Blockade of CD28-B7 Costimulation by CTLA4Ig Interrupts Chronic Allograft Rejection

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INTRODUCTION

Chronic rejection has now emerged as the leading cause of late allograft failure following solid organ transplantation[1-4]. The major manifestation in chronic rejection is an obliterative arteriosclerosis characterized by diffuse, concentric fibrointimal thickening in large and medium sized arteries and arterioles often extending throughout the entire length of the vessel wall rather than focal distribution[5-8]. Although the pathogenesis of chronic rejection is uncertain, a number of alloantigendependent and independent factors have been implicated[9-11]. The leading hypothesis is that it arises from chronic immune stimulation involving donor-derived endothelium leading to vascular and inflammatory cell activation[12].

The Lew to F344 rat heterotopic abdominal cardiac transplantation model has been favored because of its reproducibility, in this model about 80% of the cardiac allografts survive longer than three weeks and 25% survive indefinitely[1]. The vascular changes, patterns of cell migration and cytokine production have been well characterized in this model and reflect many of the changes that have been observed in human chronic cardiac allograft rejection[2, 13, 14]. One of the most striking features is the persistence of T cells within the allograft. It is clear that T cells play a central role in the acute allograft rejection and prevention of T cell activation has been shown to induce transplant tolerance in many animals models[2, 15]. The F344 to LEW rat renal allograft model is a well established model for the study of experimental chronic allograft rejection. Activation of T cells to proliferate and secrete cytokines requires two distinct signals. The first signal is provided by the engagement of the TCR with the MHC molecule complexed with peptide on APC surface, and the second costimulatory signal is provided by engagement of specific T cell surface receptors with their ligands on APC. Among the multiple costimulatory pathways identified, interaction of CD28 on T cells with either of two ligands, B7-1 or B7-2 on APC is the most important costimulatory pathway for the response to alloantigens[16-18]. The most effective way of blocking CD28 interaction with B7-1/B7-2 has proven to be by the recombinant fusion protein CTLA4Ig. This protein consists of the extracellular domain of CTLA4, a T cell coreceptor for B7-1/B7-2 fused with human IgG1 heavy chain[19]. This molecule has a 20 fold higher affinity for B7-1/B7-2 than does CD28[20].

CTLA4Ig has been reported to prevent acute rejection[21], induce donor-specific tolerance[22-26], and we have recently shown that a single injection of CTLA4Ig on day 2 posttransplant prevents development of graft arteriosclerosis, in the

Lewis to F344 chronic cardiac allograft rejection model[2]. As acute rejection has been shown to be an important contributory factor in the development of chronic rejection and tolerance induction in an acute rejection model has been shown to prevent the development of chronic rejection, it can be argued that prevention of chronic rejection is merely due to prevention of acute rejection[27]. Whether inhibiting T cell activation late post-transplantation, after initial acute rejection, will interrupt progression of chronic rejection has not been addressed. We used the same Lew to F344 heart or F344 to Lew renal transplant model to evaluate this question.

METHOD

1. Lewis to F344 rat cardiac model of chronic rejection

Adult male Lewis (RT1l) and F344 (RT1l) rats were obtained from Harlan Sprague Dawley (Indianapolis, IN). They were housed under conventional conditions and fed a standard diet (Rodent Laboatory Chow, No. 5001, Ralston Purina Company, St. Louis, MO) and water ad libitum. Rat cardiac transplantation was performed by a heterotopic abdominal approach[1]. The allogeneic combination of Lewis rats as donors and F344 rats as recipients was used to induce chronic rejction. Although the Lewis to F344 strain combination is compatible for the Ag-B locus (major histocompatibility complex RT1), there are multiple mismatches at the H loci[28]. LEW or F344 rats were used in the isogenic combination to produce control hearts exposed to the same surgical procedure. Allografts were followed by daily palpation. Allografts surviving at least four weeks were removed at the time of rejection or, if continuing to function, 120 days after transplantation. Rejection was defined as the complete cesstion of palpable heart beat.

CTLA4Ig and control human Ig were generously provided by Bristol-Myers- Squibb (Seattle, WA). All animals received a low dose cyclosporine A (Sandoz, Ltd., Basel, Switzerland) protocol (5 mg/kg/day s.c.) for 30 days starting on day of transplant.

