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Transforming growth factor β decreases the immunogenicity of rat islet xenografts (rat to mouse) and prevents rejection in association with treatment of the recipient with a monoclonal antibody to interferon γ

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ABSTRACT **Culture of rat islets of Langerhans for 1 week at 37°C with recombinant transforming growth factor β prolonged the survival of islet xenografts transplanted into diabetic mouse recipients. Treatment of diabetic recipients with a neutralizing monoclonal antibody to murine interferon γ did not affect the survival of islet xenografts cultured 7 days in control medium. However, treatment of donor islets with transforming growth factor β in combination with treatment of diabetic recipients with interferon γ antibody produced a 75% survival of the islet xenografts at 100 days. Fifty percent of the recipients who had accepted their graft for more than 100 days were immune unresponsive to a transplant of freshly isolated islets from the same donor strain.**

Preventing rejection of islet allografts has been achieved by alteration or destruction of intraislet passenger leukocytes (1, 2). Preventing rejection of closely related islet xenografts (rat to mouse) has been achieved by *in vitro* culture at low temperature to alter the function of passenger leukocytes combined with a temporary immunosuppression by anti-lymphocyte serum or anti-CD4 monoclonal antibody (3, 4). These findings have shown that islet graft rejection is initiated by major histocompatibility complex (MHC) class II-bearing, antigen-presenting lymphoid cells within the donor islets. Transforming growth factor β (TGF-β) (5), initially described as a growth regulator, has strong immunosuppressive effects *in vitro*. Interferon γ (IFN-γ) is a lymphokine secreted essentially by T cells (6). It activates macrophages (7) and induces MHC class II antigen expression on macrophages and a variety of nonlymphoid cells including pancreatic beta cells (8, 9). Monoclonal antibodies neutralizing murine IFN-γ have been produced (10).

We determined if treatment of donor islets with TGF-β in combination with treatment of recipients with IFN-γ antibody could prevent the rejection of islet xenografts. In the present communication, we report that treatment of rat islets with human recombinant TGF-β *in vitro* delayed their rejection in mice. Moreover, treatment of the recipient with neutralizing specific monoclonal IFN-γ antibody had synergistic effects with the *in vitro* TGF-β treatment and thereby prevented rejection, with a 75% survival of islet xenografts at 100 days after transplant.

MATERIAL AND METHODS

Islet Isolation and Culture. Islets were isolated from male Wistar Furth rats (Charles River Breeding Laboratories) by the collagenase technique (11). The islets were purified on a discontinuous Ficoll gradient and were hand-picked. Batches of 300 islets were cultured in 2 ml of medium in 50-mm bacteriologic Petri dishes in CMRL 1066 medium with L-glutamine (2 mM), penicillin (100 units/ml), streptomycin (100 μg/ml), glucose (150 mg/dl), and 10% fetal bovine serum (complete medium). The medium was changed on day four for 1-week culture. The cultures were maintained at 37°C, in a 95% air/5% CO_2 atmosphere.

Transplants. Ten- to 14-week-old male C57BL6/J mice (The Jackson Laboratory) received a single i.v. injection of streptozotocin at 165 mg/kg (courtesy of Upjohn) and were considered diabetic when their plasma glucose on two consecutive bleedings exceeded 400 mg/dl. Prior to transplantation, sets of 650–750 islets were repicked from the cultures. The islets were then placed under the left kidney capsule by using a previously described technique (12). After transplant, the plasma glucose was measured three times a week with a Beckman glucose analyzer on samples obtained by retroorbital puncture. After normalization of the plasma glucose, rejection was defined as when the plasma glucose exceeded 200 mg/dl on two consecutive bleedings. The animal was then sacrificed, and the graft-bearing kidney and the pancreas were preserved for histology. When normoglycemia persisted >60 days or >100 days, the graft-bearing kidney was surgically removed for histological examination. The plasma glucose was measured to confirm that the recipient would become diabetic again and hence that the graft was still functioning before the nephrectomy. In some cases the nephrectomized animals were retransplanted with freshly isolated Wistar Furth rat islets under the right kidney capsule, and the plasma glucose was monitored as described above. When the second islet transplant was tolerated for >60 days, a Wistar Furth tail skin graft was implanted on the back of the animals. The graft was staged daily and considered rejected when no remnant was detectable.

