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27. We thank M. Steinmetz for his help during the initiation of this work, K. Minard for preparation of the sequencing gels, and B. Larsh and S. Olive for preparation of the manuscript. Supported in part by NIH grants (L.H.) and by the Fondation pour la Recherche Médicale (M.M.).

16 June 1983

Pancreatic Islet Allograft Prolongation by Donor-Specific Blood Transfusions Treated with Ultraviolet Irradiation

Abstract. *Survival of allografts of islets of Langerhans in nonimmunosuppressed adult rats was prolonged by transfusions of donor blood irradiated with ultraviolet light before transplantation across a major histocompatibility barrier. This treatment is donor blood–specific and has produced greater than 160-day survival of transplanted islets without the administration of immunosuppressive agents.*

The successful transplantation of allogeneic pancreatic islets in mice depleted of cells bearing I region–associated antigens (Ia) by antiserum to Ia and the enhancement of skin allografts with this antiserum (*1*) suggest that allografted tissue depleted of Ia-bearing cells is accepted without being recognized as foreign. The type of Ia-bearing cell eliminated in such experiments is not known, but appears to be the dendritic cell; Ia-bearing dendritic cells are present in frozen tissue sections of islets and in the parenchyma of human kidneys, hearts, thyroid glands, and skin (*2*). Such a wide distribution suggests that the depletion of Ia-bearing cells from organ allografts may have clinical applicability not only to pancreatic islet transplantation but to transplantation of other organs as well.

Table 1. Effect of UV irradiation on the stimulatory activity of Lewis rat peripheral blood lymphocytes (PBL) in MLR's. Values are means ± standard deviations.

Responder	Stimulator	[^{3}H]thymidine incorporation (count/min)
ACI	ACI	465 ± 153
ACI	Lewis PBL	5371 ± 543
ACI	Lewis PBL and UV irradiation for 20 minutes	772 ± 102

Although abolition of the initial recognition of a foreign allograft by the host is critical to successful allografting without further immunosuppression, maintenance of a functioning allograft may depend on the initiation of donor-specific suppressor T lymphocytes in the host (*3*). Such a state of unresponsiveness to allogeneic tissue is seen when Ia-negative platelets and red blood cells are unable to provoke a primary immune response and attenuate the subsequent challenge with Ia-bearing cells (*3*).

This idea was further supported when treatment of diabetic mice with donor blood depleted of Ia-bearing cells allowed successful transplantation of fresh, untreated allogeneic islets of the blood donor strain (*4*). It appears, therefore, that immunization with Ia-negative donor blood cells induces immunological unresponsiveness to the donor strain in recipients by the stimulation of specific suppressor cells.

Our demonstration of a rapid and simple method of inducing donor-specific immunological unresponsiveness in adult animals that allows long-term survival of islet allografts is consistent with recent clinical studies in which donor-specific transfusions led to 1-year survival of kidney allografts in more than 90 percent of mismatched donor-recipient pairs of one haplotype (*5*). Since ultraviolet (UV) irradiation of the stimulating cell population in a primary mixed-lymphocyte reaction (MLR) leads to little or no proliferative response (*6*), we hypothesized that Ia-bearing cells may not need to be eliminated from blood before its use for immunization, but may need to be inactivated with UV light, leading to abrogation of the stimulating allogeneic signal while leaving major histocompatibility complex antigens intact for the induction of donor-specific immunological unresponsiveness.

Rats of strain ACI (RTl^a) were made diabetic with intravenous streptozotocin (60 mg/kg). A rat was used as a recipient of blood and islets only if its blood glucose concentration exceeded 300 mg/dl for more than 3 weeks. Islet allografts were considered to have been rejected when plasma glucose was greater than 200 mg/dl on two successive daily measurements.

Whole blood was obtained from normal Lewis rats (RTl^l) by intracardiac puncture. The blood was diluted 1:50 in phosphate-buffered saline (PBS), placed with a magnetic stirring bar into 250-ml petri dishes, and irradiated for 20 minutes with two Sylvania FS-20 lamps located 10 cm from the dishes. The blood cells were then centrifuged and the resulting pellet was resuspended in PBS to 50 percent packed cell volume. Each diabetic ACI rat received 1 ml of UV-irradiated blood or 1 ml of identically treated nonirradiated blood adjusted to 50 percent packed cell volume through the penile vein 3 weeks, 2 weeks, and 1 week before islet transplantation. One group of diabetic ACI rats received islets without previous transfusions.

