Regulation of the Anti-Allograft Response by Targeting the CD2 Antigen: A Potential Strategy for the Creation of Transplant Tolerance

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Activated T cells play a central role in the rejection of histoincompatible organ allografts. Studies of transmembrane signaling requirements of T cells, by identifying molecular and cellular mechanisms of T-cell activation, can lead to rational therapeutic strategies for the regulation of the anti-allograft response. A clear consensus exists that the primary signal for T-cell activation is generated as a consequence of the interactions among the T-cell receptor for antigen (TCR)/cluster designation 3 (CD3) complex and the antigenic peptide presented in the context of major histocompatibility complex (MHC) proteins expressed on the surface of the antigen-presenting cells (APCs).[1-7] The TCR/CD3-dependent signaling is necessary but insufficient in itself to fully activate normal human primary (quiescent) T cells, and additional costimulatory signals are required for full activation.[8-13]

A number of proteins expressed on the T-cell surface and upon the surface of APCs are candidate molecules for the generation of the costimulatory signal obligatory for T-cell activation.[8-17] The costimulatory signal is considered to be generated by specific physical contacts between T-cell surface receptors and their counter receptors displayed on APCs (Fig. 1). The current list of participants in humans include the following: CD2 antigen on the T-cell surface (by interacting with the CD58 [LFA-3] antigen on the APCs); CD4 or CD8 antigen (generates the costimulatory signal by interacting with the monomorphic component of class II or I antigens, respectively, of the MHC); CD11a/CD18 (LFA-1) antigen (by interacting with the

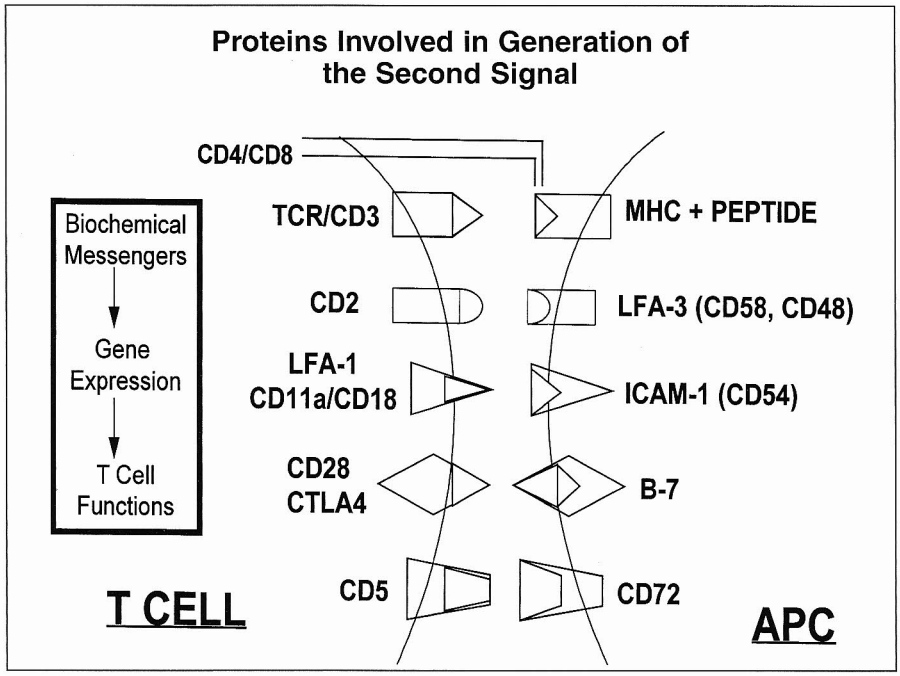

Figure 1. T-cell/antigen presenting cell contact sites. In this schema of T-cell activation, the antigenic signal is initiated by the physical interaction between the clonally variant TCR α1ß chains and the antigenic peptide presented in the context of MHC molecules; the antigenic signal is generated by the TCR/CD3 complex. Additional proteins expressed on the T-cell surface molecules generate the obligatory costimulatory signals by interacting with their counter receptors expressed on the surface of APCs. The simultaneous delivery of the antigenic signal and the costimulatory signal results in the optimum generation of second messengers (e.g., calcium), expression of nuclear transcription factors (e.g., nuclear factor of activated T cells) and T-cell growth-promoting genes (e.g., IL-2). (CD: cluster designation; MHC: major histocompatibility complex; LFA-1: leukocyte function associated antigen-1; ICAM-1: intercellular adhesion molecule-1). (Kidney Intl 1993;S3-S11, with permission.)

