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Regulated interleukin-10 expression prevents chronic rejection of transplanted hearts

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Objective: Interleukin-10 is a pleiotropic cytokine with variable effects on the alloimmune response, depending on the experimental model system. The purpose of this study was to determine the role of regulated interleukin-10 expression on the development of chronic rejection in heart transplantation, or cardiac allograft vasculopathy.

Methods: Donor hearts from B6.C-H2^{bm12} mice were transplanted into wild-type and interleukin-10 transgenic recipients. In interleukin-10 transgenic recipients, murine interleukin-10 cytokine is produced under the control of human interleukin-2 promoter. Donor hearts were sacrificed at days 7 and 24. No immunosuppression was used. Intimal proliferation was measured morphometrically. Intragraft cellular infiltrate was defined by both immunohistochemistry and flow cytometry. Intracellular cytokine staining assay was performed to determine both the type and source of intragraft cytokines.

Results: Hearts transplanted into wild-type recipients developed severe cardiac allograft vasculopathy by 24 days. Intimal lesions were absent in the donor hearts transplanted into interleukin-10 transgenic recipients. The number of graft-infiltrating T lymphocytes and the percentage of interleukin-2/interferon- γ producing T lymphocytes were markedly reduced in interleukin-10 transgenic recipients. Finally, the overexpression of interleukin-10 resulted in the decline of graft-infiltrating macrophages at all time points.

Conclusions: Regulated expression of interleukin-10 inhibits cardiac allograft vasculopathy development via reduction of mononuclear cell recruitment and alteration of their cytokine profile. This strategy may prove beneficial in controlling the alloimmune response in solid organ transplants.

Cardiac allograft vasculopathy (CAV), the primary manifestation of chronic rejection in transplanted hearts, is the leading cause of late death in heart transplant recipients. The pathogenesis of CAV remains incompletely understood. Our laboratory has characterized a major histocompatibility complex (MHC) class II mismatched model of CAV (B6C.H-2^{bm12} donors and C57BL/6 recipients) where the development of intimal lesions is absolutely contingent on the presence of CD4⁺ lymphocytes.¹ In addition to playing a role in the activation of antigen presenting cells (APCs) via CD40 signaling,² we found that graft-infiltrating CD4⁺ lymphocytes were the primary source of the proinflammatory cytokines (Th-1),

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interleukin (IL)-2 and interferon (IFN)- γ .¹ Importantly, neither CD4⁺, nor CD8⁺ lymphocytes secreted the anti-inflammatory cytokines (Th-2), IL-10, or IL-4.¹

In organ transplantation, Th-1 cytokines are thought to promote allograft rejection by activating effector cells such as cytotoxic T lymphocytes or macrophages. In contrast, Th-2 cytokines are thought to inhibit the alloimmune response, primarily by decreasing antigen presentation and antigen-specific T lymphocyte activation.^{3,4} Not surprisingly, several investigators have sought to prevent allograft rejection by overexpressing Th-2 cytokines, including IL-10. Overall, the reported outcomes have been contradictory. Qin and colleagues⁵ reported that retroviral-mediated transfer of viral IL-10 gene prolonged murine cardiac allograft survival in an acute rejection model. In contrast, Qian and colleagues⁶ illustrated that systemic administration of IL-10 paradoxically led to accelerated acute rejection. Finally, Furukawa and colleagues⁷ examined the effects of IL-10 treatment on chronic rejection and observed that exogenous IL-10 exacerbated CAV development. The contradictory nature of the cited findings is in part related to the pleiotropic properties of IL-10, and in part to the dose, timing, and duration of IL-10 therapy.

Because CAV development in our model is dependent on IFN- γ secretion,^{8,9} we hypothesized that the selective expression of IL-10 by graft-infiltrating T lymphocytes would inhibit the secretion of IFN- γ within the donor heart, and subsequently abrogate CAV development. To address this hypothesis, we used an IL-10 transgenic mouse recipient, in which mouse IL-10 is driven by the T-lymphocyte-specific human IL-2 promoter.¹⁰ Activation of IL-2 promoter in recipient T-lymphocytes (ie, in an allogeneic response) results in overexpression of IL-10, at sites of T-lymphocyte activation such as primary and secondary lymph nodes and in the allograft. This study sought to determine the effect of regulated IL-10 production on intimal thickening, mononuclear cell infiltration, and the cytokine phenotype of graft-infiltrating lymphocytes. Our findings suggest that regulated IL-10 expression markedly reduces the number of graft-infiltrating T lymphocytes and macrophages, alters their cytokine profile, and inhibits the development of CAV.

