

## **CD4<sup>+</sup> but not CD8<sup>+</sup> Cells Are Essential for Allorejection**

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### **Summary**

The generation of knockout mice with targeted gene disruption has provided a valuable tool for studying the immune response. Here we describe the use of CD4 and CD8 knockout mice to examine the role of CD4<sup>+</sup> and CD8<sup>+</sup> cells in initiating allotransplantation rejection. Pre-treatment with a brief course of depletive anti-CD4 monoclonal antibody therapy allowed permanent survival of heart, but not skin, allografts transplanted across a major histocompatibility barrier. However, skin as well as heart grafts were permanently accepted in the CD4 knockout mice. Transfer of CD4<sup>+</sup> cells into CD4 knockout recipient mice 1 d before skin engraftment reconstituted rejection, demonstrating that CD4<sup>+</sup> cells are necessary for initiating rejection of allogeneic transplants. Major histocompatibility complex disparate heart and skin allografts transplanted into CD8 knockout recipients were rejected within 10 d. This study demonstrates that CD4<sup>+</sup> but not CD8<sup>+</sup> T cells are absolutely required to initiate allograft rejection.

The relative importance of the CD4<sup>+</sup> and CD8<sup>+</sup> T cell subpopulations in mediating transplant rejection remains controversial. In certain settings, CD8<sup>+</sup> cells alone seem to be able to initiate allorejection (1). However, CD4<sup>+</sup> T cells have been shown to play a central role in transplantation rejection (2–6). Whether CD4<sup>+</sup> T cells are absolutely required to initiate allograft rejection has been the subject of a dispute. Naive CD4<sup>+</sup> and/or CD8<sup>+</sup> subpopulations have been adoptively transferred into immunoincompetent nude mice to assess their ability to reject skin allografts (7). This study indicated that skin allograft rejection was mediated by collaborations *in vivo* between T inducer and effector cells, and that two interacting T cell subsets can express different phenotypes as well as different antigen specificities. These experiments established the criterion that rejection requires two T cell subpopulations: one providing help, the other cytotoxic effector function. In certain mouse strains (e.g., B6 and B10), MHC class I-reactive CD8<sup>+</sup> cells can be activated *in vitro* independent of MHC class II-reactive CD4<sup>+</sup> cells (7–9). CD8<sup>+</sup> cells have also been shown to be the only subset effective in restoring rejection of MHC class I incompatible grafts (8, 10), and skin grafts from strains with isolated MHC class I mutations (7, 9). However, these investigators eliminated T cell subsets by treatment with specific mAbs *in vivo* to deplete either CD4<sup>+</sup> or CD8<sup>+</sup> T cells, and it was possible that reconstituted nude mice contained T cells derived either from the nude host animal or, more likely, from contaminating T cells in the reconstituting T cell population. It has recently been shown that, despite marked depletion of CD8<sup>+</sup> T cells after treatment with anti-CD8 mAbs *in vivo*, a unique subpopulation of CD8<sup>+</sup> cells remained which re-

jected MHC class I disparate skin grafts and generated allospecific CTL responses (11). Contamination of “purified” T cell subpopulations has also been shown to occur in an adoptive transfer study using mAbs to negatively select purified T cell subpopulations to determine the relative contributions of CD4<sup>+</sup> and CD8<sup>+</sup> cells from diabetic mice into NOD-scid mice, where purified donor CD4<sup>+</sup> populations revealed <2.5% contaminating CD8<sup>+</sup> T cells (12).

Targeting the CD4 or CD8 molecule with mAb to eliminate or inactivate CD4<sup>+</sup> or CD8<sup>+</sup> T cells has been a promising strategy for the induction of transplantation tolerance. Depleting regimens of anti-CD4 mAbs have been shown to induce long-term survival (tolerance) of pancreatic islet (13) and vascularized heart allografts (14–17), but only delay skin graft (18, 19) survival in rodents. A variety of mechanisms for anti-CD4-induced tolerance have been suggested (14, 20–23).