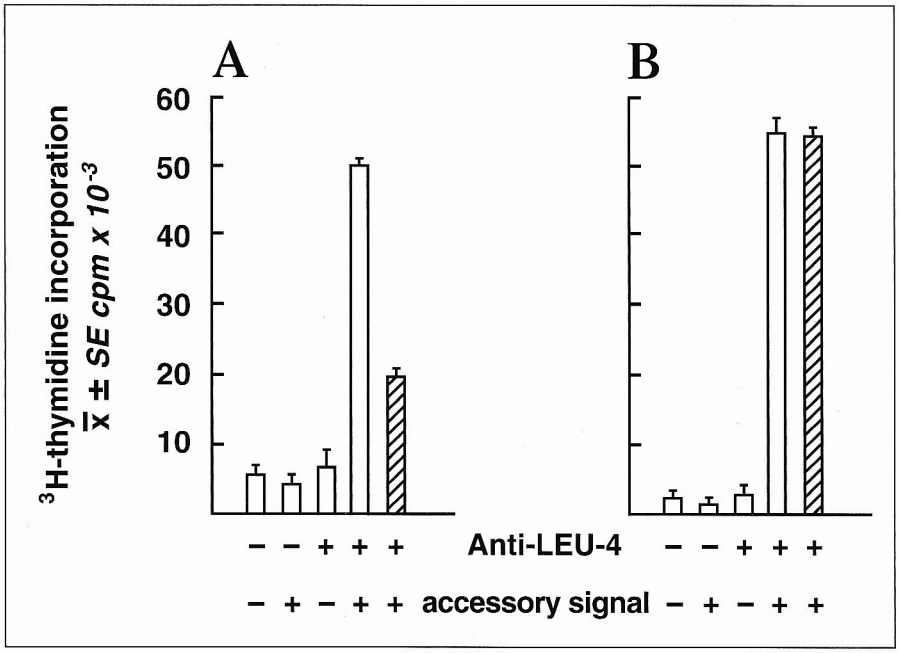

Figure 2. Inhibition of T-cell proliferation by anti-CD2 mAbs is dependent on the nature of the costimulatory signal provided. T-cell proliferation was induced by providing the antigenic signals and the costimulatory signals to highly purified T cells (1 x 10^6 cells/mL). The antigenic signal was provided with anti-Leu-4 (anti-CD3, 0.1 µg/mL) and the costimulatory signal was provided with either paraformaldehyde-fixed B-LCL-8392 (2 x 10^4 cells/mL [A]) or with TPA (0.5 ng/mL [B]). Anti-Leu-5b (anti-CD2, 0.05 µg/mL) was added at the time of initiation of cultures and 3H-thymidine incorporation into DNA during 48-64 hr of culture was determined. Anti-CD2 mAbs mediated significant inhibition of proliferation only when the APCs provided the obligatory costimulatory signal and not when a APC substitute, TPA, provided the needed costimulatory signal. (Cell Immunol 1988;112:112-22, with permission.)

CD54 (ICAM-1) antigen; CD28/CTLA 4 antigen (by interacting with the B7/BB1 antigen); CD5 antigen (by interacting with the CD72 antigen).

Reviewed in this chapter are our primary and previously published data that engendered the hypothesis that the CD2 antigen expressed upon the T-cell surface functions as a receptor for APCs and generates the costimulatory signal obligatory for antigen-dependent T-cell activation, and our preclinical studies demonstrating the ability of an anti-CD2 monoclonal antibody to prolong the survival of murine islet allografts and engender antigen-specific tolerance. The clinical implications of a novel T-cell activation model in which the CD2 antigen-derived signals complement TCR/CD3 complex-derived signals in promoting T-cell activation are also described in this synopsis.

COSTIMULATORY SIGNALING FEATURES OF CD2 ANTIGEN

CD2 Antigen Functions as a Receptor for APCs

Cell-to-cell contact between T cells and APCs are a demonstrated requirement for the effective delivery of the APC-derived costimulatory signals to antigen-specific T cells. We investigated the contribution of the CD2 antigen to the physical clustering among the T cells and the APCs with mAbs directed at the CD2 antigen. In accordance with the hypothesis that the CD2 antigen participates in the physical association between T cells and APCs, anti-CD2 (OKT11, 1.0 mg/mL) mAbs significantly inhibited the physical contact between T cells and the APCs.[8] The inhibitory effect was CD2 antigen-specific since even a 10-fold higher concentration of anti-CD4 mAb (OKT4), anti-CD8 mAb (OKT8), or anti-CD3 mAb (OKT3) mediated only minimal inhibition of the physical contact between T cells and APCs.[8]

CD2 Antigen Generates and/or Transduces APC-derived Signals

The participation of the CD2 antigen in the generation and/or transduction of APC-derived costimulatory signals was examined in our studies by determining the effects of anti-CD2 mAbs on two models of T-cell proliferation distinguished by the nature of the costimulatory signals. In one model, T-cell proliferation was dependent upon the costimulatory signals provided with APCs.[8,9] In the other

T-cell activation model, T-cell proliferation was independent of APCs but dependent upon the costimulatory signal provided with phorbol esters or cytokines. The antigenic signal, in both models of T-cell activation, was identical and was provided with either anti-CD3 mAb 8 or oxidizing mitogens (e.g., sodium periodate, galactose oxidase).[9]

The effect of anti-CD2 mAbs on T-cell proliferation was clearly dependent upon the nature of costimulatory signal provided to the T cells. In accordance with the postulate that the CD2 antigen participates in the transduction and/or generation of APC-derived costimulatory signals, anti-CD2 mAbs (anti-Leu-5b) inhibited T-cell proliferation only when the costimulatory signal was provided with the APCs but not when the costimulatory signal was provided with the mitogen, TPA, a potent APC substitute (Fig. 2).

The effect of natural ligands (MHC antigens) for the T-cell antigen receptor complex (TCR/CD3) can be mimicked with crosslinked monoclonal antibodies directed at the TCR/CD3 complex. If our hypothesis that the CD2 antigen is a receptor for APCs is correct, then we should be able to substitute APCs-derived signals with crosslinked mAbs directed at the CD2 antigen.

Signals initiated with crosslinked anti-CD2 were indeed effective substitutes for the APC-derived costimulatory signals (Table 1).[8-10] Furthermore, whereas CD2 antigen-derived signals substituted for the APC-derived signals, they did not replace the antigenic signal initiated via the TCR/CD3 complex (Table 1).