Materials and Methods

Animals

Adult female B6C.H-2^{bm12} and wild-type C57Bl/6 mice (7-10 weeks old) were purchased from Jackson Laboratories (Bar Harbor, Me). C57Bl/6 mice bearing the IL-10 transgene under the control of the human IL-2 promoter were generated as previously described.¹⁰ The B6C.H-2^{bm12} and C57Bl/6 strains differ at the I-A locus of MHC II but are identical at MHC I and minor MHC loci. The mice were housed under conventional conditions and fed water and standard rodent laboratory chow (Purina Mills, Inc., St Louis, Mo) ad libitum. All animals received humane care in compliance with UCLA guidelines and the *Principles of Labora-*

tory Animal Care, published by the National Institute of Health (NIH Publication No. 86-23, revised 1985).

Transplantation

Intra-abdominal heterotopic cardiac transplantation was performed using a modification of the method outlined by Corry and colleagues.¹¹ Donor hearts were arrested with heparinized saline (100 U/1 cc), excised, and stored on ice. Through a midline abdominal incision, the donor aorta was anastomosed to the recipient infra-renal abdominal aorta, and the donor pulmonary artery was anastomosed to the inferior vena cava with 10-0 nylon suture. Ischemia time was 60 \pm 15 minutes. The transplanted hearts spontaneously resumed contractions after reperfusion.

Experimental Groups

B6C.H-2^{bm12} strain mouse hearts were transplanted heterotopically into (1) C57Bl/6 wild-type mice or (2) IL-10 transgenic mice, which were sacrificed at days 7 or 24 post-transplant (n = 6 per group each time point). No immunosuppression was given.

Morphometric Analysis

Elastica von Gieson stains were performed on transverse sections of allografts and isografts. The morphometric analysis method as previously described by Armstrong and colleagues¹² was utilized. Briefly, the areas of the neointima, media, and lumen were measured utilizing Optimas 6 software by Media Cybernetics (Silver Springs, Md). The neointima was defined as the area bound by the internal elastic lamina and the lumen. The media was defined as the region between the internal and external elastic membranes. The lumen was defined as the clear region in the vessel. At least 3 sections, 100 μ m apart, from the middle of the heart were utilized for analysis. Vessels greater than 80 μ m in diameter were measured. More than 10 vessels per heart were examined.

Immunohistochemistry

The transplanted and native hearts were explanted in all animals. The harvested hearts were fixed in liquid nitrogen embedded in OCT compound, and kept at -70°C . The primary antibodies were as follows: rat anti-mouse CD4 monoclonal antibody clone GK1.5; rat anti-mouse CD8a monoclonal antibody clone 53-6.7; rat anti-mouse MOMA-2 monoclonal antibody for monocytes/macrophages; hamster anti-mouse CD54 monoclonal antibody for intercellular adhesion molecule-1 (ICAM-1) clone 3E2; rat anti-mouse CD106 monoclonal antibody for vascular cell adhesion molecule (VCAM)-1 clone 429; anti-mouse α smooth muscle actin monoclonal antibody clone 1A4 for smooth muscle cells. All primary antibodies were purchased from PharMingen (San Diego, Calif), with the exception of α -smooth muscle actin antibody (Sigma Chemical Co, St. Louis, Mo).

Immunohistochemistry was performed on 5- μ m-thick cryostat sections with the use of an avidin-biotin-peroxidase technique (Vector Laboratories, Inc., Burlingame, Calif) with diaminobenzidine (DAB) as the chromogen. Sections were incubated with the Avidin-Biotin Complex Solution, Vectastain Elite ABC kit, (Vector Laboratories, Inc.). DAB was applied and subsequently counterstained with Harris hematoxylin.

The perivascular and vascular region was scored by two blinded observers on a scale of 0 to 4 (0 = no staining; 1 =

