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13. In three animals of the E0.10 and E1.00 groups, epinephrine increased heart rate and EKG amplitude for 5 to 15 minutes. However, the shock and white noise elicited no EKG response.
14. During the CS, we counted the heartbeats for the 5-second period 1 to 6 seconds after onset of the white noise to allow time for the appearance of a change in heart rate to an acoustic stimulus.
15. Although there were no significant differences between groups, and although most stimuli elicited no change in heart rate, it was possible that the stimuli sometimes did elicit a slight change in heart rate. We thus analyzed heart rate of saline and E0.01 animals during "sham" trials—randomly selected, adjacent 5-second periods in intertrial intervals between test shocks. The fluctuation in heart rate between the first and second 5-second periods during the sham trials did not differ significantly from that between pretrial and shock periods, either within or between saline and E0.01 groups.
16. We thank J. L. McGaugh for beneficial discussions and for the use of testing facilities and K. Strang for assistance in analyzing the EKG records. Supported by a grant from the Monsanto Company; NIH grants AL 01642, MH 31144, and MH 12526; and a biomedical research support grant from the University of Virginia.

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Prolongation of Rat Islet Allograft Survival by Direct Ultraviolet Irradiation of the Graft

Abstract. *Ultraviolet irradiation of rat dendritic cells completely abrogated their allostimulatory capacity in a mixed lymphocyte reaction. Rat islets of Langerhans similarly irradiated remained hormonally functional when transplanted into syngeneic diabetic rats. Allogeneic transplantation across a major histocompatibility barrier of islets initially treated in vitro with ultraviolet irradiation resulted in prolonged allograft survival without the use of any immunosuppressive agents.*

Although disparity between major histocompatibility complexes (MHC's) leads to rejection of grafted tissue, it is the recognition of this incompatibility by the host that appears to be critical in initiating the rejection process. Recognition of foreignness by the host seems to require the presence of class I and class II MHC antigens on the graft, while lymphoreticular cells accompanying the graft and bearing both classes of antigens are thought to be responsible for sensitizing the host toward a primary immune response (*1*). We report here that ultraviolet (UV) irradiation (sunbeam spectrum), at a dose that can abrogate a mixed-lymphocyte reaction (MLR) after irradiation of rat dendritic stimulator cells (*2*), can also attenuate the immunogenicity of pancreatic islets without altering their endocrine function and can prolong the survival of rat islet allografts in diabetic hosts without the use of immunosuppressive agents.

The exact nature of the "passenger leukocyte" implicated in causing graft rejection is not known. Rat dendritic cells (DC's) have been demonstrated to be extremely powerful as accessory cells in T-cell proliferation and in causing rapid rejection of rat kidneys otherwise depleted of passenger leukocytes (*3*). We first investigated the ability of UV irradiation of rat DC's derived from afferent lymph (*3*) to attenuate their stimulatory activity in an MLR. Abdominal lymph nodes were removed from rats 6 weeks before thoracic duct drainage. Lymph was collected over a 36-hour period and DC's among the resultant cells were enriched to approximately 70 percent by high-density centrifugation (*4, 5*). These cells were gamma-irradiated (1600 rads) and then UV-irradiated in open petri dishes while suspended in Hanks balanced salt solution with constant stirring with a magnetic bar. The source of the UV irradiation was a bank of two FS20 lamps (Westinghouse), which have a flux of 1 mW/cm^2 at 310 nm (UVX-Radiometer, Ultra-Violet Products) measured 10 cm from the source. Using thoracic duct lymphocytes from rats of strain ACI (RT1^a) as responders and Lewis rat (RT1^l) DC's as stimulators, we obtained a high stimulation index (SI) (*6*) of > 400 with 10^5 DC's (Fig. 1). When the number of DC stimulators was decreased to 0.125×10^5 the SI remained markedly elevated at 162.

Dendritic cells subjected to UV irradiation (800 to 1000 J/m^2) were completely ineffective as stimulators in the MLR (SI < 3). Although DC's are extremely powerful allogeneic stimulators in the MLR and cause graft rejection (*2, 3*), they appear to be inactivated as stimulators by UV but not gamma irradiation.

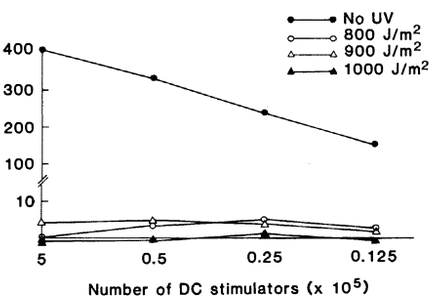

Fig. 1. Effect of UV irradiation on the stimulatory activity of Lewis rat DC's in the MLR. The proliferative response of ACI thoracic duct lymphocytes is expressed (SI) (*6*).