Table 1. Generation of costimulatory and not the primary antigenic signals with crosslinked anti-CD2 antibodies

	Test Condition		T-Cell Proliferation* (mean cpm/culture)		
	Monoclonal antibodies		Goat anti-mouse γ heavy chain		
Protocol	Anti-CD2	Anti-CD3	—	IgG	+
1	—	—	159	1253	—
	OKT11	—	371	810	—
	—	OKT3	2292	837	—
	OKT11	OKT3	1432	52,616	—
	—	anti-Leu-4	315	344	—
	OKT11	anti-Leu-4	673	39,702	—
	Anti-CD2	**B- LCL**	—		+
2	—	—	183		472
	OKT11	—	1,376		1,540
	—	B-LCL-8392	2,955		1,198
	OKT11	B-LCL-8392	1,540		1,548

T cells were incubated with agents shown and [^{3}H] thymidine incorporation (mean CPM/culture) found during 48-64 hours of culture is shown. Results are from one of four similar experiments. (T cells, 1 X 10^6 cells/mL; anti-CD2 [OKT11] or CD3 [OKT3] antibodies; 0.5 µg/mL; affinity purified goat antibodies specific for mouse γ heavy chain; 10.0 µg/mL; B-LCL-8392; 2 x 10^4 cells/mL.) From: Suthanthiran M. T cell differentiation antigen cluster 2 (CD2) is a receptor for accessory cells and can generate and/or transduce accessory signals. Cell Immunol 1988;112-122. With permission.

Signal Transduction

Ca^{2+} and PKC activation participate in signal transduction in a variety of eukaryotic cells.[18,19] We investigated the role of Ca^{2+} mobilization and PKC activation in the transduction of signals initiated via the TCR/CD3 complex and the CD2 antigen. The contribution of Ca^{2+} to T-cell proliferation was examined with EGTA, a calcium chelator, and that of PKC activation with direct or competitive inhibitors of PKC.[10]

T-cell proliferation induced by signaling via the TCR/CD3 complex and the CD2 antigen was inhibited by EGTA; dose-response experiments revealed that the half-maximal inhibitory concentration (IC_{50}) was 0.28 mM. The compound H-7, a derivative of isoquinolinesulfonamide and a competitive inhibitor of PKC, also inhibited T-cell proliferation, with an IC_{50} of 0.003 mM. Staurosporine, a microbial alkaloid and a direct inhibitor of PKC also mediated marked inhibition with an IC_{50} of 0.003 mM. An additional compound, K-252a, a competitive inhibitor of PKC whose mechanism of action is similar to that of compound H-7 but with greater potency, inhibited T-cell proliferation induced by crosslinkage of anti-TCR-1 with OKT11 and the IC_{50} for K-252a was 0.05 mM.

Our studies on the mechanisms for signal transduction following complimentary signaling via the TCR/CD3 complex and the CD2 antigen indicate that a sustained Ca^{2+} mobilization pattern is a prerequisite for full T-cell activation and that transmembrane signaling T cells via the TCR/CD3 complex and the CD2 antigen is homologous to physiological antigenic signaling with respect to utilization of Ca^{2+} mobilization and PKC activation for signal transduction.

Molecular Mechanisms

We have determined the basis for the complementary nature of signals initiated via the TCR/CD3 complex and the CD2 antigen with the aid of two molecular techniques: electrophoretic mobility shift assay[11] and reverse transcription-assisted polymerase chain reaction.[12]

The emergence of transcription factors nuclear factor kappa B (NF-kB), activator protein-1 (AP-1), and nuclear factor of activated T cells (NF-AT), following signaling T cells via the TCR/CD3 complex and/or the CD2 antigen, is shown in Table 2. It is evident that these DNA-regulatory proteins are expressed best in T cells signaled simultaneously via the TCR/CD3 complex and the CD2 antigen as compared with T cells stimulated either via the TCR/CD3 complex alone or the CD2 antigen alone.

The inducibility of mRNA encoding interleukin-2 (IL-2) and IL-2 receptors α and β following stimulation of T cells via the TCR/CD3 complex and/or the CD2 antigen are also shown in Table 2. It is clear that the best expression of IL-2, IL-2 receptor α and IL-2 receptor β mRNA is

Table 2. Signaling efficacy of CD3 and/or CD2 antigens

Experimental condition	NF-kB[c]	AP-1[c]	NF-AT1[c]	IL-2[c]	IL-2Ra[c]	IL-2Rb[c]
T cells + none	0.14	0.50	0.05	UD	UD	0.02
T cells + anti-CD2 mAb	0.31	0.59	0.06	UD	0.02	0.03
T cells + anti-CD3 mAb	0.82	0.59	0.06	UD	0.04	0.03
T cells + anti-CD2 mAb + anti-CD3 mAb	4.42	2.02	0.21	0.03	0.14	0.05
T cells + anti-CD2 mAb + anti-CD3 mAb + CsA	0.32	0.62	0.05	UD	0.06	0.03

*T-cells (1 x 10^6 cells/mL) were incubated alone or with anti-CD2 mAb (0.5 mg/mL), anti-CD3 mAb (0.5 mg/mL), or anti-CD2 mAb + anti-CD3 mAb prior to the isolation of total cellular RNA or the nuclear extracts. (The mAbs were crosslinked using 5.0 mg/mL of goat anti-mouse IgG antibodies). (Transplantation Proceedings 1993;25:104-105. With permission.)

c: The absolute absorbance values were determined by laser densitometric scanning of negatives of photographs of the gels.

UD: undetected.

Table 3. Induction of antigen-specific tolerance with RM2-2 anti-CD2 mAb.

Group	Islet Cell Graft Survival	MST ± SD
Third party donor (SJL)	12, 12, 18	14 ± 2
Same party donor (DBA/2)	24, >35, >35, >35, >35 >100, >100	52 ± 12*

$B6AF_1$ recipient mice with DBA/2 islet allografts functioning greater than 120 days following initial treatment with RM2-2 anti-CD2 mAb had their islet allograft bearing kidney removed. The removal of functioning islet allografts resulted in the return of hyperglycemia. The mice then underwent retransplantation with islets either from DBA/2 mice (original donor strain) or from SJL mice (third party donor). Graft survival was determined by monitoring of blood glucose.

MST, mean survival time.

* $p<0.0167$ when compared to third party donor islet allograft (Wilcoxon)

found in T cells signaled simultaneously via the TCR/CD3 complex and the CD2 antigen.