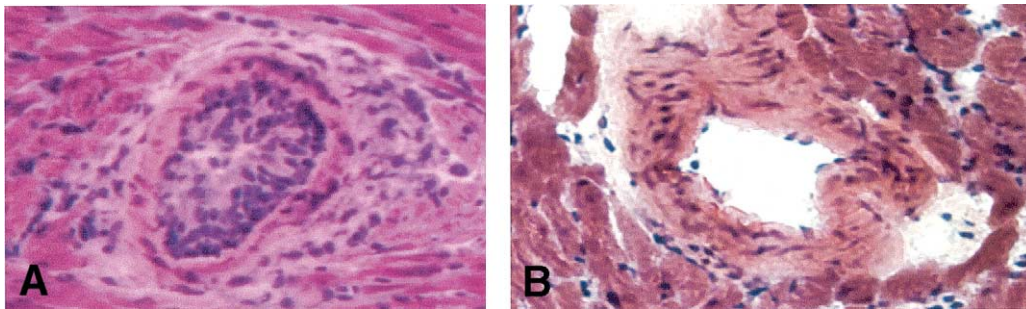


Figure 1. A, B6.CH2^{bm12} donor hearts transplanted into C57Bl/6 wild-type recipients developed severe intimal lesions at 24 days post-transplantation (hematoxylin and eosin [H&E] stain, magnification $\times 100$). B, In contrast, donor hearts transplanted into IL-10 transgenic recipients had complete absence of CAV development (H&E stain, magnification $\times 100$).

scattered individual cells; 2 = focal clusters of cells; 3 = multifocal cluster of cells; 4 = diffuse clusters of cells).

Graft-Infiltrating Cell Isolation and Fluorescence-Activated Cell Sorter (FACS) Analysis

Donor hearts from wild-type and IL-10 transgenic recipients, harvested on days 7 and 24 post-transplantation were analyzed for graft infiltrating cells. Hearts were minced and digested for 2 hours in Collagenase D (2 mg/mL; Worthington Biochemical, Lakewood, NJ) at room temperature in RPMI 1640 media (Sigma Chemical Co.) with 10% fetal calf serum (FCS; Life Technologies, Rockville, Md). Isolated cells were collected, washed twice in RPMI + 5% FCS, and counted after lysis of erythrocytes.

The isolated graft infiltrating cells ($1-2 \times 10^6$ /mL) were stimulated with PharMingen Activation Cocktail (10 μ L/6 mL) (containing PMA, ionomycin, and Brefeldin A) for 4 hours at 37°C. The samples were then stained for the presence of cell surface antigens with fluorescein isothiocyanate (FITC)-labeled anti-CD8 monoclonal antibody or FITC-labeled anti-CD4 monoclonal antibody for 30 minutes at 4°C. Samples were washed, then fixed and permeabilized with PharMingen Perm/Fix solution for 30 minutes at 4°C. The cells were washed in permeabilizing buffer, and incubated with phycoerythrin (PE)-labeled IL-2 monoclonal antibody, PE-labeled IFN- γ monoclonal antibody, PE-labeled IL-4 monoclonal antibody, PE-labeled IL-10 monoclonal antibody, or an irrelevant PE-conjugated isotype-matched control antibody for 30 minutes at 4°C. After 2 washes with permeabilizing buffer, the cells were resuspended in phosphate-buffered saline. All antibodies were purchased from PharMingen. FACS analysis of labeled cells was carried out on a COULTER EPICS XL-MCL Flow Cytometer (Coulter Corporation, Miami, Fla) The net percentage of cells positive for the cell surface markers CD4 and CD8 was calculated by subtracting the background staining (percentage of fluorochrome-positive cells labeled with irrelevant isotype-matched control antibody) from the total staining (percentage of fluorochrome-positive cells labeled with anti-CD4 or anti-CD8 antibody). The number of fluorochrome-positive cells is reported as the number of fluorochrome-positive cells per gram of transplanted heart.

Statistics

Data are presented as mean \pm SEM. Analysis of variance was used to compare mean values of cell number and intimal thickening between different time points.

Results

Regulated IL-10 Production Attenuates CAV Development

In this model of CAV, the donor hearts reproducibly develop intimal lesions in 24 days. The expanded intima consists of T lymphocytes, monocytes/macrophages, smooth muscle cells, and extracellular matrix.¹ All donor hearts had palpable contractions at the time of harvest. At Day 7, as expected, intimal thickening had not developed in the donor hearts transplanted into either wild-type or IL-10 transgenic recipients. By day 24, B6.C-H2^{bm12} donor hearts transplanted into wild-type mice developed extensive intimal thickening ($50 \pm 7\%$, Figure 1, A). In contrast with wild-type control mice, the donor hearts transplanted into IL-10 transgenic recipients did not develop any intimal thickening at day 24 ($0 \pm 0\%$; Figure 1, B).