Anti-CD8 mAb therapy, on the other hand, has had variable results. Although nondepleting anti-CD8 therapy has been shown to induce permanent survival of vascularized heart allografts in mice (16, 24), mice treated with depleting anti-CD8 rejected their allografts (16). Anti-CD8 therapy also did not prolong heart or islet allograft survival in rats (25, 26), nor did it prolong skin graft survival in mice (19, 27). However, anti-CD8 combined with anti-CD4 treatment has been shown to prolong islet (28), bone marrow (19, 27), skin (27), and vascularized heart (16) graft survival.

Although using anti-CD4 or anti-CD8 mAbs is one strategy for studying the induction of tolerance, the interaction between the mAb and the target molecule could induce multiple immunological phenomena. Not all CD4<sup>+</sup>

or CD8<sup>+</sup> cells are depleted in studies using depleting anti-CD4 or anti-CD8 therapy. Thus, the use of depleting anti-CD4 or anti-CD8 mAbs does not exclude the possibility that signals generated as a result of the interaction between the antibody and the target molecule on residual CD4<sup>+</sup> or CD8<sup>+</sup> cells are involved in unresponsiveness (29). Additionally, nondepleting anti-CD4 or anti-CD8 mAbs may potentially affect CD4<sup>+</sup> or CD8<sup>+</sup> T cell function by direct blockade, by transmitting a negative signal, or by interfering with normal signal transduction mechanisms.

To avoid inherent questions regarding the efficacy versus mechanisms of anti-CD4 or anti-CD8 induced tolerance, we studied allotransplantation in CD4 and CD8 knockout mice that were generated using homologous recombination in pluripotent embryonic stem cells (30,31). Although it has previously been shown that skin allografts from mice lacking either class I ( $\beta$ 2-microglobulin or TAP1 and  $\beta$ 2-microglobulin), class II (A $\beta$ 3) or both class I and class II ( $\beta$ 2-microglobulin and A $\beta$ 3) are rejected (32, 33), these mice contain a small number of CD4<sup>+</sup> and/or CD8<sup>+</sup> T cells. We expanded upon these studies by directly testing the hypothesis that the complete absence of CD4<sup>+</sup> (or CD8<sup>+</sup> cells) would block the initiation of all rejection and consequently allow the indefinite survival of allografts. Here we report that rejection can occur in the absence of CD8<sup>+</sup> cells, and that CD4<sup>+</sup> cells are required for all rejection.

## Materials and Methods

**Mice.** Inbred male C57BL/6 (H-2b, B6), BALB/c (H-2d), and A/J (H-2a) mice were purchased from The Jackson Laboratory (Bar Harbor, ME); BALB/c CD4 knockout and BALB/c or PL/J(H2u) CD8 knockout mice (homologous for disrupted CD4 or CD8 gene as previously described [30, 31]) were the generous gift of Dr. Tak Mak (University of Toronto). The animals were housed and bred in pathogen-free conditions in the Stanford Department of Laboratory Animal Medicine (DLAM) according to the National Institutes of Health guidelines.

**mAb and Immunosuppression.** The mAb used in these studies, GK1.5 (CD4), is a rat immunoglobulin (IgG2b) directed against mouse L3T4 (34). GK1.5 was produced from ascites in nude mice primed with pristane (Sigma Chemical Co., St. Louis, MO) followed by intraperitoneal inoculation of GK1.5 hybridoma cells. The antibody was purified via passage over an affinity-gel protein A column. Antibody content was quantified by an optical density spectrometer (DU 640; Beckman Instruments, Inc., Fullerton, CA) and quantitated by FACS<sup>®</sup> analysis and depletion studies in vivo. The supernatant was passed through a 0.22- $\mu$ m filter (Millipore Corp., Bedford, MA) before being stored at -20°C. 5 mg/kg of antibody was administered at -3, -2, -1, and 0 d relative to allograft transplantation.