Summary of In vitro Studies

Our in vitro studies demonstrated the following: (1) the CD2 antigen participates in the physical contact between T cells and APCs; (2) CD2 antigen generates and/or transduces costimulatory signals obligatory for full T-cell activation; (3) the synergism between TCR/CD3 complex-derived signals and the CD2 antigen-derived signals is demonstrable at the level of emergence of transcription factors and induced expression of genes important for T-cell proliferation; and (4) transmembrane signals of T cells via the TCR/CD3 complex and the CD2 antigen is homologous to physiologic signaling with respect to calcium dependency and PKC activation.

REGULATION OF THE ANTI-ALLOGRAFT RESPONSE BY TARGETING THE CD2 ANTIGEN

We have tested the hypothesis that (1) CD2 antigen participates in the anti-allograft rejection response, and (2) blockade of CD2 antigen-derived signals will result in engraftment. These hypotheses were tested in a murine pancreatic islet cell allograft model using RM2-2 anti-CD2 mAbs to block CD2 antigen-dependent anti-allograft responses. Herein, we summarize our preclinical data demonstrating the ability of mAbs directed at the CD2 antigen to (a) facilitate murine islet cell engraftment and (b) engender antigen-specific transplantation tolerance. Moreover, our data regarding the molecular mechanisms for the efficacy of anti-CD2 mAb therapy are provided.[32]

Prolongation of Murine Pancreatic Islet Cell Allograft Survival with RM2-2 Anti-CD2 mAb

Allogenic pancreatic islet cell graft transplantation was performed using DBA/2 mice and $B6AF_1$ mice as islet cell donors and recipients, respectively. RM2-2 anti-CD2 mAb was administered, 100 µg/day, on day -1, 0, and +1 with respect to transplantation. Islet graft recipients treated with RM2-2 anti-CD2 mAb displayed significantly longer allograft survival times compared to the untreated group; the mean ± SE survival time for the RM2-2 anti-CD2 mAb treated group was 97 ± 16 days (n=21 recipients) and the mean ± SE survival time of the untreated group was 27 ± 9 days (n=19 recipients). The cumulative survival rates (CSR) of anti-CD2 mAb treated group and the control group were calculated by the product limit (Kaplan-Meier) method, and are illustrated in Figure 3. The difference in the CSR between the two groups was highly significant ($p=0.0000$) by generalized Wilcoxon (Breslow) statistics.

The superior survival time for islet cell allografts in recipients treated with the RM2-2 anti-CD2 mAb was due to targeting of the CD2 antigen rather than due to some nonspecific effects of mAb, since an isotype-matched rat IgG2a mAb did not prolong the survival of DBA/2 islet grafts in $B6AF_1$ recipients. Peritransplant administration of a rat IgG2a mAb (100 µg/day) on day -1, 0 and +1 with respect to transplantation, was associated with a survival time of only 14 ± 2 days (mean ± SEM, n=5 recipients).

Emergence of Antigen-Specific Tolerance

To determine if antigen-specific tolerance had been achieved by the peritransplant administration of RM2-2 anti-CD2 mAb, we removed the islet allograft-bearing kidney and retransplanted the mice with islets from either the original donor strain (DBA/2) or with islets from a third

party donor strain (SJL). None of the retransplanted animals received any additional RM2-2 anti-CD2 mAb treatments. Table 3 shows that six out of seven mice retransplanted with DBA/2 islets did not reject their allografts and remained normoglycemic. In contrast, mice retransplanted with third party donor islets rejected their allografts and became hyperglycemic by 18 days post-transplantation.

Histological Confirmation of Anti-CD2 Targeted Therapy

Tissue samples, from islet allograft recipients treated with a peri-transplant course of anti-CD2 mAb and samples from untreated recipient mice, were analyzed histologically for the viability of transplanted islet cell allografts and for the intensity of mononuclear cell infiltration. Recipients in both the control and treated groups were normoglycemic at the time of graft harvest. As illustrated in Figure 4, panels A vs. B, a clear histological difference was observed between the untreated control group and the anti-CD2 mAb–treated group. Whereas viable islet cells arranged in organized patterns with good vascularization and without much mononuclear cell infiltrates were observed in the anti-CD2–treated recipients, the islet allografts from the untreated recipients had marked pleomorphic mononuclear cell infiltrates and islet graft degeneration, and necrosis.

Reduction of Intragraft Expression of mRNA-Encoding Granzyme B or IL-10 Following RM2-2 Anti-CD2 mAb Therapy

Cytotoxic T-cell–specific serine protease, granzyme B, is a molecular correlate of allograft rejection.[20-22] Data also exist that intragraft expression of IL-10 mRNA is a significant correlate of allograft rejection[23] and that IL-10–producing T cells are abundant in irreversibly rejected allografts.[24] To investigate potential molecular mechanisms for the efficacy of RM2-2 anti-CD2 mAb treatment, we determined whether intragraft expression of mRNA-encoding granzyme B and IL-10 mRNA is reduced by CD2 antigen-targeted therapy. On day 8 post-transplantation, tissue samples were obtained from RM2-2 anti-CD2 mAb–treated mice and from untreated mice. Day 8 was selected in view of an earlier demonstration that intragraft mRNA expression peaks at day 8 post-transplantation in the unmodified islet allograft recipient.[20]