Cytokines. Purified recombinant human TGF-β1 and tumor necrosis factor α (TNF-α) were generously provided by Genentech. Rat IFN-γ was purchased from Amgen Biologicals.

Monoclonal Antibody to Murine IFN-γ. The neutralizing hamster monoclonal antibody specific for murine IFN-γ (H22) has been described elsewhere (10). This antibody neutralizes all the actions of murine IFN-γ and crossreacts with rat IFN-γ. Endotoxin-free H22 was purified from culture supernatants by chromatography on protein A-agarose. For *in vivo* treatment, 500 μg of monoclonal antibody was injected subcutaneously the day before the transplant (day −1), and 250 μg was injected on days 14 and 28 posttransplant, unless rejection had already occurred. Purified normal hamster IgG (Pel-Freez Biologicals) was used as a control at the same dose. For *in vitro* treatment, donor islets were incu-

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Abbreviations: TGF-β, transforming growth factor β; IFN-γ, interferon γ; MHC, major histocompatibility complex; TNF-α, tumor necrosis factor α; MST, mean survival time.

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bated with H22 at 10 μg/ml, a concentration known to neutralize rat IFN-γ.

Immunofluorescence Staining of Islets and RINm5F Cells. Rat insulinoma RINm5F cells (13) were cultured in the presence of various cytokines before staining for MHC class I and class II antigens with the monoclonal antibodies OX18 and OX6 (Accurate Chemicals, Westbury, NY) and a fluoresceinated second antibody (14). Islets were stained with the same reagents as described (9).

Histology. The islet grafts were fixed in Bouins' solution and stained with conventional hematoxylin/eosin and with aldehyde fuschin to demonstrate beta cells in the islets. Cultured islets were fixed in glutaraldehyde and processed for electron microscopy.

Statistics. The different groups of transplanted animals were compared by the Mann–Whitney U test by using a statistics software (ABSTAT, Anderson Bell, Parker, CO). The mean survival time (MST) ± SEM of each group was also calculated.

RESULTS

Culture of Donor Islets with TGF-β. We first determined whether treatment of donor islets with TGF-β would affect xenograft survival. Wistar Furth rat islets were cultured with TGF-β at 50 ng/ml for 4 or 7 days prior to transplantation under the kidney capsule of diabetic mice. As shown in Table 1, four days of treatment of the donor islets with TGF-β did not modify the survival of the grafts as compared to islets cultured in complete medium only (TGF-β, 14.7 ± 1.6 days; controls, 15.8 ± 1.5 days). In contrast, seven days of treatment of the donor islets with TGF-β significantly prolonged the survival of the grafts (group IV, 47.9 ± 4.4 days) as compared to islets cultured for 1 week in medium only (group III, 27.6 ± 7.2 days, $P < 0.03$). This effect was transient; it resulted in a delay of the rejection process but not in permanent acceptance of the graft since, at 60 days post-transplantation, the number of grafts still functioning was similar in both groups.

Treatment of the Recipients of TGF-β-Treated Donor Islets with Monoclonal Antibody to IFN-γ (H22). We then determined whether H22 injected into diabetic recipients would affect the rejection of donor islets cultured for 7 days with TGF-β. A total of 1 mg of H22 was injected subcutaneously into the diabetic recipients in three divided doses (500 μg at day −1, 250 μg at day 14 and at day 28). The dosage and schedule of injection were chosen according to the circulating half-life of the antibody after subcutaneous injection in C57BL/6 mice (14 days; R.D.S., unpublished observation). A similar treatment regimen had been shown to inhibit immune responses to *Listeria monocytogenes in vivo* in mice (15). The combination of TGF-β *in vitro* and H22 *in vivo* produced 83.3% survival of 60 days with a mean survival time (MST) >56.6 ± 3.3 days (group V, Table 1), which was significantly different from controls (group III, $P < 0.007$) and from treatment of donor islets with TGF-β alone (group IV, $P < 0.04$). These animals were followed until 100 days after transplantation (group V, Table 2), and the survival rate was 75%, with a MST of 88.9 ± 7.2 days at this time.