**Heterotopic Heart Transplantation.** Vascularized heart grafts were transplanted using standard microsurgical techniques (35). Briefly, the harvested donor heart was placed in 4°C saline until transplantation. The mouse was anesthetized by intraperitoneal injection of Nembutal (50 mg/kg). The donor aorta was sutured to the recipient aorta and the donor pulmonary artery to the recipient inferior vena cava end to side using 10-0 suture. Transplant

**Table 1.** Anti-CD4 mAb (GK1.5) Allows Heart but not Skin Allograft Survival

GK1.5	Allograft	Survival	MST $\pm$ SEM
		<i>d</i>	
+	Heart	60, 90, >100 $\times$ 6	93.8 $\pm$ 5.0*
-	Heart	6, 7 $\times$ 3, 8, 9	7.3 $\pm$ 0.4
+	Skin	8, 10, 11, 11, 11	10.2 $\pm$ 0.6
-	Skin	7, 8 $\times$ 5	7.8 $\pm$ 0.2

Pretreatment with a brief course of GK1.5 allowed long-term survival of A/J (H-2a) heart, but not skin allografts in C57BL/6 (H-2b, B6) recipients. 5 mg/kg of antibody GK1.5 was administered at -3, -2, -1, and 0 d relative to allograft transplantation.

\*  $P < 0.002$ ; Mann-Whitney U test.

function was evaluated by daily abdominal palpation. Cessation of palpable heartbeat was used to determine the end point of rejection.

**Skin Grafts.** Skin allografts taken from donor chest skin were grafted onto the flank of the recipients with a running 6-0 suture using the uncovered skin graft technique (36). Using this method, the skin graft was visible from the day of engraftment until rejection was complete, and mice were not burdened by circumferential body dressings. Skin graft changes of shrinkage and black coloration were defined as the time of rejection.

**MINIMACS Purification of CD4<sup>+</sup> Cells.** Single cell suspensions of freshly isolated spleen and LN cells from naive BALB/c mice were counted and incubated with anti-CD4 magnetic microbeads (Miltenyi Biotec, Auburn, CA) for 20 min on ice, washed, and purified by passage through magnetic flow columns. The eluent gave a population of 90% CD4<sup>+</sup> cells by FACS<sup>®</sup> analysis (data not shown).  $5 \times 10^7$  CD4<sup>+</sup> cells were then inoculated intraperitoneally into each CD4 knockout mouse.

## Results and Discussion

**Skin but not Heart Allografts Are Rejected in anti-CD4 mAb-Treated Mice.** Mice treated with anti-CD4 mAb accepted heart but not skin allografts (Table 1). B6 mice that received a brief course of anti-CD4 showed long-term survival of A/J heart allografts (mean survival time [MST], 94 d). Skin allograft survival was not prolonged in the anti-CD4 treated recipients compared with untreated controls (MST, 10 and 8 d, respectively). Other investigators have previously shown that heart but not skin allografts were permanently accepted in mice treated with a short course of anti-CD4 therapy (16, 17, 19).

Why are skin allografts rejected in the anti-CD4-treated mice? Generally, skin allografts induce stronger allospecific cellular immunity than heart allografts (1). It has been demonstrated in the anti-CD4-treated mice that "memory" T cells persist despite depletion of peripheral CD4<sup>+</sup> cells (23, 37). These residual CD4<sup>+</sup> T cells (resistant memory cells) may mediate the induction of graft rejection in response to highly immunogenic antigens present in skin grafts. Differ-

**Table 2.** CD4 Knockout Mice Retain Skin and Heart Allografts Indefinitely

Strain combination	Allograft	Survival	MST ± SEM
		<i>d</i>	
B6 to CD4 KO BALB/c	Heart	>100 × 8	100.0 ± 0.0*
B6 to BALB/c	Heart	7, 8, 8, 9, 10	8.4 ± 0.5
B6 to CD4 KO BALB/c	Skin	>100 × 8	100.0 ± 0.0*
B6 to BALB/c	Skin	8, 8, 9, 10, 10	9.0 ± 0.4

BALB/c CD4 knockout (KO) recipients permanently accept C57BL/6 (H-2b, B6) heart and skin allografts.

\*  $P < 0.01$ , Mann-Whitney U test.

ences in graft immunogenicity of skin grafts may be due to either the number of class II passenger leukocytes (including Langerhans cells) or the MHC class I density in skin tissue. Recognition of minor or Qa differences or skin-specific alloantigens may be important as well (38). These differences may initiate a strong response that recruits the small number of residual CD4<sup>+</sup> cells in anti-CD4-treated recipients.