Reverse transcriptase-polymerase chain reaction revealed significant differences between the RM2-2 anti-CD2 mAbs–treated recipients and the control recipients. Agarose gel electrophoretic resolution of the granzyme B PCR products and the IL-10 PCR products, generated with the cDNA from the RM2-2 anti-CD2 mAbs–treated mice and with cDNA from untreated mice, are shown in Figure 5, panel A, and the absolute (mean ± SD) absorbance values, determined by laser densitometry, are shown in Figure 5, panel B. It is evident that granzyme B mRNA and IL-10 mRNA are expressed at a higher level in the untreated recipients as compared to recipients treated with the RM2-2 anti-CD2 mAbs. The mean ± SD absolute absorbance value for granzyme B mRNA was 0.009 ± 0.004 (n=4 mice) in the RM2-2 anti-CD2 mAbs–treated group and 0.025 ± 0.001 (n=4) in the untreated

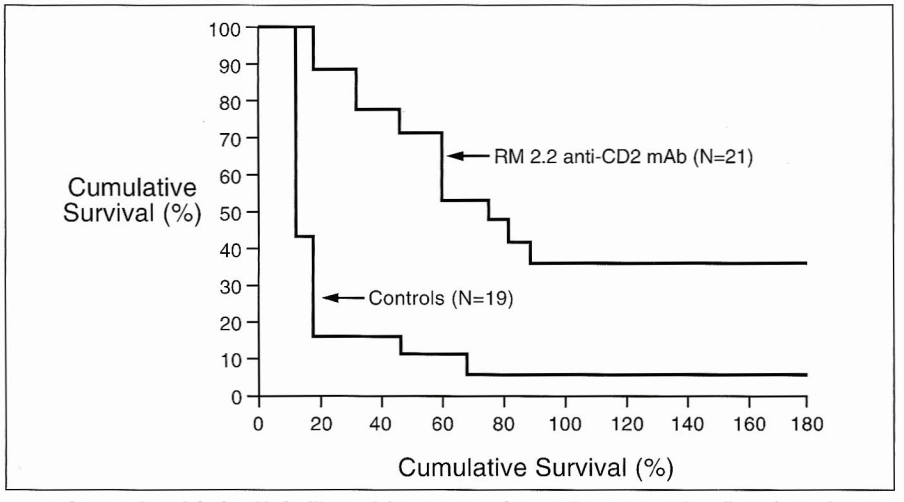

Figure 3. RM2-2 anti-CD2 mAb facilitates islet cell engraftment. Pancreatic islet allograft survival distributions were estimated by the product-limit (Kaplan-Meier) method and the equality of the survival curves tested by the generalized Wilcoxon statistic. The difference in the survival times between the RM2-2 anti-CD2 mAb group (N=21 recipients) and the control group (N=19 recipients) was significant at p=0.0000. (Kapur S, et al. Transplantation. Manuscript in press.)

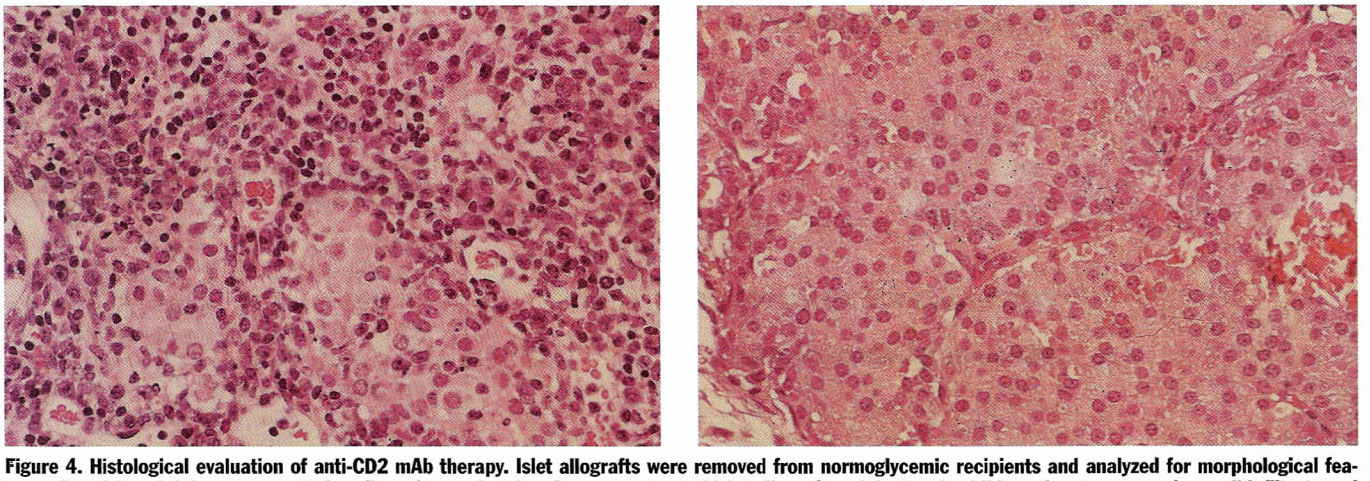

Figure 4. Histological evaluation of anti-CD2 mAb therapy. Islet allografts were removed from normoglycemic recipients and analyzed for morphological features. Panel A on left is a representative allograft sample taken from an untreated islet allograft recipient and exhibits a dense mononuclear cell infiltrate and islet allograft degeneration and necrosis. Panel B on right is a representative allograft sample from an anti-CD2 mAb–treated recipient and exhibits viable islet cells without cellular infiltration.

Figure 5. Reduction in intragraft expression of granzyme B mRNA and IL-10 mRNA following RM2-2 anti-CD2 mAb treatment. Islet cell allografts were retrieved 8 days post-transplant from untreated or RM2-2 anti-CD2 mAb-treated mice and analyzed by RT-PCR for mRNA encoding granzyme B, IL-10 or b-actin (n=4 mice from each group). Agarose gel electrophoretic resolution of granzyme B, IL-10, and b-actin PCR products are shown in Figure 5a. Note the reduced expression of granzyme B mRNA in all 4 anti-CD2 mAb treated mice, and the reduced expression of IL-10 mRNA in 3 of 4 treated mice. In Figure 5b, absorbance values, determined by laser densitometric scanning of the negatives of the photographs of the gels, are shown. Results are expressed as a mean ±SD (n=4) of the untreated or RM2-2 anti-CD2 mAb–treated mice. Statistical significance was determined by unpaired t-test. (Kapur S, et al. Transplantation. Manuscript in press.)