Treatment of the Recipient and/or the Donor Islets with H22. To understand in more detail the respective contribution of TGF-β and H22 in preventing xenograft rejection, we examined whether H22 treatment affected rejection kinetics of donor islets cultured in only medium for 7 days. In the control group (group VI, Table 2), the diabetic recipients received an equivalent amount of normal hamster IgG *in vivo*, and the donor islets were incubated with normal hamster IgG (10 μg/ml) *in vitro* for 7 days. Treatment of the recipients with H22 did not prolong the survival of cultured donor islets (group VII, Table 2). The MST was 37.3 ± 13.3 days in the H22-treated group and 39.4 ± 13.8 days in the normal hamster IgG-treated control group.

We then determined whether *in vitro* incubation of the donor islets for 7 days with IFN-γ monoclonal antibody would affect graft survival (group VIII, Table 2). The MST was 52.2 ± 9.8 days, which was slightly, but not significantly, longer than that of islets cultured with normal hamster IgG (group VI, 39.4 ± 13.8). Treatment of the donor islets *in vitro* with H22 plus administration of H22 *in vivo* did not prolong the survival of the islet xenografts as compared to the controls (group IX, Table 2; MST = 40.0 ± 13.2 vs. 39.4 ± 13.8 days).

These findings indicated that treatment of the donor islets *in vitro* with H22 alone or in conjunction with H22 *in vivo* did not produce a significant prolongation of islet xenograft survival.

Effect of Combined Use of TGF-β and H22 *in Vitro*. Since treatment of the islets with H22 *in vitro* did not produce a significant effect in prolonging survival, we determined whether culture of donor islets with both H22 and TGF-β would be effective. The donor islets were treated for 7 days with a combination of TGF-β (50 ng/ml) and H22 (10 μg/ml) and transplanted into recipients receiving no treatment ($n = 14$) or normal hamster IgG ($n = 6$). Since the MST was not significantly different with or without hamster IgG *in vivo*, these two subgroups were combined resulting in a MST of 62.4 ± 7.8 days (group X, Table 2). When compared to the controls (group VI), treatment of donor islets with TGF-β and H22 significantly prolonged the survival when analyzed at 60 days ($P < 0.05$) but not when analyzed at 100 days. These results confirmed that treatment of donor islets with TGF-β delays but does not prevent rejection. They also indicated that the addition of H22 to the *in vitro* treatment of the islets with TGF-β does not improve graft survival as compared to TGF-β alone.

Finally, the islets were cultured with both TGF-β and H22 and transplanted into recipients receiving H22 (group XI, Table 2). A significant prolongation of survival was obtained

Table 1. Islet xenograft (rat to mouse) survival

Group	*In vitro* treatment of islets	*In vivo* treatment of recipients	Graft survival, days		
			Individual	Mean ± SEM	% at 60 days
I	CM for 4 days	None	12, 13, 16, 18, 20	15.8 ± 1.5	0
II	TGF-β for 4 days	None	12, 12, 17, 18	14.7 ± 1.6	0
III	CM for 7 days	None	12, 12, 13, 19, 20, 25, >60 (2 mice)	27.6 ± 7.2	25.0
IV	TGF-β for 7 days	None	22, 24, 39, 40, 50, 55, 57, >60 (4 mice)	47.9 ± 4.4	36.4
V*	TGF-β for 7 days	H22 (1 mg)	20, 59, >60 (10 mice)	56.6 ± 3.3	83.3

Rat islets were cultured at 37°C in control medium (CM) or with recombinant human TGF-β (50 ng/ml). Group IV vs. III, $P < 0.03$; V vs. III, $P < 0.007$; V vs. IV, $P < 0.04$.

*The animals in group V were followed until day 100.