Pancreatic islets were harvested from Lewis (RTl^l) and Wistar Furth (WF) (RTl^u) rats by the collagenase technique (*7*) and Ficoll gradient separation (*8*), with subsequent handpicking under a dissecting microscope. Some 1200 to 1500 freshly prepared allogeneic islets

Table 2. Effect of UV light on the serological reactivity of Lewis rat PBL surface antigens. Values are mean counts (± standard deviations) of ^{125}I-labeled staphylococcal protein A bound per assay (background, 200 count/min).

Antigen	PBL	PBL and UV irradiation (20 minutes)
Rabbit antiserum to rat lymphocytes	2996 ± 172	3315 ± 434
Monoclonal antibodies to rat Ia (MRC/OX4)	2050 ± 421	1963 ± 268

were transplanted intraportally into four groups of diabetic ACI rats. Two groups of islet recipients (groups 1 and 4) were first transfused with UV-irradiated whole blood. One control group (group 3) was not transfused before receiving islets, while a second control group (group 2) was transfused with nonirradiated blood before allografting.

In vitro studies of Lewis rat peripheral blood lymphocytes that were either nonirradiated or irradiated in a manner identical to that of the whole blood were performed in conjunction with the above study (*9*). The binding of monoclonal antibodies to rat Ia (MRC-OX4) (*10*) and of a polyclonal rabbit antiserum to rat lymphocytes (M.A. Bioproducts) was determined with ^{125}I-labeled staphylococcal protein A on Lewis rat lymphocytes obtained from peripheral blood that was UV-irradiated or untreated with irradiation (*11*). MLR's were performed by using Lewis lymphocytes obtained from irradiated or untreated whole blood (identical treatment to that described for transfusions before islet allografting) as stimulator cells and ACI thoracic duct lymphocytes as responders (*12*).

Lewis peripheral blood lymphocytes obtained from UV-irradiated blood did not stimulate ACI thoracic duct lymphocytes significantly compared to Lewis lymphocytes obtained from nonirradiated whole blood (Table 1). In the radioimmunoassay there appeared to be no significant difference between lymphocytes obtained from irradiated Lewis peripheral blood and untreated blood (Table 2). The rabbit antiserum to rat lymphocytes and the monoclonal antibody to rat Ia (MRC/OX4) showed similar binding to Lewis peripheral blood lymphocytes, regardless of whether UV irradiation was used. Therefore we did not detect allostimulation in the MLR's by peripheral blood lymphocytes that were irradiated, despite the clear demonstration by radioimmunoassay that major histocompatibility antigens are quantitatively unchanged by previous irradiation of lymphocytes.

In the in vivo allograft experiments, diabetic ACI recipients that were transfused with UV-irradiated Lewis whole blood and subsequently transplanted with fresh Lewis islets (group 1) showed 100 percent conversion to normoglycemia. There was no tissue rejection in any of the ten animals in a period of more than 160 days after allografting. The nontransfused control group (group 3) and the control group transfused with nonirradiated blood (group 2) had similar mean survival times (8.2 ± 2.9 and 8.8 ± 4.1 days, respectively). When third-party WF islets were transplanted into diabetic ACI recipients transfused previously with irradiated Lewis whole blood (group 4), rejection of islets and return to the diabetic state occurred in a normal fashion (mean survival time, 7.5 ± 3.0 days) (Table 3 and Fig. 1).

Table 3. Survival of islet transplants in the various treatment and control groups.

Group	Treatment	Donor strain	*N*	Survival time (days)	Mean survival time ± standard deviation (days)
1	Irradiated Lewis blood	Lewis	10	> 160	
2	Lewis blood	Lewis	5	3, 7, 9, 11, 14	8.8 ± 4.1
3	None	Lewis	5	5, 7, 8, 8, 13	8.2 ± 2.9
4	Same as group 1	WF	4	6, 6, 6, 12	7.5 ± 3.0

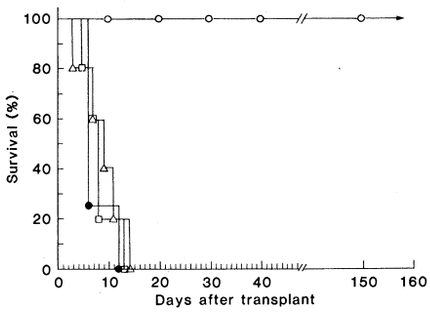

Fig. 1. Graft survival in diabetic ACI recipients of islet allografts. Symbols: (○) group 1, (△) group 2, (□) group 3, and (●) group 4.

