the cell cycle. In the second place, when a 20-minute exposure (which does not arrest the cell cycle) was used, lymphocytes from old donors incorporated less [^{3}H]thymidine than did lymphocytes from young donors; but this impairment was only 20 percent, as compared to the 46 percent impairment observed after a 24-hour exposure (*11*).

The effect of [^{3}H]thymidine on the progression of lymphocytes through the cell cycle and on chromosomal damage offers a new and potentially important probe of cell proliferation and the chromosomal stability of dividing cells in culture. The perturbation of cell cycle progression induced by [^{3}H]thymidine incorporation may be more pronounced in lymphocytes from old donors because of an increased sensitivity to radiation damage or a reduced capacity of lymphocytes from old individuals to repair DNA damage. The impaired proliferative response of lymphocytes from subjects with various diseases, as assessed by [^{3}H]thymidine incorporation, may therefore represent impaired activation of quiescent lymphocytes or increased sensitivity of the cells to the radiobiological effects of [^{3}H]thymidine.

LISA STAIANO-COICO
ZBIGNIEW DARZYNKIEWICZ
Sloan-Kettering Institute, New York 10021

JOHN M. HEFTON
REGINA DUTKOWSKI
GRETCHEN J. DARLINGTON
MARC E. WEKSLER
Department of Medicine, Cornell University Medical College, New York 10021

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H-2 Antigen Class: Effect on Mouse Islet Allograft Rejection

Abstract. *Rejection of mouse pancreatic islet allografts occurred in a high percentage of donor recipient combinations identical for H-2I–region antigens and differing at H-2K and H-2K + H-2D without I-region disparities. The results suggest that disparities in major histocompatibility complex antigens of class I (H-2K and H-2D) alone are capable of eliciting islet allograft rejection, and that lack of a stimulus from class II (I-region) alloantigens does not ensure permanent islet allograft survival.*

Prolonged survival of murine pancreatic islet allografts transplanted across a major histocompatibility barrier (H-2K + H-2I + H-2D disparate) was reported by Faustman *et al.* (*1*) after isolated islets were treated with donor-specific antiserum to I-region associated (Ia) antigens and complement. Loss of the Ia stimulus presumably led to prolonged islet allograft survival. Pancreatic islet β cells do not express class II (I-region) determinants, but do express class I (H-2K and H-2D) antigens (*2*). Protocols such as culture of islets (*3*) or treatment of islets with antiserum to lymphocytes (*4*) which lead to prolonged survival of islet allografts have been postulated to do so because they eliminate Ia-bearing lymphoid or other passenger cells within the islets that are necessary to initiate islet allograft rejection.

Evidence from studies in vitro indicates that Ia$^+$ cells are necessary for stimulation in the mixed lymphocyte reaction (MLR) regardless of whether the alloantigen differences involve either H-2K or Ia (*5*) determinants, and that Ia$^+$ stimulator cells are necessary in primary MLR's against both H-2K and H-2D gene products (*6*). Islet transplantation between congenic mice with disparities at the H-2K or H-2K + H-2D regions but with identical I-region loci can thus be used to test the requirement for allo Ia$^+$ cells to initiate an immune response to an allograft.

Previously, variable results were obtained in intrasplenic islet allograft experiments in which congenic strains differing at isolated I and S subregions of the H-2 complex were used (*7*). To define better the isolated contributions of class I and class II antigens we tested the effects of H-2K, H-2D, H-2K + H-2D, and H-2I-region disparities on islet allograft rejection in B10 congenic mice raised in the mouse colony of the Immunobiology Research Center at the University of Minnesota.

Recipient mice were made diabetic by intraperitoneal injection of streptozotocin (200 mg per kilogram of body weight). Only mice of the same sex as the donors, with nonfasting plasma glucose levels of more than 400 mg/dl, were used as recipients.

Islets were isolated by collagenase digestion as described (*8*). Briefly, for each transplant, intact pancreata were rapidly excised from seven overnight-fasted mice, cut into four pieces, and placed 2 pancreata per vial, into 8 ml of collage-

Table 1. Donor-recipient strain combinations and duration of islet allograft function after transplantation in diabetic mice according to isolated H-2 differences.