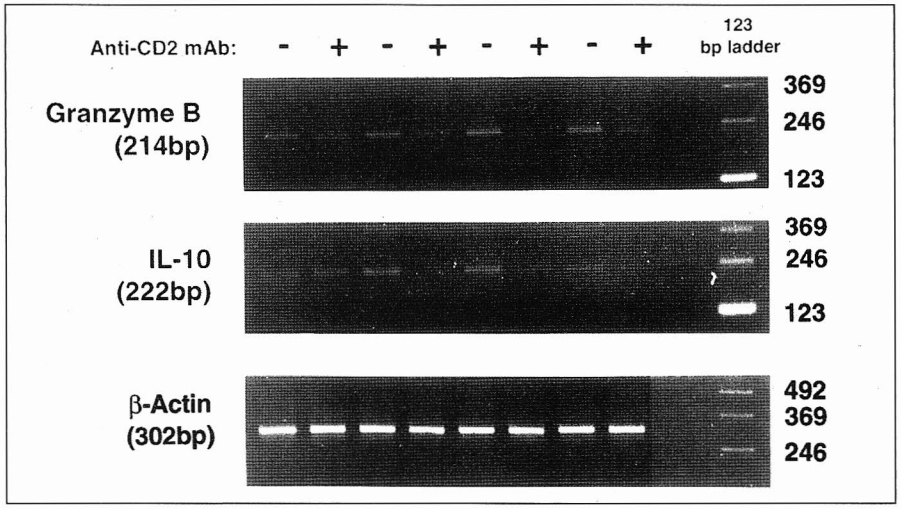

Figure 5a.

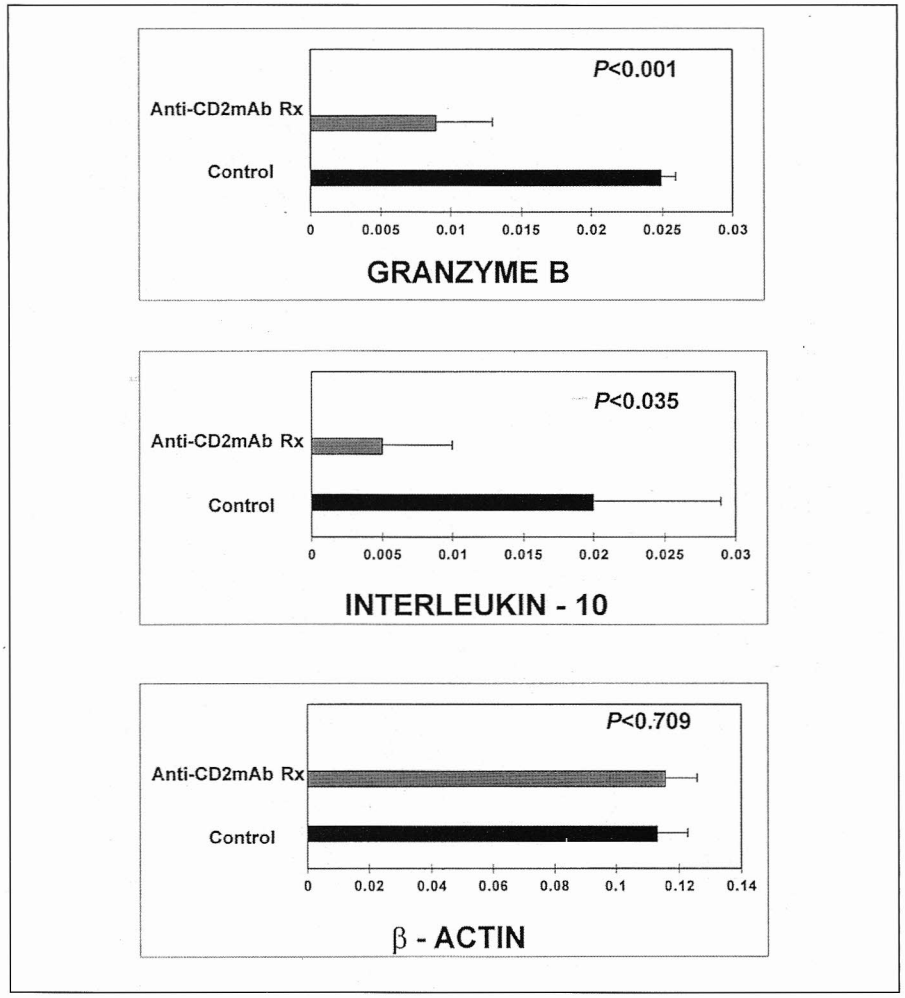

Figure 5b.

group ($p<0.001$, unpaired t-test). The mean ± SD absolute absorbance value for IL-10 mRNA was 0.005 ± 0.005 in the RM2-2 anti-CD2 mAb–treated group and 0.020 ± 0.009 in the untreated group ($p=0.035$).

The significant reduction in the intensity of induced expression of granzyme B mRNA and IL-10 mRNA following RM2-2 anti-CD2 mAb therapy was gene-specific, since the level of expression of a constitutionally expressed gene, b-actin mRNA, was quite similar in the RM2-2 anti-CD2 mAb–treated group and the untreated groups (Fig. 5).

A NEW T-CELL ACTIVATION MODEL

A unifying theme has emerged in which T cells view antigens in the context of MHC gene products expressed on antigen-presenting cells. T-cell surface proteins, in addition to the TCR, that contribute to the informative T-cell–APC interactions, however, have remained an enigma despite the paradigm that the CD4 and CD8 molecules function as associative recognition/restriction elements for the MHC gene products expressed on the surface of APCs.