These results demonstrate that transfusions of UV-irradiated whole blood of the donor type lead to a prolonged and perhaps indefinite survival of islet allografts and induction of normoglycemia in the diabetic host. These results are obtained without any immunosuppressive drugs, and the induction of unresponsiveness with UV-irradiated blood appears to be donor-specific. Parallel in vitro studies suggest that UV irradiation of blood abrogates the allostimulatory effect of blood lymphocytes in the MLR—even in the presence of UV-absorbing red blood cells—while not affecting the serological reactivity of Ia (as shown by radioimmunoassay) or antigens detected by rabbit antiserum to rat lymphocytes. These findings suggest that allostimulation requires the presence of a metabolically active Ia-bearing cell and that immunization with inactivated cells can lead to subsequent strain-specific immunological unresponsiveness to islet allografts in rats. Although immunological unresponsiveness to allografts and induction of T suppressor cells have been demonstrated with various blood transfusion protocols, the results were inconsistent and immunosuppression was generally required (*13*). We believe, as Faustman *et al.* (*4*) suggested, that in islet transplantation sensitization and subsequent rejection occurs because of "contaminating" Ia-bearing cells; however, our findings suggest that these cells need not be physically eliminated but may simply be inactivated to result in immunological unresponsiveness of the host without the need for further immunosuppressive intervention. Studies of recombinant mouse strains (*14*) support the conclusion that the Ia signal is altered by UV irradiation without a significant change in the class I antigens. This may occur through metabolic inactivation of the cell.

Thus UV irradiation offers a promising method for the induction of donor-specific immunological unresponsiveness. The use of UV irradiation for immunological inactivation of blood products could be easily applicable to allotransplantation in other species for which specific antibodies to Ia are not available or required. This approach may prove useful in the transplantation of human organs, an area where donor-specific blood transfusions are already in use, and may eliminate the possibility of sensitization to major histocompatibility antigens of the donor. Prolonged (or indefinite) islet allograft survival and correction of diabetes may be achieved by this simple maneuver without requiring immunosuppression of the diabetic host.

HENRY LAU
KEITH REEMTSMA
MARK A. HARDY*

Department of Surgery,
Columbia College of Physicians and Surgeons, New York 10032

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15. We thank Margie Prasek and Jitka Mucha for technical assistance and Renee Grobtuch for preparing the manuscript. We are grateful for the comments of Doctors Russo, Ferrone, and Pernis. This work was supported by NIH grants AM 30468 and HL 14799.
* To whom requests for reprints should be addressed.

23 May 1983

Inflammation and Collagenase Production in Rats with Adjuvant Arthritis Reduced with 13-*cis*-Retinoic Acid

Abstract. *Oral administration of 13-*cis*-retinoic acid (40 or 160 milligrams per kilogram of body weight daily) significantly reduced the inflammation associated with developing and established adjuvant arthritis, an experimentally induced arthritis in rats that resembles human rheumatoid arthritis. The amount of collagenase secreted in tissue culture by adherent cells isolated from the inflamed joints of adjuvant rats treated with 13-*cis*-retinoic acid also decreased as compared to the amount secreted by cells from vehicle-treated adjuvant rats. Collagenase is important in the joint destruction accompanying rheumatoid arthritis. The successful use of retinoids in the treatment of this proliferative but nonmalignant disorder demonstrates a new application of these compounds.*

In recent years, naturally occurring all-*trans*-retinoic acid and the synthetic retinoid 13-*cis*-retinoic acid have been used to treat a variety of disorders. All-*trans*- and 13-*cis*-retinoic acid have been effective in the prevention of experimental neoplasms (*1*); 13-*cis*-retinoic acid, the less toxic compound, has been used in the management of human tumors (*2*) and in the treatment of a number of proliferative dermatologic disorders (*3*). Rheumatoid arthritis is another proliferative but nonmalignant disease in which cartilage, tendons, and subchondral bone are progressively destroyed by a mass of proliferating and invading synovial cells. Collagenase and prostaglandin E_2 (PGE_2) are synthesized and secreted in large quantities by rheumatoid synovial cells, and these two compounds are prime mediators of the joint destruction seen in rheumatoid disease (*4*). Until recently, only corticosteroids were known to block the production of collagenase by these cells.