Four experimental groups of animals were studied. Group 1 received CTLA4Ig 0.5mg intraperitoneally twice a week from day 30 to 120 (n=6). Group 2 received same dose of CTLA4Ig from day 60 to 120 (n=5). Group 3 received same dose of control Ig from day 30 to 120 (n=10). Group 4 received no subsequent therapy after day 30 of isograft transplantation (n=3 : F344 to F344, n=5 : Lewis to Lewis).

For reverse transcription PCR analysis, surviving cardiac allografts were harvested after day 120. Serial midventricular sections were frozen in liquid nitrogen for subsequent RNA extraction. For morphometric analysis of arteriosclerotic lesion development, we harvested a group of grafts also at the time of rejection or, if continuing to function, after day 120 from the long term surviving grafts.

2. F344 to Lewis rat renal model of chronic rejection

Inbred male LEW rats were transplanted with kidneys from F344. Kidneys were transplanted orthotopically to the left renal vessels and the left ureter of the host by end to end anastomosis. The right kidney was removed 10 days later.

The control group (n=13) of LEW rats received F344 kidneys and a short course of CsA (5 mg/kg/d×10 d, subcutaneously) and no further treatment. The treated group (n=12) received the same CsA protocol, as well as a single intraperitoneal injection of CTLA4Ig (0.5 mg, intraperitoneally) at 8 wk postoperatively. Urine was collected every 2-4 wk from the time of transplantation. Protein excretion was determined by measuring precipitation after interaction with 3% sulfosalicylic acid. Turbidity was assessed by

absorbance at a wave length of 595 nm using a Coleman JuniorII spectrophotometer.

3. Morphometric analysis

Allografts were serially sectioned into approximately 2-mm slices and fixed in 10% formaldehyde. Sagittal sections of cardiac allografts stained with Verhoeff's elastin. Slides were then examined by light microscopy and allografts scored for the degree of arterial intimal thickening present and were compared by using the 0-5 grading system described by Adms et al.[9]. In brief, a score of 0 indicated a normal vessel, 3 implied 20-50% luminal occlusion, and a score of 5 implied >80% luminal occlusion. A total of vessels were analyzed with an average of scored per graft. Mean scores were pooled for allografts in each group and subjected to MANOVA. All arteries seen were examined and scored.

4. Measurement of gene transcript levels

To evaluate the effects of CTLA4Ig treatment on lymphocyte and macrophage activation, we measured gene transcript levels for factors we have previously shown to be upregulated[2]. At the time of harvest, allograft ventricular sections were snap-frozen in liquid nitrogen for subsequent RNA extractions. Total cellular RNA was extracted from ventricular sections by using RNeasy Total RNA kits® (QUIAGEN, Germany) as directed by the manufacturer. The quality of the RNA was confirmed on formaldehyde-agarose gels. The 4 cDNAs (experimental set) analyzed were prepared from day 120 cardiac allografts treated in various subgroups with (1) CTLA4Ig starting after day 30 group (n=6) (2) CTLA4Ig starting after day 60 group (n=5) (3) control Ig group (n=10) (4) isograft group (n=8). We used a published reverse-transcription PCR technique developed to measure MCP-1 transcript levels[15], modified as follows for use with IFN-γ, iNOS, TGF-β, B7-1 and the 'housekeeping' gene glyceraldehyde-3-phosphate dehydrogenase (G3PDH)[2]. Oligonucleotide primers were obtained from Clonetech or synthesized by Genosys Biotechnologies. For each primer combination, conditions were optimized to generate a single specific band. We used the PTC-100™ Programmable Thermal Controller and the "hot start" technique to increase specificity. Reaction conditions included 1.25 ml cDNA, 1 mM (each) 5′ and 3′ primers, 10 mM Tris-HCL, 50 mM KCL, 1.5 mM $MgCl_2$, 0.001% (wt/vol)gelatin, 800 mM dNTPs, and 0.625units AmpliTaq DNA polymerase in a total volume of 25ml. ^{32}P-dCTP (150,000 cpm) was included for quantitative PCR studies. The thermal cycling parameters were denaturation at 94℃ for 15 sec, annealing between 50 and 58℃ for 20 sec, and extension for 60 sec (with a final extension of 7 min at the end of all cycles). Primer sequences, annealing temperature, and number of cycles were