Regulated IL-10 Production Reduces T-Lymphocyte Recruitment

In this MHC class II mismatched model, we have previously demonstrated that CD4⁺ lymphocytes are absolutely required and that CD8⁺ lymphocytes augment CAV development.¹³ In this model of CAV, CD4 and CD8 lymphocytes infiltrate the donor heart in nearly equal numbers at day 7 post-transplantation, whereas at 24 days, CD4 lymphocytes are the predominant graft infiltrating lymphocytes.² Therefore, we sought to determine whether regulated IL-10 production prevented CAV by reducing the accumulation of CD4⁺ and/or CD8⁺ lymphocytes in the donor heart. The number of graft infiltrating lymphocytes in the donor hearts transplanted into IL-10 transgenic recipients was decreased significantly at both 7 and 24 days after

transplantation, when compared to the donor hearts in wild-type recipients (Figure 2). Immunohistochemical stains also confirmed that there was a marked decline in the number of graft infiltrating CD4 and CD8 lymphocytes in the donor hearts of IL-10 transgenic recipients (Figures 3, B, D) versus control recipients (Figures 3, A, C), at 24 days post-transplantation.

Regulated IL-10 Production Attenuates Th-1 Response of Graft Infiltrating T Lymphocytes

Intracellular cytokine assays were performed on graft-infiltrating CD4⁺ and CD8⁺ lymphocytes to determine the effect of regulated IL-10 expression on the intra-graft cytokine profile. Representative cytokines from both Th-1 type (IL-2 and IFN- γ) and Th-2 type (IL-4, IL-10) were studied.

In wild-type mice, during the course of CAV development, both graft-infiltrating CD4⁺ and CD8⁺ lymphocytes predominately secreted the Th-1 type cytokines (ie, IL-2 and IFN- γ) (Figures 4, A,B). In IL-10 transgenic recipients, there was a significant decrease in the percentage of IL-2 producing graft-infiltrating CD4⁺ lymphocytes; however, the percentage of IFN- γ producing CD4⁺ lymphocytes remained unchanged (Figure 4, A). Moreover, regulated production of IL-10 significantly affected graft-infiltrating CD8⁺ lymphocytes; there was a decline in the percentage of both IL-2 and IFN- γ producing graft-infiltrating CD8⁺ lymphocytes (Figure 4, B). The reduction in the number of graft-infiltrating CD4/CD8 lymphocytes and the alteration in the cytokine profile of the residual graft infiltrating cells resulted in marked decline in the intra-graft IL-2 and IFN- γ level.

Regulated IL-10 Production Reduces Monocyte/Macrophage Recruitment

The above findings suggested that the overall expression of Th-1 type cytokines are reduced in the donor hearts transplanted into IL-10 transgenic recipients. Th-1 type cytokines, including IFN- γ , lead to the recruitment of monocytes/macrophages, which are thought to participate in CAV development. Therefore, donor hearts were tested immunohistochemically for the presence of graft-infiltrating monocytes/macrophages. Interestingly, correlating with the absence of intimal thickening, there was a significant reduction in monocyte/macrophage recruitment in donor hearts transplanted into IL-10 transgenic compared with wild-type recipients (wild-type: 3.4 ± 0.4 vs IL-10 transgenic: 0.7 ± 0.6 , $P < .05$) (Figures 3, E,F).

Regulated IL-10 Production Does Not Alter Adhesion Molecule Expression

IL-10 has been reported to decrease the expression of adhesion molecule expression and consequently mononuclear cell adherence.¹⁴ Therefore, donor hearts were examined immunohistochemically for ICAM-1 and VCAM-1 expres-