Brinckerhoff *et al.* have reported that all-*trans*- and 13-*cis*-retinoic acid also inhibited the production of collagenase by monolayer cultures of rheumatoid synovial cells; PGE_2 production was affected little or not at all (*5*). We report here the effect of oral treatment with 13-*cis*-retinoic acid on the inflammation associated with adjuvant arthritis (AA) in rats and on the production of collagenase and PGE_2 by adherent cells taken from inflamed ankle joints of these rats (*6*). Adjuvant arthritis, which can be induced by the injection of adjuvant into one hind paw of a rat, shares a number of features with rheumatoid arthritis, for example, proliferating synovitis, joint swelling, and erosion of cartilage and bone (*7*). The condition is characterized by an initial inflammatory response that peaks in the adjuvant-injected paw by day 5; a systemic response that is characterized in part by the development of chronic inflammatory lesions in both the injected and the contralateral hind paw develops at about day 14. The secondary stage of AA is thought to result from a cell-mediated immune response either to disseminated *Mycobacterium* antigens or, more likely, to some endogenous antigen (*8*). The concentrations of acute-phase proteins, for example, fibrinogen (*9*), in plasma from rats with AA are elevated as they are in plasma from patients with rheumatoid arthritis (*10*). Adjuvant arthritis is widely used as a model in which to identify and to test the efficacy of anti-inflammatory compounds. Nonsteroidal anti-inflammatory drugs and corticosteroids markedly inhibit the development of AA when administered from the time of adjuvant injection or when given to animals with established AA (*11*).

The data illustrated in Fig. 1 (combined from three experiments) demonstrate that treatment with 13-*cis*-retinoic acid (40 or 160 mg/kg) for 25 days suppressed the development of AA. Treatment with 13-*cis*-retinoic acid suppressed the secondary lesions that developed in the arthritic paws (both adjuvant-injected and contralateral paws) between days 11 and 25 (Fig. 1, A and B) to a greater extent than it did the acute lesion that developed in the injected paw during the first days of the disease (Fig. 1A).

The concentration of plasma fibrinogen in normal rats was 298 ± 16 mg per deciliter of plasma. In vehicle-treated rats with AA, this rose to 586 ± 48 mg/dl but decreased significantly to 373 ± 34 mg/dl in rats with AA treated with 13-*cis*-retinoic acid at a dosage of 160 mg/kg. The rats with AA gained significantly less weight than normal rats during the 25-day experimental period, and this effect was not reversed by treatment with 13-*cis*-retinoic acid.

13-*cis*-Retinoic acid was also effective in the treatment of established AA. The AA was allowed to develop for 21 days, and then rats with equal mean paw volumes were divided into three groups for treatment. Drugs administered once a day by intubation for 8 days were either vehicle, indomethacin (3 mg/kg), or 13-*cis*-retinoic acid (100 mg/kg). Changes in paw volume and in the concentration of

Table 1. Effect of treatment for 8 days with 13-*cis*-retinoic acid or indomethacin on established adjuvant arthritis. Means ± the standard errors of the means are reported for the combined data from two experiments. In these experiments, arthritis was induced by the subcutaneous injection of adjuvant into the base of the tail. Changes in paw volume (the volume on day 28 minus the volume on day 21) are the combined values for both hind paws. Drugs were administered from day 21 through day 28.

Treatment (*N*)	Dose (mg/kg)	Change in paw volume (ml)	Plasma fibrinogen* (mg/dl)
Vehicle (11)		+1.14 ± 0.25	1482 ± 92
13-*cis*-Retinoic acid (11)	100	−0.25 ± 0.11†	965 ± 97†
Indomethacin (11)	3	−1.92 ± 0.28†	369 ± 61†

*Measured as described by Exner *et al.* (*22*). †Significantly different from the value for vehicle-treated arthritic animals ($P < 0.05$).