*Mice Lacking CD4<sup>+</sup> Cells but with Functional CD8<sup>+</sup> Cells Permanently Accept Heart and Skin Allografts.* C57BL/6 heart and skin allografts were permanently accepted in the BALB/c CD4 knockout recipients (MST >100 d) (Table 2). Why do CD4 knockout mice not reject allotransplants? Mice lacking CD4<sup>+</sup> cells through targeted gene disruption have previously been shown to have normal numbers of T and B cells, with peripheral expansion of the CD8<sup>+</sup> compartment (31). The CD4 knockout mice possess an expanded subpopulation of CD4-CD8-TCR- $\alpha/\beta$ <sup>+</sup> (double negative) T cells in the thymus and periphery that is not normally found in significant numbers in conventional mice (31). These mice have been shown to have intact Ig isotype class switch from IgM to IgG in response to sheep erythrocytes and vesicular stomatitis virus in vivo (30). It was also demonstrated (using depletive regimens of mAbs to various subpopulations of T cells in vivo) that the double negative cells were responsible for providing help in the antibody response of CD4 knockout mice to vesicular stomatitis virus infection (30). These cells were demonstrated to be class II MHC-restricted in responses against the T cell-dependent antigen KLH. CTLs were also shown to be generated against lymphocytic choriomeningitis and vaccinia virus, suggesting that CD8<sup>+</sup> cells in these CD4 knockout mice can mount an immune response in the absence of CD4<sup>+</sup> cells (30).

Double-negative T cells have been previously shown to have suppressive properties (39). It is possible that these CD4-CD8-TCR- $\alpha/\beta$ <sup>+</sup> T cells are not only unable to initiate but may actively suppress a response against the allograft. The activity of these double-negative cells in CD4

and CD8 knockout mice, and in CD4-CD8- double knockout mice, however, has been variable. In CD4 knockout mice, CD4-CD8-TCR- $\alpha/\beta$ <sup>+</sup> cells have been shown to provide MHC class II-restricted help in vitro as stated above (30). Although naive CD8 knockout mice have normal numbers of CD4-CD8-TCR- $\alpha/\beta$ <sup>+</sup> cells, double-negative cells are increased in CD8 knockout mice engrafted with an MHC class I-disparate skin graft (40). Double-negative cells also significantly increase when thymocytes from these CD8 knockout mice are transferred to nude mice who receive and subsequently reject MHC class II-deficient skin grafts. CD4 depletion with anti-CD4 mAbs in CD8 knockout mice has no effect on rejection of MHC class I-disparate skin allografts; thus residual nondepleted CD4<sup>+</sup> or CD4-CD8-TCR- $\alpha/\beta$ <sup>+</sup> cells may play a role in this rejection. In CD4-CD8- knockout mice, CD4-CD8-TCR- $\alpha/\beta$ <sup>+</sup> cells have been shown to generate alloreactive cytolytic T cells, and recognize MHC class I antigens in vitro (41). These CD4-CD8- double-knockout mice have been shown to reject skin grafts with major H-2 histocompatibility disparities, but accepted grafts with only minor antigen differences (41). To address the possibility that CD4-CD8-TCR- $\alpha/\beta$ <sup>+</sup> cells in CD4 knockout recipients serve as "suppressor cells,"  $3.0\text{--}4.0 \times 10^7$  spleen cells from CD4 knockout mice bearing B6 hearts for over 100 d were transferred into irradiated (200 rads) BALB/c hosts along with a fresh donor-matched B6 heart allograft. Tolerance was not adoptively transferred to these naive recipients; all B6 heart allografts were rejected within 18 d, similar to irradiated controls (Table 3). These data suggest that unresponsiveness in the CD4 knockout recipients was not due to the presence of suppressor CD4-CD8-TCR- $\alpha/\beta$ <sup>+</sup> T cells. That double-negative cells do not actively suppress allojection is consistent with previous results in both CD8 and CD4-CD8- knockout mice that demonstrated that double-negative cells tend to play a role in skin allograft rejection rather than suppression.