Donor strain	Recipient strain	H-2 disparity	Day islets rejected by individual mice	Time mice followed with continuing islet function (days)	Number functioning > 100 days (%)
B10.AQR	B10.AQR	None		> 110, > 110 > 130, > 190 > 190	100
B10.T(6R)	B10.AQR	I	21	> 100, > 100, > 100 > 100, > 100, > 100 > 100, > 100, > 100 > 110, > 100, > 110	92
B10.AQR	B10.A	K	8, 11, 13, 17, 19, 23, 23, 25	> 100, > 100, > 110	27
B10.A(2R)	B10.A	D	10	> 100, > 100, > 100 > 100, > 110, > 110 > 110, > 110	89
B10.AQR	B10.M(17R)	K + D	10,12, 17, 20, 25	> 100, > 110	29
B10.S	B10.G	K + I + D	12, 13, 13, 14, 18, 24, 26, 40	> 100, > 100	20

nase (1 mg/ml) solution. After being shaken vigorously (200 cycle/min) in a 37°C water bath for 12 minutes, the partially digested pancreata were combined in 6 ml of collagenase (0.5 mg/ml) for sequential 5-minute digestion periods. After each digestion period the liberated tissue in suspension was washed with Hanks balanced salt solution (HBSS) and the islets were meticulously hand-picked under a dissecting microscope. The islets, 700 to 800 in number, were examined by reflected green light to remove lymph nodes (*9*) and were then transplanted beneath the left renal capsule by means of a butterfly needle. Normoglycemia (plasma glucose < 200 mg/dl) appeared in diabetic recipients within 1 to 3 days after islet transplantation. The mice were weighed and blood samples were obtained from the orbital sinus three times per week. Rejection of islet allografts was defined as the first day of permanent reversion of nonfasting plasma glucose to more than 200 mg/dl.

Transplantation of freshly isolated islets was performed in congenic mice differing at the H-2K region only, H-2D region only, H-2I region only, H-2K + H-2D only, or for the entire H-2 complex (Table 1). Most of the islet recipients that were disparate at H-2K only, H-2K + H-2D, and H-2K + H-2I + H-2D regions rejected the islet allografts (Table 1). The mean (± standard error) survival time (MST) of rejected grafts (N = 8) in the K only disparate group was 17.3 ± 2.2 days; however, three out of 11 animals (27 percent) had islet allografts surviving after 100 days. In recipients of islet allografts differing at the H-2K + H-2D region the MST of rejected grafts (N = 5) was 16.8 ± 2.7 days, with two out of seven animals (29 percent) remaining normoglycemic after 100 days. Transplants of islets disparate at the entire H-2 complex (K + I + D) were rejected between days 12 and 40 (MST, 20 ± 3.4 days), but two animals (20 percent) remained normoglycemic after 100 days. In contrast, most mice disparate at H-2D only or at the I region only did not reject the islet allografts. One of 13 animals (8 percent) with an I-region disparity only rejected (at day 21); histology showed mononuclear infiltration around and in the subcapsular graft with islet membrane disintegration. In Fig. 1 we show the graft survival rate with time after transplantation in all groups. In Fig. 2, A to C, we show the mean glucose levels and patterns of rejections of islet allografts in the H-2K only, K + D, and K + I + D disparate groups, respectively. Removal of the kidney bearing the islet transplant resulted in prompt return to hyperglycemia (> 400 mg/dl) within 4 days in all mice with grafts surviving more than 100 days: isografts (N = 5), I only (N = 3), K only (N = 1), D only (N = 4), and K + D (N = 1).

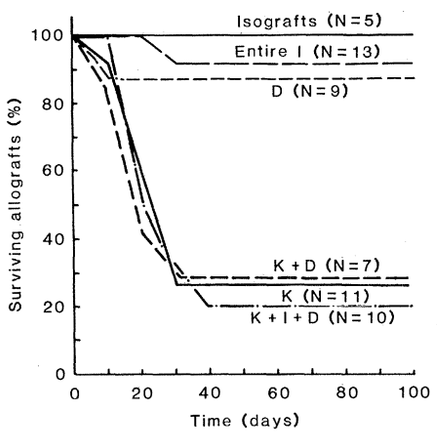

Fig. 1. Percentage of mouse islet allografts surviving and functioning in recipients differing from the donor at the H-2K, H-2D, entire I region, H-2K + H-2D, and H-2K + H-2I + H-2D encoded histocompatibility loci. Graft function was defined by maintenence of plasma glucose levels below 200 mg/dl. All mice showed glucose levels higher than 400 mg/dl before transplantation and lower than 200 mg/dl within 3 days after islet transplantation. Loss of function was defined as reversion of plasma glucose to more than 200 mg/dl after at least two consecutive days of normoglycemia.