IFN-γ 5′ ATC TGG AGG AAC TGG CAA AAG GAC G
3′ CCT TAG GCT AGA TTC TGG TGA CAG C (60℃, 32 cycles)

MCP-1 5′ ATG CAG GTC TCT GTC ACG
3′ CTA GTT CTC TGT CAT ACT (50℃, 28 cycles)

iNOS 5′ TGC CAG GGT CAC AAC TTT ACA GG
3′ GGT CGA TGT CAC ATG CAG CTT GTC (60℃, 35 cycles)

TGF-β 5′ TGA ACC AAG GAG ACG GAA TAC AGG
3′ TAC TGT GTG TCC AGG CTC CAA ATG (57℃, 26 cycles)

G3PDH 5′ TGA AGG TCG GTG TCA ACG GAT TTG GC
3′ CAT GTA GGC CAT GAG GTC CAC CAC (58℃, 22 cycles)

B7-1 5′ GGC ATT GCT GTC CTG TGA TTA C
3′ ACT CAG TTA TGT TGG GGG TAG G (56℃, 28 cycles)

PCR products (10μl) were analyzed on 1% aga-

rose gels. ^{32}P-dCTP incorporated into PCR product bands was measured from dried gels on a Molecular Dynamics Phosphor Imager as described elsewhere[29]. PCR amplification with the G3PDH housekeeping gene was performed to assess variations in total RNA or cDNA loading between samples. Corrected values were derived by dividing the measured ^{32}P value for the transcript of interest by the mean G3PDH value for the sample. Relative transcript levels were then determined from cDNA panels that included negative control samples (for which reverse transcriptase had been omitted during cDNA synthesis or water had been used instead of cDNA). PCR analysis was performed all samples in a study panel to identify relative differences, and each analysis was performed at least three times. The mean value for the corrected levels was obtained by pooling measurements from all animals in a subgroup. Results were subjected to Kruskal-Wallis non-parametric ANOVA without replication. If the ANOVA was significant, individual comparisons were made by the Welch alternate t test, as standard deviations could be assumed to be equal.

RESULTS

1. Cardiac allografts survival

None of the animals treated with CTLA4Ig after day 30 or 60 posttransplant rejected their allografts and the heart beat as assessed by daily palpation was similar to isograft controls. Survival rates of these animals longer than day 120 after transplantation was 84.0% In the group of animals treated with control Ig after day 30 posttransplant, the survival rate longer than day 120 after transplantation was 45.5% . All isografts survived longer than 120 days (Fig.1).

2. Renal allografts survival and function

36 animals were transplanted, 6 of which died by day 16 before the animals were randomly assigned to receive no further treatment (n=18) or CTLA4Ig at 8 wk after transplant (n=12). One animal in the control group died at 20 wk and another developed severe hematuria and was excluded from the analysis. A total of 11 LEW into LEW isografts was also set up. Proteinuria has been reported to be one of the best surrogates for chronic kidney dysfunction. As the interruption group did not receive CTLA4Ig until 8 wk after transplantation, the protein excretion for all animals was graphed together as treatment up to this time was identical (Fig. 2). After this time point, the proteinuria was plotted separately. In allograft recipients treated with CsA alone, animals usually start to show an increase in their 24-h protein excretion after

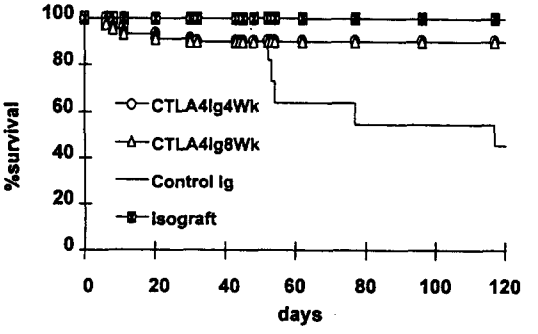

Fig. 1. Survival Rate of Allograft..