It is therefore most likely that the mechanism of unresponsiveness to allografts in the CD4 knockout mice was due to the complete absence of CD4<sup>+</sup> T cells which would

**Table 3.** Adoptive Transfer of Spleen Cells from "Tolerant" CD4 Knockout Mice Does Not Prolong Allograft Survival

Strain combination	3-4 × 10 <sup>7</sup> Splenocytes	200 rads	Survival	MST ± SEM
			<i>d</i>	
B6 to BALB/c	Tolerant	+	17, 18 × 3	17.8 ± 0.4
	CD4 KO			
B6 to BALB/c	None	+	16, 18 × 3	17.5 ± 0.8

Transfer of  $3\text{--}4 \times 10^7$  spleen cells from CD4 KO mice bearing allografted hearts for over 100 d into irradiated (200 rads) syngeneic hosts did not prevent allojection of fresh B6 heart allografts in the naive recipients of adoptive transfer.

**Table 4.** *CD4<sup>+</sup> Cells Reconstitute Allorejection*

Strain combination	Transferred cells	Mice with rejected grafts	Survival
B6 to CD4 KO	None	0/5	<i>d</i> 100 × 8
B6 to CD4 KO	CD4 <sup>+</sup> cells	5/7	13, 14, 15 × 2, 16, >30 × 2
B6 to CD4 KO	CD8 KO cells	3/3	15, 16, 17

CD4 KO BALB/c mice were given  $5 \times 10^7$  purified CD4<sup>+</sup> cells from conventional naive BALB/c mice. 1 d after this adoptive transfer, the CD4 KO BALB/c mice received a B6 skin graft

suggest that CD4<sup>+</sup> cells are required for initiation of allograft rejection. To address this possibility, we reconstituted CD4 knockout mice with naive CD4<sup>+</sup> cells just before engraftment to see whether the addition of CD4<sup>+</sup> cells would allow graft rejection. 1 d after adoptive transfer of  $5 \times 10^7$  CD4<sup>+</sup> cells from conventional naive BALB/c mice obtained by MINIMACS purification, the CD4-reconstituted CD4 knockout BALB/c mice received a B6 skin graft. Control CD4 knockout mice received identical B6 skin grafts but did not receive CD4 cells before engraftment. 5 of 7 mice reconstituted with CD4<sup>+</sup> cells rejected their grafts (Table 4). This experiment was then modified to avoid the possibility that the CD4<sup>+</sup> cells isolated by MINIMACS purification were "activated." CD4 knockout mice were reconstituted with cells from CD8 knockout mice which have functionally intact CD4<sup>+</sup> cells (31). Three of three CD4 knockout recipients reconstituted with "CD4 cells" from CD8 knockout mice rejected their skin grafts (Table 4).

**CD8 Knockout Mice Reject Heart and Skin Allografts.** Although we have demonstrated that CD4<sup>+</sup> cells are essential for allorejection, what is the role of the CD8<sup>+</sup> cell? In certain settings, CD8<sup>+</sup> cells seem capable of initiating rejection in concert with MHC class I disparity (1). However, as demonstrated in the current study, CD8<sup>+</sup> cells alone, although present in the CD4 knockout mice, could not initiate allorejection. It is possible that the CD8<sup>+</sup> cells which

may normally play a role in graft rejection were unable to respond to alloantigens in the complete absence of CD4<sup>+</sup> cell-mediated help. This question has been previously addressed in CD8 knockout mice, which lack CD8<sup>+</sup> cells but have functional CD4<sup>+</sup> cells (31). It has been previously shown that CD8 knockout mice reject MHC class I-or MHC class II-disparate skin grafts without delay compared with wild-type mice, suggesting that CD8<sup>+</sup> cells are not necessary for allorejection of either MHC class I or class II grafts (40). More recent studies have demonstrated that adoptive transfer of naive or sensitized CD4<sup>+</sup> cells from these CD8 knockout mice into nude mice that had been grafted with allogeneic skin from mice deficient in MHC class I or class II (MHC class II or MHC class I allogeneic, respectively) reconstituted rejection, suggesting that CD4<sup>+</sup> cells were sufficient to mediate rejection (42). Although MHC class I skin allografts were rejected, CD4<sup>+</sup> cells did not display alloantigen-specific cytotoxic activity, though they proliferated in vitro in response to allogeneic targets. We also studied CD8 knockout mice as recipients of MHC disparate allografts. C57BL/6 skin allografts transplanted into BALB/c CD8 knockout mice were rejected in  $8.7 \pm 0.3$  d (compared to  $9.0 \pm 0.4$  d for BALB/c controls), which concurs with the results of Dalloul et al. (42). C57BL/6 heart and skin allografts transplanted into PL/J CD8 knockout mice were rejected within 10 d for each graft separately ( $n = 14$ , data not shown). Collectively these results suggest that elimination of cells bearing the CD8 molecule does not prevent allorejection. These data also demonstrate that CD4<sup>+</sup> cells can initiate rejection. Thus, our results demonstrate that the initiation of allorejection requires CD4<sup>+</sup> and not CD8<sup>+</sup> cells.