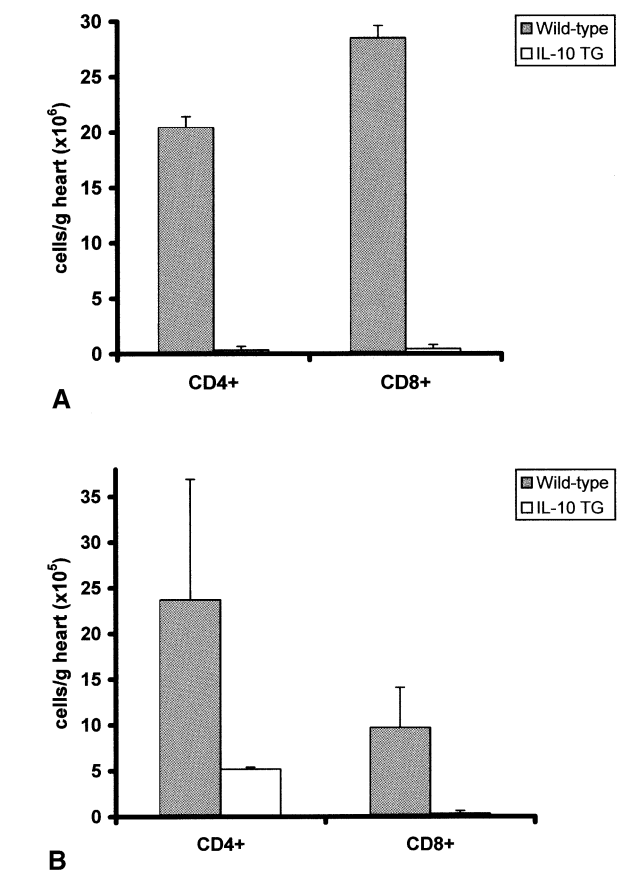


Figure 2. Flow cytometric analysis of graft infiltrating lymphocytes in B6.C-H2^{bm12} donor hearts transplanted into either C57Bl/6 wild-type or IL-10 transgenic recipients. **A,** At 7 days post-transplantation, there was a significant decrease in CD4⁺ and CD8⁺ lymphocyte recruitment to the donor hearts transplanted into IL-10 transgenic compared to wild-type recipients ($P < .05$). **B,** At day 24, there is a persistent decrease in intra-graft T lymphocyte subset recruitment in IL-10 transgenic recipients ($P < .05$ when compared to donor hearts harvested from control recipients).

sion. Notably, there were similar amounts of both ICAM-1 and VCAM-1 expression in the donor hearts transplanted into both wild-type and IL-10 transgenic recipients (data not shown).

Discussion

The findings of this study demonstrate that regulated production of IL-10 inhibits the development of intimal thickening, or CAV, in this model. The possible mechanisms of this beneficial effect of IL-10 include: (1) reduction in the number of graft-infiltrating T lymphocytes, (2) attenuation of Th-1 type response in the graft-infiltrating T lymphocytes, and (3) reduction in the recruitment of intra-graft monocytes/macrophages.

Because of their anti-inflammatory nature, Th-2 type cytokines such as IL-10 are believed to play a protective

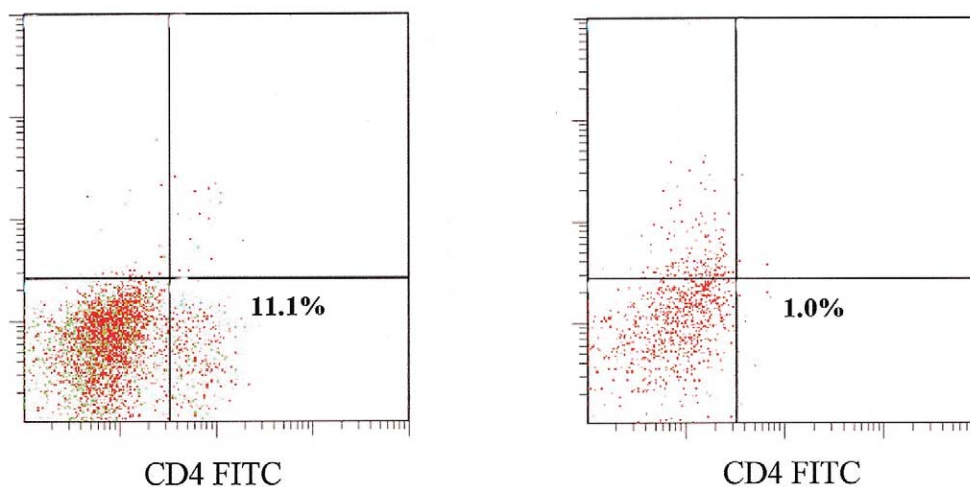


Figure 2. Cont'd. C, Representative flow cytometry dot plots gated on graft infiltrating cells at day 24 post-transplantation. There was a marked reduction in the number of infiltrated CD4 lymphocytes into the donor hearts of IL-10 transgenic recipients (right) when compared to wild-type control recipients (left).

role in the prevention of chronic rejection.⁵⁻⁷ A number of immunologic tools have been used to test the effect of IL-10 overexpression on allograft rejection, including (1) treatment of transplant recipients with either cytokine blocking antibodies or recombinant proteins, (2) transfection of donor hearts with cytokine gene constructs, and (3) transplantation of donor hearts into cytokine knockout recipients.^{5-7,15-17} Notably, these studies have relied upon unphysiologic expression of IL-10. The IL-10 transgenic mouse employed in the current study provides a unique immunologic tool for assessing the role of IL-10 in the prevention of chronic rejection. IL-10 synthesis is transient, local, and related to T lymphocyte activation, thereby avoiding the toxic effects of either constitutive expression, or very high levels of IL-10.¹⁰ It is important to mention that prior studies have demonstrated that the IL-10 transgenic mice do not exhibit any defects in growth/development and have normal numbers/phenotypes of T and B lymphocytes. Furthermore, the amount of IL-10 synthesized in these mice is not sufficient to cause a complete suppression of Th-1 cytokines.¹⁰