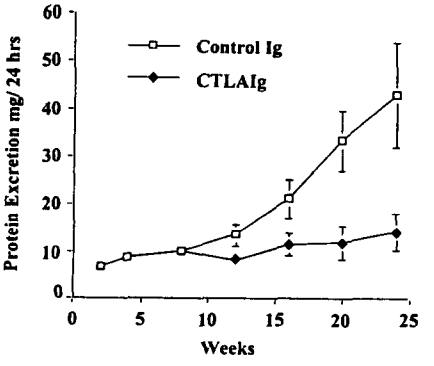

Fig. 2. Effect of CD28-B7 blocakade at 8 weeks on protein excretion.

8-12 wk after transplantation. Animals treated with CsA plus CTLA4Ig at 8 wk after transplant did not develop progressive proteinuria throughout the follow up period of 24 wk (14.3 ±4.1 mg/24 h versus 41.0±12.0 mg/24 h at 24 wk, p=0.043). Control isograft proteinuria was 9.88±0.68 mg/24 h at the same time point.

3. Inflammatory cell activation patterns

Given the differences in expression of cytokines and growth factors found in our previous studies we looked at the relative gene transcript levels in the 120 day cardiac allografts, reverse transcriptase PCR assays optimized for IFN-γ, TGF-β, iNOS, MCP-1 and B7-1. All of these factors were reduced in both CTLA4Ig treated groups compared with the control Ig treated animals. The difference only attained significance with B7-1 and iNOS. In keeping with the previous studies MCP-1 was not significantly different, and this is not suprising given the low expression of MCP-1 in the control animals. Although significance could not be proven with IFN-γ, this was likely due to the relatively high variance in the control and CTLA4Ig 8 week groups, as the expression of IFN-γ in control Ig treated group was 10 fold higher than that of the CTLA4Ig 4 week treated group (Fig. 3).

The expression of B7-1 was significantly different in both the CTLA4Ig at 4 weeks and F344 isografts groups when compared with the control Ig group. As this is the only one of the two known ligands for CTLA4Ig that is significantly expressed at this time point it would appear that even late blockade of CD28-B7 has an effect on B7-1 expression[30].

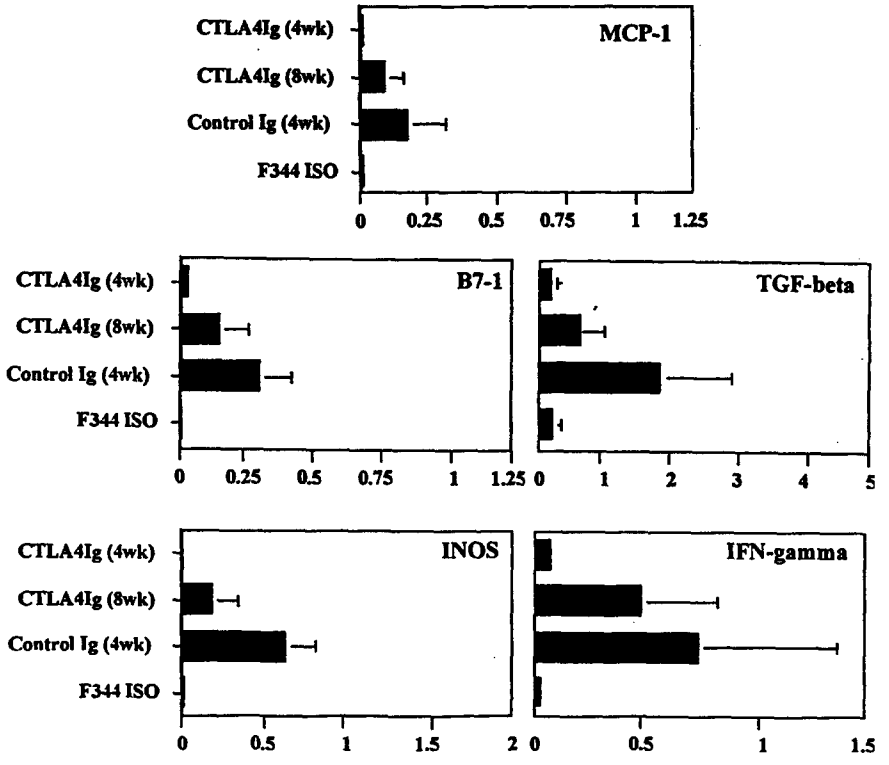

Fig. 3. Intragraft cytokine and adhesion moleule gene transcript levels.