Current theories of allograft rejection propose a two-signal hypothesis wherein H-2I-region encoded antigens induce a population of T helper cells needed for cytotoxic lymphocytes to respond to class I (H-2K and H-2D) antigens (*10*). Alternatively, H-2I antigens may activate T cells directly via stimulator cells when the T cells are simultaneously exposed to H-2K and H-2D antigens (*11*). Finally, alternative pathways have been postulated including precursor lymphocytes to explain cytotoxic responses against H-2K or H-2K + H-2D alloantigen disparities in the absence of I-region encoded differences on the stimulator cells (*12*).

Although pancreatic islets treated with donor-specific antiserum to Ia antigens and complement survived more than 200 days after transplantation across a major histocompatibility barrier (*1*), our results suggest that genetic absence of the allogeneic stimulus from I-encoded antigens does not result in predictable islet allograft survival. Recipients of islets from donors disparate at H-2K alone and at H-2K + H-2D (and therefore identical at the I region), rejected islet allografts. Allograft rejections were thus entirely due to the class I disparities, since congenic mice were used and no known differences existed in the Qa or Tla loci for the combinations tested.

In our experiments, we eliminated I-region antigens genetically, rather than with treatment of islets with antiserum to Ia antigen. The fact that islets disparate for class I antigen only are rejected under our conditions suggests that when Faustman *et al.* (*1*) treated islets with antiserum to Ia, the antiserum did not act by eliminating I-region (class II) antigens only, but that the allo-Ia stimulus (and activation of helper T lymphocytes reactive to such a stimulus) was eliminated—one of the hypotheses entertained by those authors (*1*). It seems more likely that it is the Ia positive cells that are most effective in presenting an allo disparity even when that disparity is a class

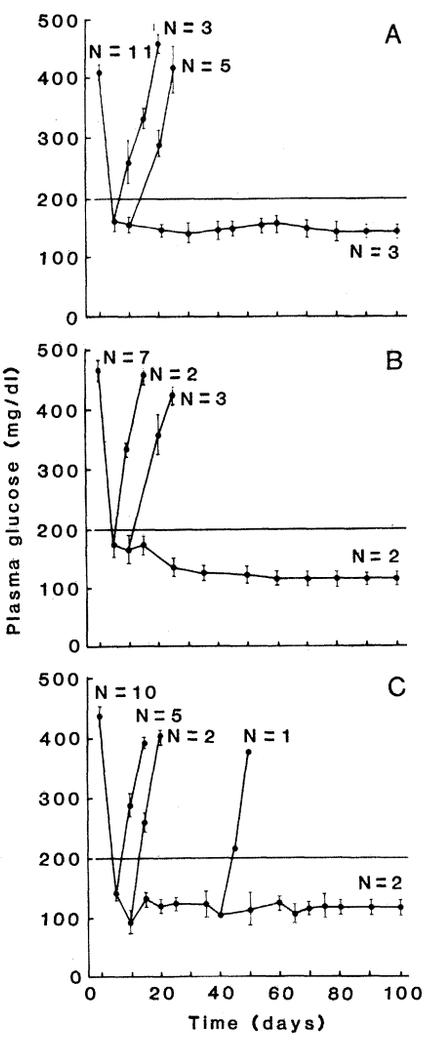

Fig. 2. Nonfasting glucose values after transplantation of islet allografts in mice disparate with the donor at (A) H-2K; (B) H-2K + H-2D; and (C) H-2K + H-2I + H-2D regions. Vertical bars represent standard error of the mean. Mice that showed recurrence of hyperglycemia are separated from those that remained normoglycemic at the time rejection occurred.

I antigen alone. In the experiments of Faustman *et al.* (*1*), the immunogenic cells were eliminated by the treatment with antiserum to Ia; in our experiments Ia positive cells were left intact (even though the Ia antigens were identical between donor and recipient) with class I disparities alone and thus the class I antigenic disparity could be presented in an immunogenic manner leading to graft rejection. We cannot rule out that treatment with antiserum to Ia (*1*) also reduced the load of class I antigens in the transplanted tissues since passenger cells eliminated by such treatment would also express class I antigens.

It is not clear why the K-region disparity studied by us (B10.AQR to B10.A) and the K + D region disparities evaluated led to rejection in the great majority of grafts whereas the D-region disparity [B10.A(2R) to B10.A] did not. Further experiments will have to be performed to evaluate the relative immunogenicity of K- or D-encoded antigens and the effect of both recipient and donor genotype on transplant success.