Once the dose range of UV irradiation necessary to attenuate the MLR was defined, we examined the ability of islets irradiated at the same dose range to reverse the diabetic state in syngeneic diabetic rats (Fig. 2). Rats were made diabetic with intravenously administered streptozotocin (60 mg/kg) and used as recipients if blood glucose was > 300 mg/dl on three weekly successive measurements. Lewis rat islets were isolated by collagenase digestion, Ficoll gradient separation (*7*), and subsequent handpicking under a stereomicroscope. Isolated islets were suspended in Hanks balanced salt solution in open petri dishes and irradiated while being constantly stirred with a magnetic bar. The UV source was the same as for the DC's. After irradiation the islets were incubated for 24 hours at 37°C and 5 percent CO_2 in CMRL medium 1066 with 10 percent fetal calf serum and transplanted through the portal vein. Islets irradiated with 1000 J/m^2 converted diabetic recipients to a normoglycemic state for less than 5 days; islets irradiated with 1200 J/m^2 failed to convert them. Irradiation of syngeneic islets with 600 or 900 J/m^2 resulted in permanent conversion of all diabetic recipients to normoglycemia. Thus the dose of UV irradiation that can abrogate the proliferative response in the MLR with 10^5 DC stimulators has no deleterious effect on the in vivo endocrine function of syngeneic islet grafts irradiated with 900 J/m^2.

To determine whether the immunogenicity of allogeneic islets was reduced after such irradiation, Lewis islets were transplanted into ACI rats made diabetic with streptozotocin (Fig. 3 and Table 1). All the control ACI animals receiving Lewis islets cultured for 24 hours at 37°C rejected their grafts and became diabetic again after 6.8 ± 2.7 days (mean ± standard deviation). When Lewis islets were exposed to UV irradiation at 900 J/m^2, cultured for 24 hours, and transplanted into diabetic ACI recipients, the islets survived for more than 80 days in 8 of 11 animals (more than 110 days in four rats), and all eight remained normoglycemic. These results indicate that UV irradiation of allogeneic rat islets at a level that is not deleterious to their endocrine function reduces the islets' immunogenicity and permits prolonged allograft survival without immunosuppression.

The importance of passenger leukocytes in initiating allograft rejection has been a recurring theme in transplantation immunology (*8*). In islet transplantation various in vitro culture techniques (*9*) to

Table 1. Effect of direct UV irradiation on the survival of Lewis islet allografts in diabetic ACI recipients.

Islet treatment	*N*	Survival time (days)
24-hour culture	10	4, 4, 4, 6, 6, 7, 7, 8, 9, 13*
Irradiation and 24-hour culture	11	10, 10, 18, > 75, > 75, > 75, > 110, > 110, > 110, > 110†

*Mean ± standard deviation, 6.8 ± 2.7 days. †Mean, > 80 days.

prolong allograft survival have been used, all relying on the assumption that long-term culture selectively depletes the islet of lymphoreticular elements (*10*). Recently, the use of antiserum to class II MHC antigen and complement to remove cells bearing I region–associated antigens permitted prolonged survival of allogeneic islet grafts in the mouse (*11*).

The identity of passenger leukocytes remains unknown. Investigators have been studying the role of interstitial DC's in the primary alloactivation of T cells in MLR's and in the rejection of rat kidney allografts (*3, 12*). The ubiquitous class II MHC antigen–bearing cells in rat islets, kidneys, and hearts may be the passenger leukocytes responsible for direct sensitization of the host toward the allograft antigens (*13*). We have demonstrated that DC's are powerful activators of relevant T responder cells in the primary MLR and that their stimulatory activity can be completely inactivated with appropriate UV irradiation.

Many studies have shown that UV irradiation has a selective effect on antigen-presenting cells (APC's) (*14*) and that passive transfer of UV-irradiated APC's can induce antigen-specific T suppressor cells (*15*). These and other studies (*16*) suggest that improper presentation of antigens may either induce preferential production of T suppressor cells or effect nonrecognition of foreignness until the antigens are represented to the host's T cells by APC's, at which point production of donor-specific T suppressor cells may also occur. We previously showed that UV irradiation of peripheral blood lymphocytes does not quantitatively alter cell-surface antigens, including class II MHC antigens (*17*). It therefore appears that primary allostimulation not only requires class I and II antigen–bearing lymphoreticular cells (*11, 18*), but that they must be metabolically active and are susceptible to inactivation by UV irradiation (*19*).