4. Morphometric analysis

In control Ig treated recipients, intimal thickening score and the percentage of number of affected vessels were 1.68±0.81, 55.93±26.06%, which were significantly higher than isograft 0.14 ±0.14, 2.78±4.81%. Control Ig group was also significantly higher than CTLA4Ig starting on day 30 group 0.39±0.30, 16.32±13.21% and day 60 group 0.57±0.35, 25.35±12.76% (Table 1). Thus, blocking T cell costimulation by CTLA4Ig late after acute graft injury is effective in preventing chronic graft loss. Lewis to F344 cardiac allografts older than day 120 showed more advanced and frequent arteriosclerotic

1. Vessel Morphometry at 120 day

	Vessel score	Diseased vessel (%)
	mean±S.D.	mean±S.D.
Control Ig	1.68±0.81	55.93±26.06
CTLA4Ig 4wk	0.39±0.30*	16.32±13.21*
CTLA4Ig 8wk	0.57±0.35*	25.35±12.76*
Isograft	0.14±0.14*	2.78± 4.81

$p<0.05$ in comparison with control Ig

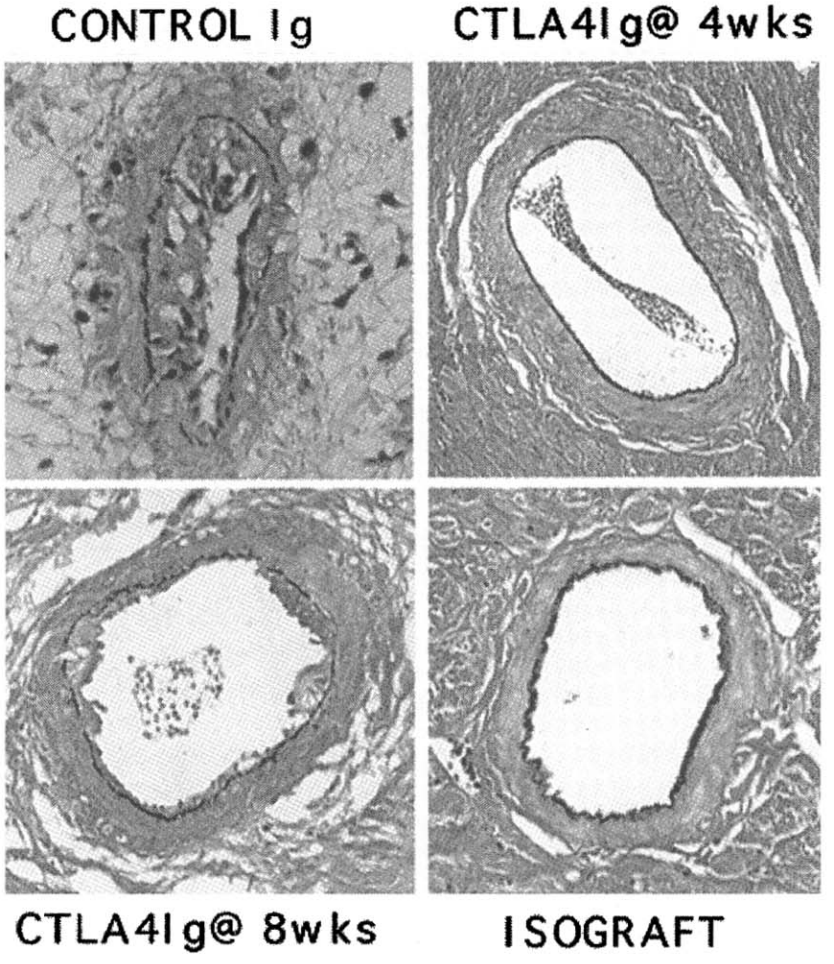

Fig. 4. *Intimal thickening in representative arteries from LEW heart grafts (>120 days old).*

lesions after control Ig treatment compared with the group that received CTLA4Ig or isografts. Fig. 4 depicts representative vessels from the control Ig treated group with moderately severe luminal obstruction due to intimal thickening, from the CTLA4Ig starting on day 30 after transplantation group with minimal intimal thickening, from the CTLA4Ig starting on day 60 after transplantation group with mild intimal thickening and from the isograft group without intimal thickening.