Table 2. Islet xenograft (rat to mouse) survival

Group	*In vitro* treatment of islets	*In vivo* treatment of recipients	Graft survival, days		
			Individual	Mean ± SEM	% at 100 days
V	TGF-β for 7 days	H22 (1 mg)	20, 59, 88, >100 (9 mice)	88.9 ± 7.2	75.0
VI	Hamster IgG (10 μg/ml)	Hamster IgG (1 mg)	9, 11, 12, 13, 28, 42, >100 (2 mice)	39.4 ± 13.8	25.0
VII	CM for 7 days	H22 (1 mg)	13, 17, 30, 32, 32, >100	37.3 ± 13.0	16.6
VIII	H22 for 7 days (10 μg/ml)	None	12, 18, 18, 20, 22, 23, 32, 43, 62, 81, >100 (4 mice)	52.2 ± 9.8	28.6
IX	H22 for 7 days (10 μg/ml)	H22 (1 mg)	13, 14, 26, 39, 48, >100	40.0 ± 13.2	16.6
X	TGF-β + H22 for 7 days	Hamster IgG (1 mg; n = 6) or none (n = 14)	9, 12, 24, 24, 31, 33, 35, 38, 38, 63, 74, 76, 91, >100 (7 mice)	62.4 ± 7.8	35.0
XI	TGF-β + H22 for 7 days	H22 (1 mg)	26, 45, 49, >100 (10 mice)	86.2 ± 7.4	76.9

Rat islets were cultured for 7 days in various conditions and transplanted to recipients treated with H22 or with control hamster IgG. The animals were followed until day 100 and then nephrectomized. Groups V vs. VI and XI vs. VI, $P < 0.01$; XI vs. X, $P < 0.02$.

with a MST of 86.2 ± 7.4 days as compared to the controls (group VI, 39.4 ± 13.8 days, $P < 0.01$). The survival time was almost identical to that obtained with only TGF-β *in vitro* and H22 *in vivo* (group V, 88.9 ± 7.2 days). This finding confirmed that *in vitro* treatment with TGF-β combined with H22 *in vivo* would prevent rejection of the xenografts and also indicated that the addition of H22 *in vitro* had no further enhancing effect in preventing rejection.

Morphological Studies of Grafts and TGF-β-Treated Islets. The grafts accepted for 100 days were vascularized and contained well-granulated beta cells. They were always surrounded but not infiltrated by foci of lymphocytes as reported in other established rat-to-mouse islet xenografts (1). The grafts that had rejected were infiltrated by lymphocytes, with no or few surviving islet cells.

Since TGF-β increases intercellular matrix protein accumulation and fibroblast proliferation, we examined the morphology of islets treated with TGF-β for 7 days. No difference between treated and control islets was demonstrable either by routine examination of the culture by transmission microscopy or by electron microscopy (data not shown).

Induction of MHC Class II Antigens on TGF-β-Treated Islets and on RINm5F Cells. A possible effect of TGF-β in delaying rejection was an inhibition of MHC class II antigen expression on the islets. To determine the effect of TGF-β, rat islets were incubated with TGF-β (50 ng/ml) for 7 days and then exposed to rat IFN-γ (100 units/ml) and murine TNF-α (50 units/ml) for 3 or 6 days, still in the presence of TGF-β. No MHC class II antigen was detectable on the islets before the addition of IFN-γ and TNF-α (day 7). TGF-β did not impair the ability of these lymphokines to induce MHC class II antigens since at day 10 there were an average of 12 Ia-positive cells per islet in the TGF-β group vs. 8 in the control group; at day 13, there were 29 vs. 30 Ia-positive cells per islet. Similarly TGF-β did not decrease constitutive or lymphokine-induced expression of MHC class I antigens on the islets.

To be able to detect more subtle differences in MHC class II antigen expression after TGF-β treatment, the rat insulinoma cell line RINm5F was also used. RINm5F cells were cultured with or without TGF-β for 7 days and then exposed to IFN-γ (50, 100, or 400 units/ml) or IFN-γ (100 units/ml) and TNF-α (50 units/ml), still in the presence of TGF-β. After 3 or 6 days of culture with the MHC class II antigen-inducing lymphokines, the cells were stained for MHC class I and class II antigens. No difference could be detected between the controls and the TGF-β-treated cells (data not shown).