In these experiments we have explored the role of CD4 and CD8 cells in transplant allorejection using knockout mice as recipients of MHC disparate allografts. The results demonstrate that heart and skin allografts are permanently accepted in CD4 knockout mice, but are rejected in CD8 knockout mice. Thus, lack of CD4<sup>+</sup> cells allows permanent survival of heart and skin allografts in mice, whereas lack of CD8<sup>+</sup> cells does not prevent allorejection. These results demonstrate that CD4<sup>+</sup> cells, not CD8<sup>+</sup> T cells, are absolutely required in initiating allorejection. Our results also demonstrate that allorejection does not require both CD4<sup>+</sup> and CD8<sup>+</sup> T cell subpopulations.

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## References

- Hall, B.M. 1991. Cells mediating allograft rejection. *Transplantation*. 51:1141–1151.
- Burdick, J.F., and L.W. Clow. 1986. Rejection of murine cardiac allografts. I. Relative role of major and minor antigens. *Transplantation*. 42:67–72.
- Peugh, W.N., R.A. Superina, K.J. Wood, and P.J. Morris. 1986. The role of H-2 and non-H-2 antigens and genes in the rejection of murine cardiac allografts. *Immunogenetics*. 23: 30–37.
- Stepkowski, S.M., A. Raza-Ahmad, and W.R. Duncan. 1987. The role of class I and class II MHC antigens in the rejection of vascularized heart allografts in mice. *Transplantation*. 44:753–759.
- Wang, Y., A. Mayne, M.K. Sell, and A. Ahmed-Ansari. 1990. The influence of MHC non-MHC genes on the nature of murine cardiac allograft rejection. I. Kinetic analysis of mononuclear cell infiltrate and MHC-class I/class II expression in donor tissue. *Transplantation*. 50:313–324.
- Roser, B.J. 1989. Cellular mechanisms in neonatal and adult tolerance. *Immunol. Rev.* 107:179–202.
- Rosenberg, A.S., T. Mizouchi, S.O. Sharrow, and A. Singer. 1987. Phenotype specificity and function of T cell subsets and T cell interactions involved in skin allograft rejection. *J. Exp. Med.* 165:1296–1315.
- Sprent, J., M. Schaefer, D. Lo, and R. Korngold. 1986. Properties of purified T cell subsets. II. In vivo responses to class I vs class II H-2 differences. *J. Exp. Med.* 165:1296–1303.
- Rosenberg, A.S., T. Mizouchi, and A. Singer. 1988. Evidence for involvement of dual function T cells in rejection of MHC class I disparate skin grafts: assessment of MHC class I alloantigens as in vivo helper determinants. *J. Exp. Med.* 168: 33–45.
- Lowry, R.P., R.D. Forbes, J.H. Blackburn, and D.M. Marghresco. 1985. Immune mechanisms in organ allograft rejection. V. Pivotal role of the cytotoxic-suppressor T cell subset in the rejection of heart grafts bearing isolated class I disparities in the inbred rat. *Transplantation*. 40:545–550.
- Rosenberg, A.S., T.I. Munitz, T.G. Maniero, and A. Singer. 1991. Cellular basis of skin allograft rejection across a class I major histocompatibility barrier in mice depleted of CD8<sup>+</sup> T cells in vivo. *J. Exp. Med.* 173:1463–1471.
- Christanson, S.W., I.D. Shultz, and E.H. Leiter. 1993. Adoptive transfer of diabetes into immunodeficient NOD-scid/scid mice. Relative contributions of CD4<sup>+</sup> and CD8<sup>+</sup> T-cells from diabetic versus prediabetic NOD.NON-Thy-1<sup>+</sup> donors. *Diabetes*. 42:44–55.
- Shizuru, J.A., A.K. Gregory, C. Tien-Bao Chao, and C.G. Fathman. 1987. Islet allograft survival after a single course of treatment of recipient with antibody to L3T4. *Science (Wash. DC)*. 237:278–280.
- Yin, D., and C.G. Fathman. 1995. CD4-positive suppressor cells block allotransplant rejection. *J. Immunol.* 154:6339–6345.