DISCUSSION

As the risks of infection have diminished and early rejection has become better controlled, chronic rejection has now emerged as the dominant reason for late allograft failure in organ transplantation[31].

We[2] and others[15] have shown previously that CD28-B7 blockade by CTLA4Ig prevents acute rejection, induces donor-specific tolerance and prevents development of chronic rejection. It can be argued that prevention of chronic rejection is merely due to prevention of acute rejection. The question is whether inhibiting T cell activation late posttransplantation can interrup progression of chronic rejection.

The vessels in allografts of control Ig group demonstrated significant coronary intimal lesions. Arteriosclerotic lesion develop in stages. The earliest change (before day 30) noted involved focal disruption of the arterial internal elastic lamina and minimal intimal thickening along the perimeter of the arterial lesion. In allografts undergoing early severe rejection, arterial lesions consisted of intimal and perivascluar mononuclear cells with occasional vacuolation and sparing of the media. Other arterial lesions demonstrated significant vasculitis, with massive mononuclear cell infiltration in all layers of the vascular wall. In the intermediate stage (between days 30 and 90), a actin-positive smooth muscle cells appear interposed among the inflammatory cells in the neointima. In the late stage (after day 90), when neointimal expansion is most striking, smooth muscle cells predominate and the number of mononuclear inflammatory cells diminishes.

Our data show that blocking of CD28-B7 T cell costimulation late after acute graft injury by administering CTLA4Ig twice a week continuously from day 30 or day 60 after transplantation increased cardiac allograft survival, decreased graft mononuclear cell infiltration, an significantly reduced gene transcript for key T cell and macrophage activation markers in the Lew to F344 rat model of chronic rejection.

Of interest, are our observations that a vessel of allograft from animals treated with CTLA4Ig 60 days after transplantation, has an intermediate degree of luminal obstruction between a vessel of isograft and allografts from animals treated CTLA4Ig 30 days after transplantation. This observation shows that the severity of chronic rejection correlates with duration of T cell recruitment and activation. In this study we demonstrate a higher level of transcript levels for four cytokines-INF-γ, MCP-1, iNOS, TGF-β in allografts of recipients treated with control Ig than CTLA4Ig.

Our finding that gene transcript levels increase in cardiac allografts for the potent macrophage-activating factor IFN-γ, and the early competence gene MCP-1 suggests that similar transcriptional pathways for macrophage activation are involved in chronic cardiac rejection in vivo. An increase in cytokine expression confirms the presence of activated lymphocytes and macrophages. It also provides support for the idea that sustained cytokine release may be an important immunoregulatory step in the development of chronic rejection. One of the known consequences of CD28-B7 signaling is the regulation of cytokine production by T cells[18, 21, 32-38]. Little is known, however, about the effects of the CD28-B7 cos-

timulatory pathway on macrophage activation. In this report we demonstrate that blocking CD28-B7 T cell costimulation late posttransplantation also disrupts increases in gene transcript levels associated with macrophage activation (iNOS). For those factors whose expression levels were reduced, this might have been caused by a direct inhibition of macrophage through the B7 axis or, more likely, by an indirect effect related to a decrease in levels of T cell cytokines (such as IFN-γ) required for induction of macrophage expression. We reported previously that early blockade of CD28-B7 disrupts increases in gene transcript levels associated with macrophage activation as well as T cells. From our data, Blocking T cell costimulation late after acute graft injury is effective in interruption of increase of gene transcript levels associated with macrophage activation as well as T cells.

These studies show that CTLA4Ig blockade of CD28-B7 costimulatory T cell activation pathway late after acute graft injury is effective in preventing chronic graft loss, indicating that continuous T cell recruitment and activation play an important role in progression of chronic rejection.