Transplantation of Fresh Wistar Furth Rat Islets Under the Controlateral Kidney Capsule After Nephrectomy. Studies were also done to determine if an immune unresponsiveness had been achieved in the animals that had accepted their graft for >100 days. The graft-bearing kidney was removed, which resulted in the animals becoming diabetic again, and freshly isolated Wistar Furth rat islets were transplanted under the controlateral kidney capsule. The results were grouped according to the regimen used for prevention of rejection of the first graft. The number of animals is less than expected from Tables 1 and 2 due to a significant mortality after the third surgical procedure.

As shown in Table 3, when the combination of TGF-β *in vitro* and H22 *in vivo* was used for the first graft, the survival of the second xenograft varied from 10 to >238 days, with 46% surviving more than 60 days. This is in marked contrast with freshly isolated Wistar Furth rat islets transplanted into naive diabetic recipients, which have a MST of 14.5 ± 1.8 days with the longest survival of 23 days (4). Hence the animals with a second graft surviving more than 60 days are

Table 3. Transplant of a second noncultured Wistar Furth xenograft in nephrectomized recipients having accepted their first graft for over 100 days

Group	Regimen used for the first graft		Survival of the second xenograft, days	
	In vitro treatment of islets	*In vivo* treatment of recipients	Individual animals	% at 60 days
V	TGF-β	H22	10, 11, 11, 19, 24, 40, 45, >71*, >100†, >189†, 235, >238†, >238† (V and XI combined)	46
XI	TGF-β + H22	H22		
Control‡	None	None	11, 12, 13, 14, 14, 23	0

The graft recipients still normoglycemic at 100 days after transplantation were nephrectomized to control that they would become hyperglycemic again and hence that the first graft was still functional. They were then retransplanted with fresh, noncultured Wistar Furth rat islets. The animals normoglycemic after this second transplant received a Wistar Furth rat skin graft.

*Died normoglycemic 71 days after the second islet graft.

†Rejected their second islet graft after an average of 20.2 ± 0.6 days after receiving a skin graft.

‡Fresh Wistar Furth rat islets were transplanted into naive C57B1/6 recipients (4).

apparently unresponsive to a new, more immunogeneic graft from the same donor strain.

When the other regimens were used for the first graft, the survival of the second graft also varied widely, ranging from 14 to >160 days, with 50% surviving at 60 days ($n = 8$, data not shown). No difference in the survival rate of the second graft could be detected according to the regimen used for the first graft. However, the very low number of animals from these groups prohibits drawing any firm conclusion as to whether treatment of the islets with TGF-β *in vitro* and the recipient with H22 *in vivo* induced this immune unresponsiveness more often than the other regimens.

A skin graft (Wistar Furth) was done in four recipients that had a second islet xenograft surviving >60 days. In each instance, the skin graft was rejected in <17 days and induced rejection of the established second islet xenograft, and the recipients became diabetic again. These findings indicate that the immune unresponsive state could be broken by a skin graft from the same donor strain as the islets. It also indicates that the recipient was dependent upon the islet graft to maintain normoglycemia.

DISCUSSION

The findings indicate that *in vitro* treatment of donor rat islets with TGF-β for 7 days will prolong the survival of islet xenografts in mice. The addition of temporary treatment of the recipient with a monoclonal antibody to IFN-γ (H22) to this regimen produced indefinite survival of the xenografts, with 75% surviving at 100 days after transplantation.

The mechanism by which TGF-β decreases the immunogenicity of the donor islets is not clear from these studies. Previous studies have shown that destruction or alteration of macrophages or dendritic cells in islets will prevent rejection of islet allografts (1, 2) and that these regimen in conjunction with temporary immunosuppression of the recipients will also prevent rejection of rat-to-mouse islet xenografts (3, 4). TGF-β has been shown to decrease interleukin 2-induced T-cell proliferation *in vitro* (16), decrease the generation of cytotoxic T lymphocytes (17), decrease H_2O_2 release from activated murine macrophages (18), and prevent IFN-γ-induced MHC class II antigen expression on human melanoma cells (19). Thus one possibility is that TGF-β impairs the function of intraislet macrophages or decreases MHC class II antigen expression on these cells. We were unable to determine the effects of TGF-β on MHC class II expression on intraislet macrophages since we had found that *in vitro* culture alone for 7 days almost completely eliminates MHC class II antigen expression on these cells (P.E.L. and E. Finke, unpublished observation). We did determine that TGF-β did not inhibit the induction of MHC class II antigen expression on islet endocrine cells or RINm5F cells by IFN-γ and TNF-α.

