft acceptance is promptly terminated by the injection of donor splenocytes into established transplant recipients, thus demonstrating the critical role of donor antigen–presenting cells in eliciting rejection (*1*).

Earlier studies on rats with established islet allografts provided evidence that tolerance had been induced in the recipients (*3*). Recently we reported that strain-specific tolerance, probably mediated by T suppressor cells, was present in mice bearing established allografts of histoincompatible islets (*4*). Since transplants of islet tissue depleted of Ia-bearing cells apparently induced tolerance in the recipients, we decided to determine whether immunization of recipient mice with Ia negative cells would permit the successful transplantation of fresh, untreated islets. We used donor blood treated with antiserum to Ia and with complement for immunization since red blood cells are Ia negative in the mouse.

Male B6 mice (C57B1/6J, H-$2^b$) were made diabetic by the intravenous injection of streptozotocin (180 mg per kilogram of body weight). Only B6 mice with plasma glucose levels of greater than 400 mg per 100 ml on three successive determinations were used as recipients. Rejection was defined as a plasma glucose concentration greater than 200 mg per 100 ml in three subsequent blood samples.

Whole blood was obtained from the orbital sinus of B10.BR (H-$2^k$) and B10.S (H-$2^s$) mice with heparinized capillary tubes. The blood was washed once with phosphate-buffered saline containing 10 percent citric acid and then washed twice in Hanks solution. The washed blood cells were centrifuged and the pellet was incubated for 30 minutes at room temperature with an equal volume of antiserum to Ia. Antiserum to $Ia^k$ (A.TH-anti-A.TL) was used to treat B10.BR blood cells; antiserum to $Ia^s$ (A.TL-anti-A.TH) was used to treat B10.S blood cells. The antiserum-treated red blood cells were washed with Hanks solution and then incubated with a 1 in 3 dilution of guinea pig serum as a source of complement for 30 minutes at 37°C. The preparations were diluted with Hanks solution and $5 \times 10^7$ red blood cells (0.5 ml) were injected via the tail vein into B6 recipients according to the schedule shown in Table 1.

Donor B10.BR (H-$2^k$) islets were isolated by the collagenase technique (*5*), separated on a Ficoll gradient (*6*), and then meticulously handpicked with the use of a Pasteur pipette under a dissecting microscope. A total of 550 to 750 freshly isolated B10.BR islets (H-$2^k$) were immediately transplanted by the portal vein technique (*7*) into diabetic B6 (H-$2^b$) animals. Four groups of B6 mice received these freshly prepared allogeneic islets (Table 2). Two groups of recipients were immunized with Ia-depleted blood cells prior to transplantation. Group 1 received donor B10.BR red blood cells (H-$2^k$); group 2 received irrel-


0036-8075/82/0709-0157$01.00/0 

Table 1. Schedule of red blood cell (RBC) injections.

| Interval between | Average time (days) | Range (days) |
|---|---|---|
| First RBC injection and induction of diabetes | 5 | 2 to 9 |
| Induction of diabetes and second RBC injection | 4 | 2 to 10 |
| Second RBC injection and transplantation | 6 | 2 to 14 |
| Total time between first RBC injection and transplantation | 15 | 8 to 26 |

Table 2. Effect of immunization with red blood cells (RBC) on subsequent allograft survival [B10.BR (H-$2^k$) to B6 (H-$2^b$)].

| Group | Treatment prior to transplantation | Transplant survival (days) | |
|---|---|---|---|
| | | Individual | Mean ± standard error |
| 1 | RBC (H-$2^k$) | 16, 16, > 100, > 100, > 100, > 100, > 100, > 100, > 100, > 100, > 100, > 100 | > 86.0 ± 9 |
| 2 | RBC (H-$2^s$) | 7, 7, 7, 13, > 100 | 26.8 ± 18 |
| 3 | Whole blood (H-$2^k$) | 2, 2, 2, 2, 2, 3, 3, 3, 5 | 2.7 ± 0.3 |
| 4 | None | 5, 5, 6, 6, 8, 12, 35, 39, > 100, > 100 | > 31.6 ± 12 |

evant B10.S red blood cells (H-$2^s$); group 3 received untreated whole blood from the donor strain B10.BR (H-$2^k$); and group 4 was not immunized.

Immunization of the recipients with purified preparations of red blood cells from an irrelevant strain had no effect on the survival time of islet allografts compared to controls that had not been immunized with red blood cells. As shown in Fig. 1, 20 percent of the islet allografts were surviving at 100 days after transplantation in both groups; the mean survival time (MST) was > 26.8 ± 18 days in the immunized animals (group 2) and > 31.6 ± 12 days in the controls (group 4 in Table 2). In contrast, immunization of the recipients with purified red blood cells from the donor strain resulted in a marked prolongation of islet allograft survival. The MST in this group was > 86.0 ± 9 days (group 1 in Table 2) and 10 of the 12 recipients were still normoglycemic at 100 days after transplantation (Fig. 1). Rejection was accelerated when untreated whole blood from the donor strain was used for immunization of the recipients. The MST of the islet allografts in these animals was only 2.7 ± 0.3 days (group 3 in Table 2).