Thus we have found that mice differing at the H-2K or H-2K + H-2D histocompatibility loci (encoding class I antigens) alone are capable of rejecting pancreatic islet allografts. It appears that the elimination of allogeneic stimulation from I-region (class II) antigens alone is not sufficient to ensure islet allograft survival. Rather, we believe that the results of our experiments in vivo are consistent with the hypothesis that it is the Ia^+ cells in a graft that are immunogenic whether the Ia antigens are foreign to the recipient or not.

CHARLES E. MORROW*
DAVID E. R. SUTHERLAND
Department of Surgery, University of Minnesota Health Sciences Center, Minneapolis 55455
MICHAEL W. STEFFES
Department of Laboratory Medicine and Pathology, University of Minnesota Health Sciences Center
JOHN S. NAJARIAN
Department of Surgery, University of Minnesota Health Sciences Center
FRITZ H. BACH
Immunobiology Research Center, University of Minnesota Health Sciences Center

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* Address correspondence to Box 314, University of Minnesota Hospital, 420 Delaware Street S.E., Minneapolis 55455.

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Tunicamycin Enhances the Antiviral and Anticellular Activity of Interferon

Abstract. *The inhibitory effects of interferon on virus multiplication and cell growth are significantly enhanced by treatment with tunicamycin. Potentiation of antiviral activity was found only with enveloped viruses and not with nonbudding viruses. Changes in the plasma membrane of treated cells may account for this effect, since enveloped viruses bud from the cell surface as a terminal step.*

Interferons are proteins secreted by animal cells in response to viruses and a number of other stimulating substances. Originally described as antiviral (*1, 2*), interferons have many other biological effects, such as the formation of defective virus particles (*3–5*), inhibition of cell growth (*6*), and regulation of the immune system (*7, 8*).

The cell surface (*9*) is important in several phases of interferon's action. Binding of interferon to specific receptors on the surface results in chemical, morphological, physical, and immunological alterations in the plasma membrane. These include changes in plasma membrane density (*10*) and cell surface charge (*11*), an increase in the number of intramembranous particles (*10*), an altered capacity to bind thyroid-stimulating hormone or cholera toxin (*12*), an increase in the binding of lectin (*13*) and in the toxicity of lymphocytes to target cells (*14*), an increase in the expression of cell surface antigens (*14*), an altered exposure of surface gangliosides (*15*), an increase in the concentration of intracellular adenosine 3′5′-monophosphate (*16, 17*), changes in thymidine uptake (*18*), inhibition of cap formation (*19*), cytoskeletal changes, and a decrease in plasma membrane fluidity (*20*). The relation between these diverse effects on cell membranes and the antiviral and antigrowth activities of interferons remains to be established.

Tunicamycin, a glucosamine-containing antibiotic (*21*) produced by *Streptomyces lysosuperficus*, inhibits lipid biosynthesis by blocking the transfer of *N*-acetylglucosamine-1-phosphate from uridine diphosphate *N*-acetylglucosamine to dolichylmonophosphate (*22*). Many important biochemical functions are inhibited by tunicamycin, such as glycoprotein synthesis in yeast (*23*); the bacterial synthesis of polyisoprenol sugars (*24*), peptidoglycans (*25*), procollagen (*26*), and polymeric cell walls (*27*); cell division in *Tetrahymena pyreformis* (*28*); secretion of immunoglobulins A and E by plasma cells (*29*); and virus multiplication (*30*). Tunicamycin also has profound effects on the morphology and surface properties of prokaryotes and eukaryotes and causes changes in membrane organization (*31*). We have reported (*32, 33*) that treatment of L_B cells with interferon or tunicamycin reduces the production of infectious vesicular stomatitis virus (VSV) particles, decreases the amount of glycoprotein and membrane protein in VSV released from treated cells, and inhibits an early step in the formation of asparagine-linked oligosaccharide chains, that is, the incorporation by membrane preparations from treated cells of *N*-acetylglucosamine into glycolipids with the properties of dolichol derivatives.

The cell surface–altering properties of tunicamycin and interferon, coupled with the biological activities mentioned above, prompted us to investigate the role of these substances in the inhibition of virus multiplication and cell growth. We found that the antiviral and antigrowth effects of interferon are significantly enhanced by tunicamycin. Potentiation of antiviral activity was found only with enveloped viruses. Tunicamycin had no effect on the antiviral activity of interferon against encephalomyocarditis (EMC) virus, which is nonbudding.

The Indiana strain of VSV, Sindbis (SB) virus, and EMC virus (originally