TGF-β also decreases the adhesion of neutrophils to cultured human endothelial cells and the expression of the adhesion molecule ICAM 1 on endothelial cells (20). The molecule ICAM 1 can be induced on human islets by IFN-γ and TNF-α (21). The role of ICAM 1 in islet graft rejection has not been investigated, but, as a ligand for lymphocyte function-associated antigen 1 on lymphocytes (22), it could play a role in nonspecific recognition of the islet cells by recipient lymphocytes or monocytes for a period of time following transplantation.

TGF-β increases the accumulation of intercellular matrix proteins and stimulates fibroblast proliferation at concentrations as low as 100 pg/ml (5). We considered the possibility that TGF-β could have induced the formation of a collagen matrix around the islets, but this was not evident from electron microscopic studies of the treated islets.

IFN-γ is secreted essentially by activated T cells (6) and also by natural killer cells (23). *In vivo* studies with monoclonal antibodies to IFN-γ have shown its role in the clearance of various intracellular pathogens (15, 24, 25) and in the pathogenesis of autoimmune diseases such as lupus-like nephritis in the NZB $\times$ NZW mouse (26) and collagen-induced arthritis in rats (27). The role of IFN-γ in graft rejection remains poorly understood. *In vivo* IFN-γ antibody treatment prevents the rejection of allogeneic tumors in mice (28) whereas in a rat cardiac allograft model (29), IFN-γ antibody has no effect when used alone but markedly prolongs graft survival when combined with multiple cycles of cyclosporin A therapy.

In the present study, *in vitro* treatment of the donor islets with IFN-γ antibody (H22) produced a slight but not significant prolongation of islet xenograft survival. When H22 was used both *in vitro* and *in vivo*, no prolongation of islet xenograft survival was obtained. However the combination of TGF-β treatment of the donor islets *in vitro* with H22 temporary therapy *in vivo* produced indefinite survival of the xenografts. Presumably, the diminished immunogenicity of the TGF-β-treated islets made it possible for the antibody to IFN-γ to inhibit the immune response of the recipient and prevent the generation of cytotoxic T lymphocytes. Further studies on islet xenografts will be needed to determine whether other procedures that have been shown to alter or destroy intra-islet macrophages will also prevent rejection when H22 is administered *in vivo*.

A most interesting finding was that an immune unresponsive state has been induced in ≈50% of the recipients with a first islet graft surviving >100 days. After removal of the first graft, a second transplant of freshly isolated rat islets survived more than 60 days in ≈50% of the recipients in contrast with a survival of 14.5 ± 1.8 days using freshly isolated rat islets transplanted into naive recipients (4). The largest sample of recipients with a long-term accepted first graft was in the groups in which the islets had been treated with TGF-β and the recipient with H22 (groups V and XI). We could not ascribe the induction of an immune unresponsive state to TGF-β exposure of the islets since a few animals in the other groups also had a prolonged survival of their second xenograft. This unresponsiveness may have been induced only to the original donor strain. We did demonstrate that the immune unresponsiveness could be broken by using Wistar Furth skin grafts, which induced rejection of long-term surviving second islet grafts.

Achievement of indefinite survival of closely related islet xenografts with TGF-β *in vitro* and an antibody to IFN-γ *in vivo* raises the possibility of using this approach for prolongation of survival of islet xenografts transplanted across a wide species barrier as well as determining whether this approach will prevent rejection of islet allografts. The elucidation of the mechanism of action of TGF-β in decreasing the immunogenicity of the islets should provide further insights into the immune mechanisms involved in graft acceptance, and further studies with antibodies to certain lymphokines may provide more specific immunotherapeutic approaches to the prevention of allograft and xenograft rejection.