The findings in this study indicate that immunization with donor red blood cells depleted of Ia-bearing cells induces unresponsiveness and markedly prolongs the survival of transplants of freshly isolated islets. In contrast, immunization with donor blood containing Ia-bearing cells results in sensitization of the recipients and acceleration of islet allograft rejection. These observations add further support to the concept that Ia-bearing passenger lymphoid cells in islet allografts are responsible for the initiation of immune rejection. In addition, the results are consistent with our earlier studies that indicated that transplants of mouse islet cells depleted of Ia-bearing cells by antiserum to Ia induced unresponsiveness in the recipient mice (*1*). Thus, it appears that either transplantation of Ia-negative mouse islet cells or immunization with Ia-negative donor red blood cells induces immunologic unresponsiveness in the recipients by mechanisms that remain to be defined. Immunization with purified red blood cells apparently raises the threshold level of contaminating passenger cells which can be present in the untreated graft and yet permit successful transplantation.

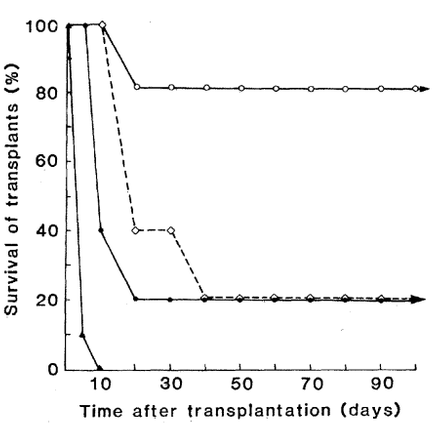


Fig. 1. Survival of fresh allogeneic islets (B10.BR) transplanted into nonimmunosuppressed diabetic recipients (B6). Group 1 (○) recipients were immunized with purified red blood cells from the donor strain. Group 2 (●) recipients were immunized with purified red blood cells from an irrelevant strain (B10.S). Group 3 (▲) recipients were immunized with untreated whole blood from the donor strain. Group 4 (◇) recipients were control mice that were not immunized with any blood cells.

Many investigators have immunized animals with various blood cell products prior to transplantation in attempts to prolong allograft survival (*8*). Although this approach was partially successful in many instances, the results were inconsistent. We believe that this was because the blood product preparations were contaminated with variable numbers of viable Ia-positive lymphoid cells and because immunosuppressive drugs were used in the recipients, a procedure demonstrated to be detrimental to the induction and expression of tolerance (*9, 10*).

Thus we have demonstrated a rapid and simple method of inducing donor-specific unresponsiveness in adult animals which allows long-term survival of islet allografts. This procedure, which eliminates the need for immunosuppression of the recipient, may have important applicability to the treatment of diabetes in humans and the transplantation of other clinically relevant tissues.

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**References and Notes**

1. D. Faustman, V. Hauptfeld, P. Lacy, J. Davie, *Proc. Natl. Acad. Sci. U.S.A.* **78**, 5156 (1981).
2. D. Faustman, V. Hauptfeld, J. Davie, P. Lacy, D. Shreffler, *J. Exp. Med.* **151**, 1563 (1980).
3. I. Zitron, J. Ono, P. Lacy, J. Davie, *Transplantation* **32**, 156 (1981).
4. D. Faustman, V. Hauptfeld, P. Lacy, J. Davie, *Proc. Natl. Acad. Sci. U.S.A.*, in press.
5. P. Lacy and M. Kostianovsky, *Diabetes* **16**, 35 (1967).
6. A. Lindall, M. Steffes, R. Sorenson, *Endocrinology* **85**, 218 (1969).
7. C. Kemp, M. Knight, D. Scharp, W. Ballinger, P. Lacy, *Diabetologia* **9**, 486 (1973).
8. G. Opelz and P. Terasaki, *Transplantation* **29**, 153 (1980); T. Fuller, F. Delmonico, A. Cosimi, C. Huggins, M. King, P. Russell, *Ann. Surg.* **187**, 211 (1978); G. Opelz, M. Mickey, P. Terasaki, *Transplant. Proc.* **13**, 136 (1981); L. Brent, *Cell. Immunol.* **62**, 264 (1981); E. K. Spees, W. K. Vaughn, G. M. Williams, R. S. Filo, J. C. McDonald, G. Mendez-Picon, G. Niblack, *Transplantation* **30**, 455 (1980); G. Persijn, B. Cohen, Q. Lansbergen, J. van Rood, *ibid.* **28**, 396 (1979); D. Salvatierra *et al.*, *Ann. Surg.* **192**, 542 (1980).
9. P. Kilshaw, L. Brent, M. Pinto, *Transplant. Proc.* 7, 225 (1975).
10. M. Wood and A. Monaco, *Transplantation* **27**, 186 (1979).
11. We thank M. L. Chivetta, S. M. Place, and K. Frederick for technical assistance. Supported in part by PHS grants AM-01226, AI-12734, and GM-07200 and by the Kroc Foundation.

25 January 1982; revised 13 April 1982