Regulated expression of IL-10 reduces T lymphocyte subset graft infiltration. The central finding of this study is that localized, transient, and regulated expression of IL-10 prevents CAV development in this murine model. Analysis of the graft-infiltrating mononuclear cells illustrated that there was a significant reduction in the number of CD4⁺ and CD8⁺ lymphocytes at all time points. Development of CAV in this murine strain combination is strictly dependent on cellular alloimmunity.¹ Our prior studies have shown that CD4⁺ lymphocytes were absolutely required for the development of chronic rejection, whereas CD8⁺ lymphocytes augmented CAV.¹ The reduction in graft-infiltrating lymphocytes in the donor hearts in the IL-10 transgenic recip-

ients may be due to (1) decreased recruitment or (2) alteration in priming of the recipient T cells.

IL-10 has been shown to block upregulation of adhesion molecules, thereby reducing T lymphocyte emigration.¹⁴ In this model, we could not detect a significant downregulation in the adhesion molecules ICAM-1 and VCAM-1, which are known to mediate mononuclear cell recruitment. Alternatively, decreased T-lymphocyte recruitment in IL-10 transgenic recipients may be due to the attenuation of chemotactic mediators. We have recently observed an important role for the CXC chemokines, and specifically for monokine induced by IFN- γ (Mig/CXCL9), in T-lymphocyte recruitment in this strain combination.¹⁹ Neutralization of Mig/CXCL9 significantly reduced T-lymphocyte recruitment and CAV development.^{19a} The effects of IL-10 on Mig/CXCL9 production was recently studied by Aliberti and colleagues,²⁰ who showed that IL-10 inhibited Mig/CXCL9 production. Additionally, given that Mig/CXCL9 production is strictly dependent on IFN- γ , IL-10 may have additional regulatory effects on Mig/CXCL9 production via its inhibitory effects on IFN- γ .

Aside from a decrease in recruitment, the number of graft infiltrating T lymphocytes may be reduced due to the inhibitory effects of IL-10 on T lymphocyte priming/proliferation. IL-10 reduces antigen-specific T-lymphocyte proliferation by diminishing the antigen presenting capabilities of APCs; IL-10 downregulates the expression of MHC class II, co-stimulatory molecules, and cytokine production by APCs.²⁰ Li and colleagues¹⁸ have shown that IL-10 inhibited T-lymphocyte proliferation by impairing the function of APCs, prior to antigen exposure. Furukawa and colleagues⁷ also illustrated that IL-10 caused a dose-dependent decrease in alloantigen specific T-lymphocyte proliferation in mixed