On the basis of our studies, we have advanced the thesis that instructive T-cell–APC interaction proceeds not only via molecular contacts between the TCR and the MHC-peptide but also via the CD2 antigen and the LFA-3 and/or other sites on the APCs (step 1). These cell-surface molecular interactions, then, lead to the coclustering of the TCR with the CD2 antigen (step 2). The antigen- and APC-induced association between the TCR and the CD2 antigen is necessary for the optimum generation of second messenger molecules (Ca^{2+}, DAG, etc.) and transcriptional activation and translation of genes (IL-2, IL-2 receptor, etc.) responsible for the antigen-dependent clonal expansion of normal human T cells (step 3).

CLINICAL IMPLICATIONS

The formulation that T-cell surface molecules in addition to the TCR/CD3 complex need to be engaged for full T-cell activation offers new opportunities for the regulation of the anti-allograft response and for the induction of T-cell clonal anergy. Our studies, by identifying a critical role for the CD2 antigen in antigen-dependent T-cell activation, suggest the hypothesis that the CD2 antigen might be targeted for the regulation of the anti-allo-

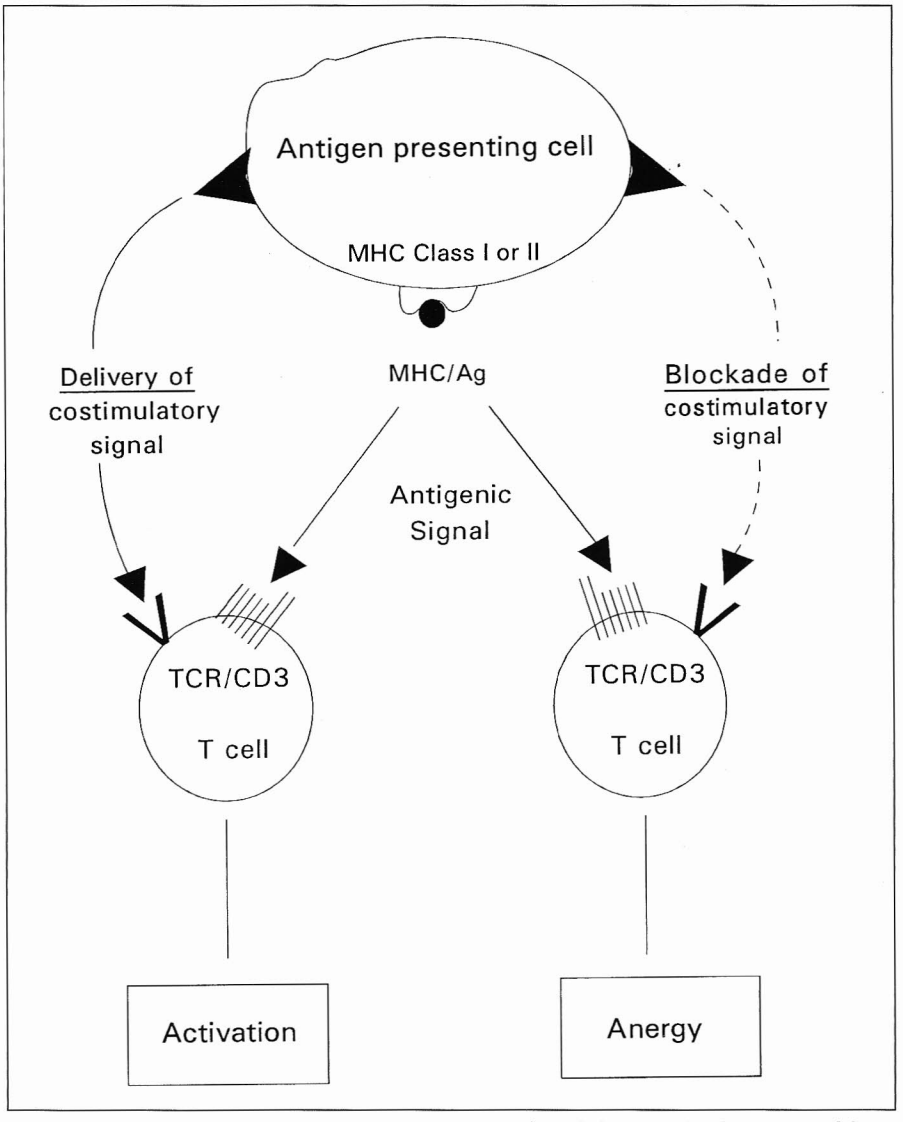

Figure 6. T-cell activation/anergy pathways. In this schema of T-cell signaling, the simultaneous delivery of the antigenic signal and the costimulatory signals results in T-cell activation; the delivery of the antigenic signal in the absence of costimulatory signals to the T cells results in T-cell paralysis/clonal anergy.[25,26] (Kidney Intl 1993;44:S3-S11, with permission.)

graft response. Furthermore, transplantation tolerance might also be accomplished by CD2 antigen-targeted therapy since the delivery of the antigenic signal to the T cells in the absence of the APC-derived costimulatory signals has been demonstrated to result in T-cell clonal anergy[25,26] (Fig. 6). Additional molecules expressed on the T cells and/or the APCs (Fig. 1) might also be suitable targets for the creation of donor-specific non-responsiveness in the clinical setting. Indeed, tolerance to organ allografts has been induced by therapy directed at the cell-surface molecules, LFA-1 and ICAM-1[27] or B7/BB1[28] in experimental models of transplantation. Our preclinical data demonstrating the efficacy of mAbs directed at the CD2 antigen, as well as elegant studies of others,[29-31] provide the experimental underpinnings for the consideration of CD2 antigen-targeted therapy in the clinic.