- Herbert, J., and B. Roser. 1988. Strategies of monoclonal antibody therapy that induce permanent tolerance of organ transplants. *Transplantation*. 46(Suppl.):128S–134S.
- Chen, Z., S. Cobbold, S. Metcalfe, and H. Waldmann. 1992. Tolerance in the mouse to major histocompatibility complex-mismatched heart allografts, and to rat heart xenografts, using monoclonal antibodies to CD4 and CD8. *Eur. J. Immunol.* 22:805–810.
- Han, W.R., P.L. Mottram, and I.F.C. McKenzie. 1993. Comparison of CD4 depleting and nondepleting monoclonal antibodies in the mouse heart allograft model. *Transplant. Proc.* 25:2933–2934.
- Cobbold, S.P., A. Jayasuriya, A. Nash, T.D. Prospero, and H. Waldmann. 1984. Therapy with monoclonal antibodies by elimination of T cell subsets in vivo. *Nature (Lond.)*. 312: 548–551.
- Cobbold, S.P., G. Martin, S. Qin, and H. Waldmann. 1986. Monoclonal antibodies to promote marrow engraftment and tissue graft tolerance. *Nature (Lond.)*. 323:164–169.
- Alters, S.E., H.K. Song, and C.G. Fathman. 1993. Evidence that clonal anergy is induced in thymic migrant cells after anti-CD4-mediated transplantation tolerance. *Transplantation*. 56:633–638.
- Qin, S., S. Cobbold, H. Tighe, R. Benjamin, and H. Waldmann. 1987. CD4 monoclonal antibody pairs for immunosuppression and tolerance induction. *Eur. J. Immunol.* 17: 1159–1165.
- Bushell, A., P.J. Morris, and K.J. Wood. 1995. Transplantation tolerance induced by antigen pretherapy and depletive anti-CD4 antibody depends on CD4<sup>+</sup> T cell recognition during the induction phase of the response. *Eur. J. Immunol.* 25:2643–2649.
- Pearson T.C., C.R. Darby, A.R. Buchell, L.S. West, P.J. Morris, and K.J. Wood. 1993. The assessment of transplantation tolerance induced by anti-CD4 monoclonal antibody in the murine model. *Transplantation*. 55:361–367.
- Mottram, P.L., L.J. Purcell, G.A. Pietersz, and I.F.C. McKenzie. 1993. Tolerance induction with anti-CD8 monoclonal antibodies in the mouse heart transplant model. *Transplant. Proc.* 25:2935–2936.
- Yin, D., and C.G. Fathman. 1995. Tissue-specific effects of anti-CD4 therapy in induction of allograft unresponsiveness in high and low responder rats. *Transplant. Immunol.* 3:258–264.
- Seydel, K., J.U. Shizuru, D. Grossman, A. Wu, S. Alters, and C.G. Fathman. 1991. Anti-CD8 abrogates effect of anti-CD4-mediated islet allograft survival in rat model. *Diabetes*. 40:1430–1434.
- Cobbold S.P., G. Martin, and H. Waldmann. 1990. The induction of skin graft tolerance in major histocompatibility complex-mismatched or primed recipients: primed T cells can be tolerized in the periphery with anti-CD4 and anti-CD8 antibodies. *Eur. J. Immunol.* 20:2747–2755.
- Yamamoto, H., M. Monden, M. Kawai, A. Uenaka, M. Gotto, T. Mori, M. Sakurai, H. Shiku, and E. Nakayama. 1990. The role of CD8<sup>+</sup> and CD4<sup>+</sup> cells in islet allograft rejection. *Transplantation*. 50:120–125.
- Shizuru, J.A., S.E. Alters, and C.G. Fathman. 1992. Anti-CD4 monoclonal antibodies in therapy: creation of nonclassical tolerance in the adult. *Immunol. Rev.* 29:1–24.
- Rahemtulla, A., W.-P. Fung-Lcung, W. Schilham, T.M. Kundig, S.R. Sambhara, A. Narendran, A. Arabian, A. Wakeham, C.J. Page, R.M. Zinkernagel, R.G. et al. 1991. Normal development and function of CD8<sup>+</sup> cells but marked decreased helper cell activity in mice lacking CD4. *Nature (Lond.)*. 353:180–184.
- Fung-Lcung, W., M.W. Schilham, A. Rahemtulla, T.M. Kundig, M. Vollenweider, J. Potte, W. van Ewijk, and T.W. Mak. 1991. CD8 is needed for development of cytotoxic T cells but not helper T cells. *Cell*. 65:443–449.
- Hergueux, J., H. Bodmer, S. Cardell, S.H. Chan, D. Cosgrove, C. Benoist, and D. Mathis. 1993. Knock-out mice: a