The prolonged survival of irradiated islets suggests that their exposure to a dose of UV irradiation that is effective in abrogating the MLR response selectively attenuates the stimulatory activity of the interstitial DC's or of other allostimulatory cells in the islet preparation. Since islets are not single-cell suspensions, some allostimulatory leukocytes may escape complete inactivation, which might explain a 27 percent failure rate in prolongation of islet allograft survival in our experiment. A more precise quantitative method of delivering UV irradiation and determining its ultimate effect on islet function and simultaneously on the allostimulatory activity of DC's and other lymphoreticular cells in the islet preparation is necessary before this approach can be uniformly successful.

In conclusion, our approach offers distinct advantages over long-term culture techniques and does not require the use of specific antisera to the class II MHC antigen. We believe that this study may form the basis for islet transplantation in other animals and ultimately in man.

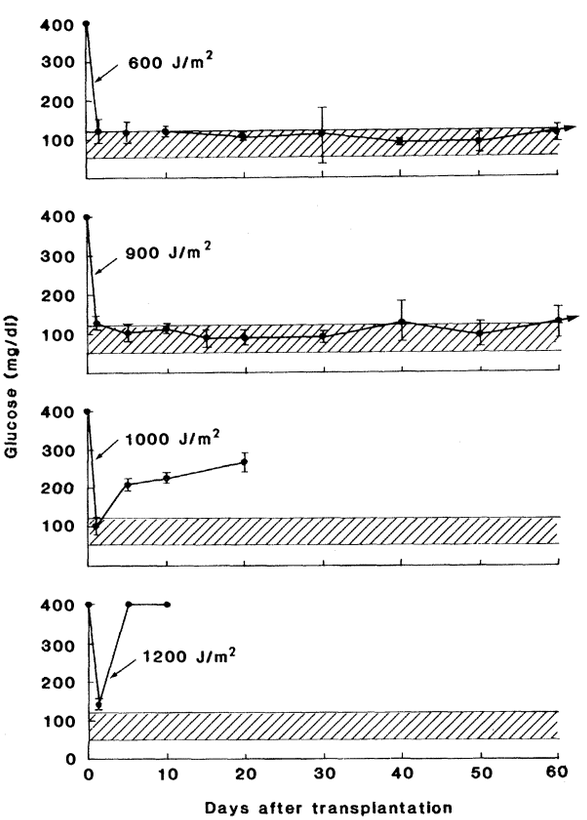

Fig. 2. Effect of direct UV irradiation on islet function after syngeneic transplantation. Shading shows the normal range of blood glucose concentrations.

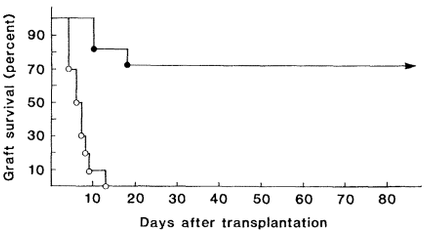

Fig. 3. Survival of UV-irradiated (●) and nonirradiated (○) Lewis rat islets in diabetic ACI recipients. Islets were isolated as described for syngeneic transplants and were irradiated with 900 J/m². All islets were cultured for 24 hours before their intraportal transplantation. Rejection of islets was considered to have occurred if the level of blood glucose exceeded 200 mg/dl on two consecutive daily measurements.

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20. We thank M. Prasek and J. Mucha for technical assistance, R. Grobtuch for editorial assistance, J. Dulin for streptozotocin, and C. Russo and B. Pernis for valuable advice.
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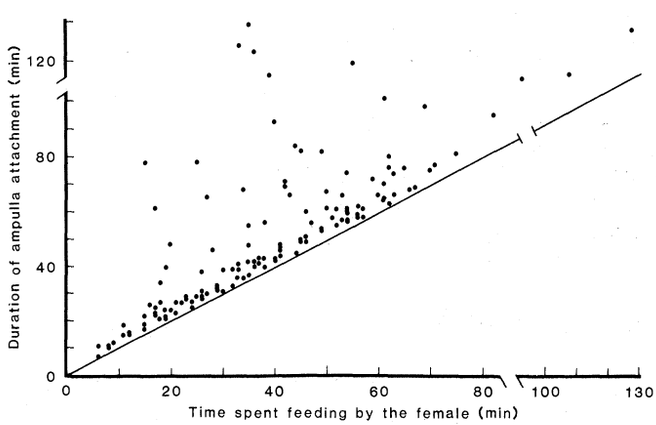
Male Crickets Feed Females to Ensure Complete Sperm Transfer