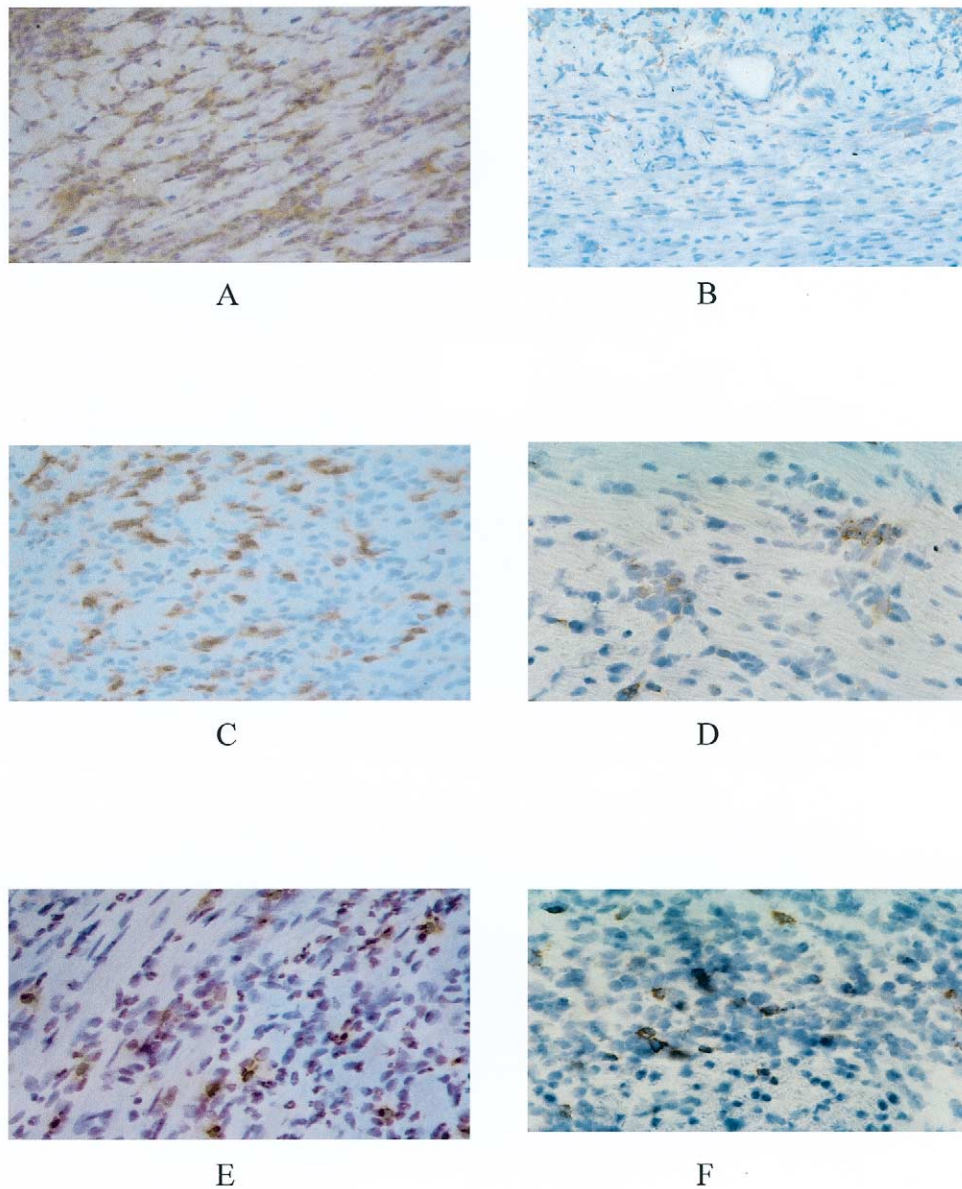


Figure 3. Representative immunohistochemical sections of donor hearts harvested at 24 days post-transplantation from wild-type control recipients and IL-10 transgenic recipients. Donor hearts transplanted into C57Bl/6 wild-type recipients developed severe intimal lesions, associated with (A) CD4⁺ lymphocyte, (C) CD8⁺ lymphocyte, and (E) monocyte/macrophage recruitment. In contrast, there was a marked decline in the number of graft infiltrating (B) CD4⁺ lymphocytes, (D) CD8⁺ lymphocytes, and (F) monocyte/macrophages in the donor hearts transplanted in the IL-10 transgenic recipients. Representative sections are from donor hearts harvested at 24 days post-transplantation. All panels in this figure are immunohistochemical stains using the avidin-biotin-peroxidase technique. Positive cells display a brown stain.

lymphocyte reactions. Hence, the decrease in the number of graft-infiltrating T lymphocytes in the donor hearts in the IL-10 transgenic recipients may be due to a reduction in T-lymphocyte recruitment as well as inhibition of T-lymphocyte proliferation.

Regulated expression of IL-10 suppresses IFN- γ production. IL-10 inhibits Th1 cytokine production (ie, IFN- γ) by CD4 lymphocytes both in vivo and in vitro.²¹ IL-10 overexpression in this model resulted in marked reduction in the number of graft infiltrating lymphocytes as