- new tool for transplantation immunologists. *Transplant. Proc.* 25:30–32.
33. Ljunggren, H., L. Van Kaer, M.S. Sabatine, H. Auchincloss, S. Tonegawa, and H.L. Ploegh. 1995. MHC class I expression and CD8<sup>+</sup> T cell development in TAP1/B2-microglobulin double mutant mice. *Int. Immunol.* 7:975–984.
  34. Dialynas, D.P., D.B. Wilde, P. Marrack, K.A. Pierres, K.A. Wall, W. Havran, G. Otten, M.R. Loken, M. Pierres, J. Kappler, and F.W. Fitch. 1983. Characterization of the murine antigenic determinant designated L3T4a recognized by monoclonal antibody GK1.5: expression of L3T4a by functional T cell clones appears to correlate primarily with class II MHC antigen-reactivity. *Immunol. Rev.* 74:29–56.
  35. Corry, R.J., H.J. Winn, and P.S. Russell. 1973. Primarily vascularized allografts of heart in mice. The role of H-2D, H-2K and non-H-2 antigens in rejection. *Transplantation.* 16:343–350.
  36. Sugarbaker, P.H., and A.E. Chang. 1979. Uncovered skin grafts in mice. *J. Immunol. Meth.* 31:167–175.
  37. Chace, J.H., J.S. Cowdery, and E.H. Field. 1984. Effect of anti-CD4 on CD4 subsets. 1. Anti-CD4 preferentially deletes resting, naive CD4 cells and spares activated CD4 cells. *J. Immunol.* 152:405–412.
  38. Steinmuller, D. 1984. Tissue-specific and tissue-restricted histocompatibility antigens. *Immunol. Today.* 5:234–240.
  39. Schmidt-Wolf, I.G., S. Dejbakhsh-Jones, N. Ginzton, and S. Strober. 1992. T-cell subsets and suppressor cells in human bone marrow. *Blood.* 80:3242–3250.
  40. Dalloul, A.H., K. Ngo, and W.-P. Fung-Leung. 1996. CD4-negative cytotoxic T cells with a T cell receptor  $\alpha/\beta^{\text{intermediate}}$  expression in CD8-deficient mice. *Eur. J. Immunol.* 26:213–218.
  41. Schilham, M.W., W.-P. Fung-Leung, A. Rahemtull, T. Kuendig, L. Zhang, J. Potter, R.G. Miller, H. Hengartner, and T.W. Mak. 1993. Alloreactive cytotoxic T cells can develop and function in mice lacking both CD4 and CD8. *Eur. J. Immunol.* 23:1299–1304.
  42. Dalloul, A.H., E. Chmouzis, K. Ngo, and W.-P. Fung-Leung. 1996. Adoptively transferred CD4<sup>+</sup> lymphocytes form CD8<sup>-/-</sup> mice are sufficient to mediate the rejection of MHC class II or class I disparate skin grafts. *J. Immunol.* 156: 4114–4119.