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1. Lacy, P. E. (1984) *Annu. Rev. Immunol.* **2,** 183–198.
2. Lafferty, K. J., Prowse, S. J. & Simeonovic, C. J. (1983) *Annu. Rev. Immunol.* **1,** 143–173.
3. Lacy, P. E., Davie, J. M. & Finke, E. H. (1980) *Science* **209,** 283–285.
4. Lacy, P. E., Ricordi, C. & Finke, E. H. (1989) *Transplantation* **47,** 761–766.
5. Sporn, M. B., Roberts, A. B., Wakefield, L. M. & de Crombrugghe, B. (1987) *J. Cell Biol.* **105,** 1039–1045.
6. Trincheri, G. & Perussia, B. (1985) *Immunol. Today* **6,** 131–136.
7. Unanue, E. R. (1984) *Annu. Rev. Immunol.* **2,** 395–428.
8. Unanue, E. R. & Allen, P. M. (1986) *Lab. Invest.* **55,** 123–125.
9. Wright, J. R., Lacy, P. E., Unanue, E. R., Muszynski, C. & Hauptfeld, V. (1986) *Diabetes* **35,** 1174–1177.
10. Schreiber, R. D., Hicks, L. J., Celada, A., Buchmeier, N. A. & Gray, P. W. (1985) *J. Immunol.* **134,** 1609–1618.
11. Lacy, P. E. & Kostianovsky, M. (1967) *Diabetes* **16,** 35–39.
12. Sullivan, F. P., Ricordi, C., Hauptfeld, V. & Lacy, P. E. (1987) *Transplantation* **44,** 465–468.
13. Gazdar, A. F., Chick, W. L., Oie, H. K., Sims, H. L., King, D. L., Weir, G. C. & Lauris, V. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 3519–3523.
14. Varey, A.-M., Lydyard, P. M., Dean, B. M., van der Meide, P. H., Baird, J. D. & Cooke, A. (1988) *Diabetes* **37,** 209–212.
15. Buchmeier, N. A. & Schreiber, R. D. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 7404–7408.
16. Kehrl, J. H., Wakefield, L. M., Roberts, A. B., Jakowlew, S., Alvarez-Mon, M., Derynck, R., Sporn, M. B. & Fauci, A. S. (1986) *J. Exp. Med.* **163,** 1037–1050.
17. Ranges, G. E., Figari, I. S., Espevik, T. & Palladino, M. A. (1987) *J. Exp. Med.* **166,** 991–998.
18. Tsunawaki, S., Sporn, M. B., Ding, A. & Nathan, C. (1988) *Nature (London)* **334,** 260–262.
19. Czarniecki, C. W., Chiu, H. H., Wong, G. H. W., McCabe, S. & Palladino, M. A. (1988) *J. Immunol.* **140,** 4217–4223.
20. Gamble, J. R. & Vadas, M. A. (1988) *Science* **242,** 97–99.
21. Campbell, I. L., Cutri, A., Wilkinson, D., Boyd, A. W. & Harrison, L. C. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 4282–4286.
22. Springer, T. A., Dustin, M. L., Kishimoto, T. K. & Marlin, S. D. (1987) *Annu. Rev. Immunol.* **5,** 223–252.
23. Bancroft, G. J., Schreiber, R. D., Bosma, G. C., Bosma, M. J. & Unanue, E. R. (1987) *J. Immunol.* **139,** 1104–1107.
24. Suzuki, Y., Orellana, M. A., Schreiber, R. D. & Remington, J. S. (1988) *Science* **240,** 516–518.
25. Schofield, L., Villaquiran, J., Ferreira, A., Schellekens, H., Nussenzweig, R. & Nussenzweig, V. (1987) *Nature (London)* **330,** 664–666.
26. Jacob, C. O., van der Meide, P. H. & McDevitt, H. O. (1987) *J. Exp. Med.* **166,** 798–803.
27. Jacob, C. O., Holoshitz, J., van der Meide, P. H., Strober, S. & McDevitt, H. O. (1989) *J. Immunol.* **142,** 1500–1505.
28. Landolfo, S., Cofano, F., Giovarelli, M., Prat, M., Cavallo, G. & Forni, G. (1985) *Science* **229,** 176–179.
29. Didlake, R. H., Kim, E. K., Sheehan, K., Schreiber, R. D. & Kahan, B. D. (1988) *Transplantation* **45,** 222–223.