Abstract. *The spermatophore transferred by the male decorated cricket* Gryllodes supplicans *to the female during copulation includes a large gelatinous portion (spermatophylax), which the female removes and feeds on immediately after mating. Females usually removed and ate the smaller sperm-containing portion (ampulla) within 1 to 7 minutes after fully consuming or losing the spermatophylax. Complete sperm transfer requires that the ampulla remain attached for a minimum of 50 minutes; this corresponds to the average time at which females actually removed ampullae, 52.0 ± 2.2 minutes after mating. These results indicate that nuptial feeding of the female cricket functions to deter females from removing the sperm ampulla before sperm transfer is complete.*

Nuptial feeding of females by males occurs in various insects although its adaptive significance is often unclear. Food gifts presented to females before, during, or after mating include prey captured by the male, glandular products and, in some instances, the male's own body (*1*). The bipartite spermatophore which the male decorated cricket, *Gryllodes supplicans*, transfers to a female during, copulation consists of a large gelatinous mass or spermatophylax attached to a smaller sperm-containing ampulla (*2*). Immediately after mating, the female detaches the spermatophylax and feeds on it before removing and eating the sperm ampulla. Alexander and Otte (*2*) proposed that the spermatophylax of *G. supplicans* might prevent the female from removing the sperm ampulla before it was emptied of sperm. Although this "sperm protection" function has been attributed to glandular feeding in other gryllids as well (*3–5*), it never has been empirically demonstrated. I now report that nuptial feeding of female crickets deters females from removing the sperm ampulla until complete sperm transfer has occurred.

After mating, the spermatophylax was normally removed by a female *G. supplicans* within 1 to 5 seconds of dismounting the male (202 of 228 matings) (*6*). Alexander and Otte's hypothesis (*2*) predicts that the female should remove the sperm ampulla shortly after the spermatophylax is fully consumed. Females finished eating the spermatophylax 39.8 ± 0.7 minutes (mean ± standard error) after mating and subsequently removed and ate the ampulla 12.2 ± 1.5 minutes later (N = 143) (Fig. 1) (*7*). In 64 percent of the matings (91 of 143), females removed ampullae less than 7 minutes after fully consuming the spermatophylax. These results reveal that a female removes the sperm ampulla shortly after feeding regardless of the feeding-phase duration.

Loss of the spermatophylax to the female should result in her removing the sperm ampulla within a similar time period as occurs after normal feeding. In many cases, the female only ate part of the spermatophylax before dropping what remained. This normally occurred 10.5 ± 2.0 minutes after mating (N = 85). In these cases, the female removed the ampulla 10.1 ± 1.6 minutes later, regardless of the amount of time that had



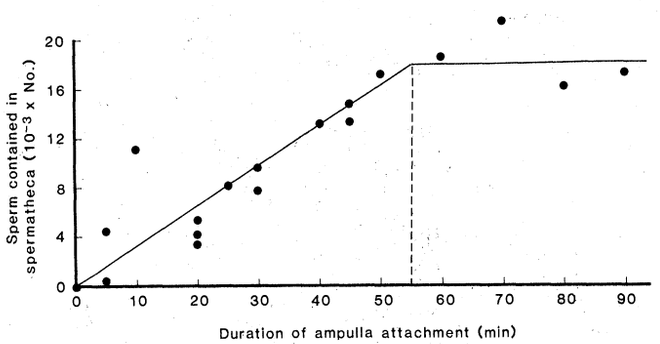


Fig. 1 (left). The time after mating at which female *G. supplicans* removed the sperm ampulla as a function of the time at which they fully consumed the spermatophylax. The line describes those points where the duration of ampulla attachment equals the time spent feeding by the female. Females removed the ampulla 12.2 ± 1.5 minutes (mean ± standard error) after fully consuming the spermatophylax (median time, 5 minutes; range, 1 to 105 minutes). Fig. 2 (right). The number of sperm transferred to the female's spermatheca as a function of the duration of ampulla attachment (N = 18). The point at which the dotted line intersects the x-axis (abscissa) represents the time at which sperm transfer is probably complete. The lines drawn are based on the assumption that the 55-minute cutoff after sperm ejaculation ceases would lead to a constant value after that time, thus: $y = 0.322x$ for $y < 55$ minutes.