REFERENCES

1) Adams DH, NL Tilney, JJ Collins, MJ Karnovsky : Experimental graft arteriosclerosis. *Transplantation*(Balitimore) **53**:1115-1119, 1992
2) Russell ME, WW Hancock, E Akalin, AF Wallace, TG Jensen, TA Willet, MH Sayegh : Chronic cardiac rejection in the LEW to F344 rat model : Blockade of CD28-B7 costimulation by CTLA4Ig modulates T cell and macrophage activation and attenuates arteriosclerosis. *J Clin Invest* **97**:833-838, 1996
3) Russell ME, AF Wallace, WW Hancock, MH Sayegh, DH Adams, NES Sibinga, LR Wyner, MJ Karnovsky : Upregulation of cytokines associated with macrophage activation in the Lew to F344 rat transplantation model of chronic cardiac rejection. *Transplantation*(Baltimore) **59**:572-578, 1995
4) Paul LC, B Fellstrom : Chronic vascular rejection of the heart and the kidney : Have rational treatment options emerged? *Transplantation*(Baltimore) **53**:1169-1179, 1992
5) Russell ME, M Fujita, MA Masek, RA Rowan, ME Billingham : Cardiac graft vascular disease : Nonselective involvement of large and small vessels. *Transplantation*(Baltimore) **56**:1599-1601, 1993
6) Johnson DE, SZ Gao, JS Schroeder, WM DeCampli, ME Billingham : The spectrum of coronary pathologic findings in human cardiac allografts. *J Heart Transplant* **8**:349-359, 1989
7) Cramer DV, GD Wu, FA Chapman, E Cajulis, HH Wang, L Makowka : Lymphocytic subsets and histopathologic changes associated with the development of heart transplant arteriosclerosis. *J Heart Lung Transplant* **11**:458-466, 1992
8) Russell R : The pathogenesis of atherosclerosis : A perspective for the 1990s. *Nature* **362**:801-809, 1993
9) Tullius SG, NL Tilney : Both alloantigen-dependent and -independent factors influence chronic allograft rejection. *Transplantation*(Baltimore) **59**:313-318, 1995
10) Tullius SG, U Heemann, WW Hancock, H Azuma, NL Tilney : Long-term kidney isografts develop functional and morphologic changes that mimic those of chronic allograft rejection. *Ann Surg* **220**:425-435, 1994
11) Tullius SG, UW Heemann, H Azuma, WW Hancock, P Pradham, NL Tilney : Antigen-independent events mimic characteristic changes of chronic allograft rejection. *Trans Proc* **27**:561-563, 1995
12) Plissonnier D, D Nochy, P Poncet, C Mandet, N Hinglais, J Bariety, J Michel : Sequential immunological targeting of chronic experimental arterial allograft. *Transplantation* **60**:414-424, 1995
13) Sayegh MH, LA Turka : T cell costimulatory pathways : Promising novel targets for immunosuppression and tolerance induction. *J Am Soc Nephrol* **6**:1143-1150, 1995
14) Adams DH, LR Wyner, MJ Karnovsky : Experimental graft arteriosclerosis II. Immunocytochemical analysis of lesion development. *Transplantation*(Baltimore) **56**:794-799, 1993
15) Lin H, SF Bolling, PS Linsley, RQ Wei, D Gordon, CB Thompson, LA Turka : Long-term acceptance of major histocompatibility complex mismatched cardiac allografts induced by CTLA4