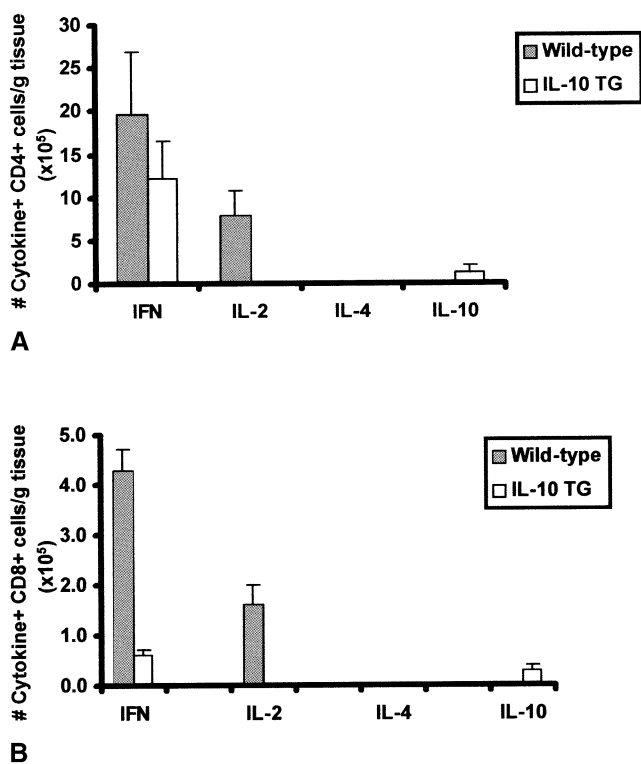


Figure 4. Intracellular cytokine staining of graft-infiltrating lymphocytes in B6.C-H2^{bm12} donor hearts transplanted into either C57Bl/6 wild-type or IL-10 transgenic recipients (24 days). **A**, IL-10 overexpression lead to a significant decrease in intragraft IL-2 producing CD4⁺ lymphocytes. **B**, Similarly, IL-10 overexpression resulted in a significant decrease in intragraft IL-2 and IFN- γ by graft-infiltrating CD8⁺ lymphocytes. $P < .05$ when the number of IL-2 producing CD4 and IFN- γ /IL-2 producing CD8 lymphocytes harvested from the donor hearts of IL-10 transgenic recipients were compared with that of wild-type control recipients.

well as suppression of Th1-like cytokine production by the residual infiltrated T lymphocytes. The net effect was a significant reduction in the total intragraft IFN- γ level in the donor hearts transplanted into IL-10 transgenic recipients when compared to wild-type recipients. IFN- γ is known to play a central role in the development of CAV in this strain combination.^{8,9} Nagano and colleagues^{8,9} previously reported that the serological neutralization or genetic absence of IFN- γ , markedly reduced the extent of chronic rejection. IFN- γ may contribute to CAV development through a number of mechanisms. Tellides and colleagues²² have reported that IFN- γ alone could induce arteriosclerotic changes in either swine or human arterial loops that were transplanted into immunodeficient mouse recipients.²² Alternatively, IFN- γ is known to play a role in the activation of macrophages, which have been suggested as key effector cells in CAV development.²³⁻²⁶ Thus, suppression of IFN- γ production by local expression of IL-10 can affect CAV devel-

opment in this model via 2 pathways: (1) directly, and (2) by controlling macrophage activation.

Regulated IL-10 Expression Reduces Monocyte Recruitment. Immunohistochemically, we observed a significant decrease in both macrophage recruitment and lesion development in the presence of IL-10 expression. The biological effect(s) of IL-10 on macrophage recruitment and activation have been studied by several investigators. Mostafa Mtairag and colleagues²⁷ illustrated that IL-10 decreased monocyte/endothelial cell adhesion via the modulation of monocyte/macrophage adhesion molecules, CD18 and CD62L. O'Farrell and colleagues²⁸ reported that IL-10 inhibited macrophage proliferation in vitro in a dose-dependent fashion; inhibition was due to G1 cell cycle growth arrest, and not apoptosis. Finally, because IFN- γ is known to play a role in the activation of macrophages, IL-10 can control macrophage activation, indirectly, via a reduction in intragraft IFN- γ levels.²³⁻²⁶ The net effect of local and transient IL-10 production is a reduction in monocyte/macrophage recruitment.

A limitation of this study is that CAV development in IL-10 transgenic recipients was assessed up to 24 days after transplantation. It is conceivable that regulated IL-10 expression may delay CAV development; examination of later time points will be necessary to document eradication of CAV development in the donor hearts of IL-10 transgenic recipients.

This study expands the current body of knowledge on the role of IL-10 in the alloimmune response; it emphasizes the importance of the location, duration, and timing of IL-10 expression on its biologic effect in vivo. It also supports IL-10 gene-based therapy directed at the recipients. A potential strategy may include transfer of IL-10 gene under IL-2 promoter to the recipient's hematopoietic stem cells while on the waiting list. The transduced recipient cells will produce IL-10 only when activated (ie, IL-2 promoter is activated). However, it is important to note that the effect of immunosuppressive medications on the overexpression of IL-10 and its beneficial effects are unknown.

In conclusion, this study provides important insight on the role of IL-10 in alloimmune responses. It suggests that regulated IL-10 expression can attenuate chronic rejection in a murine model. The abrogation of CAV appears to correlate with the reduction of graft-infiltrating T lymphocytes and macrophages, and consequently, intragraft IFN- γ production. The results of this study justify and support ongoing IL-10 gene-based therapies in solid organ transplantation.

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