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REFERENCES

1. Schwartz RH. T-lymphocyte recognition of antigen in association with gene products of the major histocompatibility complex. Ann Rev Immunol 1985;3:237-61.
2. Weiss A, Imboden J Hardy K, et al. The role of the antigen receptor complex in T-cell activation. Annu Rev Immunol 1986;4:593-619.
3. Clevers H, Alarcon B, Wileman T, et al. The T-cell receptor/CD3 complex: a dynamic protein ensemble. Annu Rev Immunol 1988; 6: 629-62.
4. Bjorkman PJ, Saper MA, Samraoui B, et al. The foreign antigen binding site and T-cell recognition regions of class I histocompatibility antigens. Nature 1987;329:512-8.
5. Davis MM, Bjorkman PJ. T-cell antigen receptor genes and T-cell recognition. Nature 1988;334:395-402.
6. Townsend A, Bodmer H. Antigen recognition by class I-restricted T lymphocytes. Ann Rev Immunol 1989;7:601-624.
7. Unanue ER, Cerottoni J-C. Antigen presentation. FASEB 1989;3:2496-502.
8. Suthanthiran M. T-cell differentiation antigen cluster 2 (CD2) is a receptor for accessory cells and can generate and/or transduce accessory signals. Cell Immunol 1988;112:112-22.
9. Suthanthiran M. Oxidative mitogenesis: participation of CD2 antigen in the generation and/or transduction of obligatory accessory signals. Cellular Immunology 1988;114:117-25.
10. Suthanthiran M. A novel model for antigen-dependent activation of normal human T cells. Transmembrane signaling by crosslinkage of the CD3/T-cell receptor-a/b complex with the cluster determinant 2 antigen. J Exp Med 1990;171:1965-79.
11. Sehajpal PK, Sharma VK, Ingulli E, et al. Synergism between the CD3 antigen and CD2 derived signals: exploration at the level of induction of DNA binding proteins and characterization of the inhibitory activity of cyclosporine. Transplantation 1993;55:1118-24.
12. Sehajpal PK, Li B, Zanker B, et al. The molecular basis for the synergism between the CD3/ab T-cell receptor and the CD2 antigen-derived signals in promoting T-cell proliferation. Transplantation 1991;51:468-74.
13. Sehajpal PK, Sharma VK, Stenzel KH, et al. Two signal model for T-cell activation: molecular mechanisms. Transplantation Proceedings 1993;25:104-5.
14. Springer TA, Dustin ML, Kishimoto TK, et al. The lymphocyte function-associated LFA-1, CD2, and LFA-3 molecules: Cell adhesion receptors of the immune system. Ann Rev Immunol 1987;5:223-52.
15. Linsley PS, Clark EA, Ledbetter JA. T-cell antigen CD28 mediates adhesion with B cells by interacting with activation antigen B7/BB-1. Proc Natl Acad Sci 1990;87:5031-5035.
16. Linsley PS, Brady W, Urnes M, et al. CTLA-4 is a second receptor for the B cell activation antigen B7. J Exp Med 1991;174:561-9.
17. DeFranco AL. Between B cells and T cells. Nature 1991;351:603-4.
18. Nishizuka Y: Intracellular signaling by hydrolysis of phospholipids and activation of protein kinase C. Science 1992;258:607-614.
19. Subramaniam A, Sehajpal P, Murthi VK, et al. Activation of human T cells with the physiologic regulatory of protein kinase C. Cell Immunol 1988;116:439-49.
20. O'Connell PJ, Pacheco-Silva A, Nickerson PW, et al. Unmodified pancreatic islet allograft rejection results in the preferential expression of certain T cell activation transcripts. J Immunol 1993;150:1093.

21. Steiger J, Nickerson PW, Steurer W, et al. IL-2 knockout recipient mice reject islet cell allografts. J Immunol 1995;155:489-98.
22. Lipman ML, Stevens AC, Strom TB. Heightened intragraft cytotoxic T lymphocyte gene expression in acutely rejecting renal allografts. J Immunol 1994;152:5120-7.
23. Xu G-P, Sharma VK, Li B, et al. Intragraft expression of IL-10 messenger RNA: a novel correlate of renal allograft rejection. Kidney Int 1995;48:1504-7.
24. Merville P, Lambert C, Durand I, et al. High frequency of IL-10-secreting CD4+ graft-infiltrating T lymphocytes in promptly rejected kidney allografts. Transplantation 1995;59:1113-9.
25. Jenkins MK, Schwartz RH. Antigen presentation by chemically modified splenocytes induces antigen-specific T-cell unresponsiveness in vitro and in vivo. J Exp Med 1987;165:302-19.
26. Schwartz RH. A cell culture model for T lymphocyte clonal anergy. Science 1990; 248: 1349-56.
27. Isobe M, Yagita H, Okumura K, et al. Specific acceptance of cardiac allograft after treatment with antibodies to ICAM-1 and LFA-1. Science 1992;255:1125-7.
28. Lenschow J, Zeng Y, Thistlethwaite R, et al. Long-term survival of xenogeneic pancreatic islet grafts induced by CTLA4Ig. Science 1992; 257:789-92.
29. Bromberg JS, Chavin KD, Altevogt P, et al. Anti-CD2 monoclonal antibodies alter cell-mediated immunity in vivo. Transplantation 1991;51:219-25.
30. Chavin KD, Qin L, Woodward JE, et al. Anti-CD2 monoclonal antibodies synergize with FK506 but not with cyclosporine or rapamycin to induce tolerance. Transplantation 1994;57:736-40.
31. Chavin KD, Qin L, Lin J, et al. Anti-CD2 and anti-CD3 monoclonal antibodies synergize to prolong allograft survival with decreased side effects. Transplantation 1993;55:901-8.
32. Kapur et al. Transplantation. In press.