Ig plus donor-specific transfusion. *J Exp Med* **178**:1801-1806, 1993
16) Linsley PS, JA Ledbetter : The role of the CD28 receptor during T cell response to antigen. *Annu Rev Immunol* **11**:191-212, 1993
17) Zhao XM, WH First, TK Yeoh, GG Miller : Expression of cytokine genes in human cardiac allografts : Correlation of IL-6 and transforming growth factor-beta (TGF-beta) with histological rejection. *Clin Exp Immunol* **93**:448-451, 1993
18) Linsley PS, JA Ledbetter : The role of CD28 receptor during T cell response to antigen. *Ann Rev Immunol* **11**:191-212, 1993
19) Linsley PS, W Brady, M Urnes, LS Grosmaire, NK Damle, JA Ledbetter : CTLA- 4 is a second receptor for the B cell activation antigen B7. *J Exp Med* **174**:561-569, 1991
20) Sayegh, M. H., E. Akalin, W. W. Hancock, M. E. Russell, C. B. Carpenter, P. S. Linsley, and L. A. Turka : CD28-B7 blockade after alloantigenic challenge in vivo inhibits Th1 cytokines but spares Th2. *J Exp Med* **181**:1869-1874, 1995
21) Lenschow DJ, Y Zeng, JR Thistlethwaite, A Montag, W Brady, MG Gibson, PS Linsley, and JA Bluestone : Long-Term Survival of Xenogeneic Pancreatic Islet Grafts Induced by CTLA-4Ig. *Science* **257**:789-792, 1992
22) Orloff MS, EM Demara, ML Coppage, N Leong, MA Fallon, J Sickel, X Zuo, J Prehn, SC Jordan : Prevention of chronic rejection and graft arteriosclerosis by tolerance induction. *Transplantation* **59**:282-288, 1995
23) Boussiotis VA, JG Gribben, GJ Freeman, LM Nadler : Blockade of the CD28 costimulatory pathway : A means to induce tolerance. *Curr Opin Immunol* **6**:797-807, 1994
24) Pearson TC, DZ alexander, KJ Winn, PS Linsley, RP Lowry, CP Larsen : Transplantation tolerance induced by CTLA4Ig. *Transplantation* **57**:1701-1706, 1994
25) Yin D, CG Fathman : Induction of tolerance to heart allografts in high responder rats by combining anti-CD4 with CTLA4Ig. *J Immunol* **155**:1655-1659, 1995
26) Ramsdell F, Fowlkes BJ : Maintenance of *in vivo* tolerance by persistence of antigen. *Science* **257**: 1130-1134, 1992
27) Someren A, RP Lowry, T Takeuchi, H Cremisi, B Konieczny : Chronic rejection of organ allografts may arise from injuries sustained in recurring foci of acute rejection that resolve spontaneously. *Trans Proc* **25**:2103-2105, 1993
28) Barker CF, RE Billingham : Compsarison of the fates of Ag-B locus compatible homografts of skin and hearts in inbred rats. *Nature* **225**:851, 1970
29) Russell ME, DH Adams, LR Wyner, Y Yamashita, NJ Halnon, MJ Karnovsky : Early and persistent induction of monocyte chemoattractant protein 1 in rat cardiac allografts. *Proc Natl Acad Sci USA* **90**:6086-6090, 1993
30) Akalin E, Chandraker A, Sayegh, MH, LA Turka : Role of the Cd28 : B7 costimulatory interaction in alloimmune response. *Kidney Int* **51** Suppl 58:S1-8, 1997
31) Shin YT, DH Adams, LR Wyner, E Akalin, M H Sayegh, MJ Karnovsky : Intrathymic tolerance in the Lewis-to-F344 chronic cardiac allograft rejection model. *Transplantation* **59**:1647-1653, 1995
32) Qian SW, P Kondaiah, AB Roberts, and MB Sporn : cDNA cloning by PCR of rat transforming growth factor beta-1. *Nucleic Acids Res* **18**:3059, 1990
33) Russell ME, U Utans, AF Wallace, P Liang, RJ Arceci, MJ Karnovsky, LR Wyner, Y Yamashita, and C Tarn : Identification and upregulation of galactose/N-acetylga; actosamine macrophage lectin in rat cardiac allografts with arteriosclerosis. *J Clin Invest* **94**:722-730, 1994
34) June CH, JA Blustone, LM Nadler, CB Thompson : The B7 and CD28 receptor families. *Immunol Today* **15**:321-331, 1994
35) Russell ME, AF Wallace, JB Newell, LR Wyner, MJ Karnovsky : Upregulation and modulation of inducible nitric oxide synthesis in rat cardiac allografts with chronic rejection and transplant arteriosclerosis. *Circulation* **92**:457-464, 1995
36) Watschinger B, MH Sayegh, WW Hancock, ME Russell : Upregulation of endothelin-1 mRNA and peptide expression in rat cardiac allografts with rejection and arterioscleosis. *Am J Pathol* **146**: 1065-1072, 1995
37) Russell ME, WW Hancock, AF Wallace, LR Wyner, MJ Karnovsky : Modulation of inflammatory activation pathways in the Lewis-to-F344 rat chronic cardiac rejection model. *Trans Proc* **220**:425-435, 1995
38) Sharma VK, RM Bologa, G Xu, B Li, J Mouradian, J Wang, D Serur, V Rao, M Suthanthiran : Intragraft TGF-β, mRNA : A correlate of interstitial fibrosis and chronic allograft nephropathy. *Kidney Int* **49**:1297-1303, 1996