

The role of CD8⁺ T cells during allograft rejection

V. Bueno and
J.O.M. Pestana

Disciplina de Nefrologia, Escola Paulista de Medicina,
Universidade Federal de São Paulo, São Paulo, SP, Brasil

Abstract

Correspondence

V. Bueno
Disciplina de Nefrologia
EPM, UNIFESP
Rua Botucatu, 740
04023-900 São Paulo, SP
Brasil
Fax: +55-11-5573-9652
E-mail: valquiriabueno@hotmail.com

Research supported by FAPESP
(No. 98/13340-2).

Received July 31, 2001
Accepted August 20, 2002

Organ transplantation can be considered as replacement therapy for patients with end-stage organ failure. The percent of one-year allograft survival has increased due, among other factors, to a better understanding of the rejection process and new immunosuppressive drugs. Immunosuppressive therapy used in transplantation prevents activation and proliferation of alloreactive T lymphocytes, although not fully preventing chronic rejection. Recognition by recipient T cells of alloantigens expressed by donor tissues initiates immune destruction of allogeneic transplants. However, there is controversy concerning the relative contribution of CD4⁺ and CD8⁺ T cells to allograft rejection. Some animal models indicate that there is an absolute requirement for CD4⁺ T cells in allogeneic rejection, whereas in others CD4-depleted mice reject certain types of allografts. Moreover, there is evidence that CD8⁺ T cells are more resistant to immunotherapy and tolerance induction protocols. An intense focal infiltration of mainly CD8⁺CTLA4⁺ T lymphocytes during kidney rejection has been described in patients. This suggests that CD8⁺ T cells could escape from immunosuppression and participate in the rejection process. Our group is primarily interested in the immune mechanisms involved in allograft rejection. Thus, we believe that a better understanding of the role of CD8⁺ T cells in allograft rejection could indicate new targets for immunotherapy in transplantation. Therefore, the objective of the present review was to focus on the role of the CD8⁺ T cell population in the rejection of allogeneic tissue.

Key words

- Transplantation
- Rejection
- T cells
- Cytokines
- Chemokines

CD8 as a co-receptor/accessory molecule

T lymphocytes can be separated into two subsets based on their expression of the CD4 and CD8 molecules on the cell surface. Approximately 65% of peripheral αβ-positive T cells express CD4 and 35% express CD8. CD4⁺ T cells are restricted to major histocompatibility complex (MHC) class II and act as helper cells for various immune responses, whereas CD8⁺ T cells recognize antigens in the context of MHC class I and

develop into cytotoxic effector cells. The present data support the view that T cell activation requires CD8 or CD4 and T cell receptor (TCR) binding to the same peptide/MHC (pMHC) molecule, leading to the classification of these cells as co-receptors. CD8 is expressed on the cell surface in two forms: a CD8αβ heterodimer and a CD8αα homodimer. CD8αβ is the prevalent form on the surfaces of the T cell population and is believed to enhance cytotoxic T lymphocyte (CTL) activation better than CD8αα. CD8, either α or β chain, consists of four discrete

functional domains that can be related to the primary sequence as follows: the Ig-like ectodomains, the membrane proximal stalk region, the transmembrane domain, and the cytoplasmic domain. The extracellular Ig-like domain is involved in the binding to MHC. The stalk region is flexible and highly glycosylated, a fact which is believed to be important in extending the region to reach the MHC, and is also postulated to interact with TCR. The cytoplasmic domain of CD8 α consists of a p56^{lck} binding motif important for signal transduction.

CD8 enhances T cell recognition of pMHC on the surface of antigen-presenting cells (APC) since binding of CD8 and TCR to the same pMHC would increase the overall avidity between the surfaces of APC and T cells. Second, CD8 binding to pMHC recruits p56^{lck} tyrosine kinase through its cytoplasmic domain into the T cell signaling complex and thus enhances signal transduction. Third, CD8 binding to pMHC possibly reduces the overall flexibility of pMHC on the cell surface, positioning the pMHC more favorably for TCR binding. It is more likely that CD8 is recruited to the pMHC-TCR complex by intracellular binding of the α chain-associated p56^{lck} to TCR-associated ZAP-70. Once recruited, CD8 would enhance pMHC binding by adding to the much stronger TCR-pMHC interaction (1).

The term accessory molecule has been used to describe the activities of CD4 and CD8 when they are unable to bind to the same MHC molecule as the TCR. There are conflicting data as to whether the binding of MHC molecules in an accessory manner contributes to T cell activation. However, Smith and Potter (2) showed that transgenic mouse skin grafts expressing a disparate class I molecule that does not engage CD8 are rejected as vigorously as wild-type grafts. Rejection was caused by a CD8⁺ class I reactive CTL, which required CD8 engagement for cytolysis and secretion of interferon γ (IFN- γ), although the co-engagement

with the same MHC class I molecule as TCR was not necessary. As an accessory molecule, CD8 would increase the overall avidity of the T cell-target cell interaction, or transduce signals through p56^{lck} that either act independently of or intersect downstream from TCR-mediated signal transduction.

CD8⁺ T cells and allograft rejection

The previous belief that CD8⁺ T cells are a homogenous population of CD4-dependent cells and produce a limited number of cytokines such as IFN- γ , tumor necrosis factor α (TNF- α) and lymphotoxin has changed. It is now accepted that CD8⁺ T cells can polarize in the same way as CD4⁺ T cells into cytotoxic T (Tc) cells - Tc1 (IFN- γ) and Tc2 (IL-4, IL-5) - and in some situations CD4-independent responses by CD8⁺ T cells occur. CD8⁺ T cell helper independence might be related to the avidity of the interaction between TCR on these cells and the antigen presented by class I molecules on the APC. High avidity T cells (multiple interactions of TCR and CD8 molecules on the T cell with pMHC complexes on the APC) may receive a strong signal that induces both IL-2R and IL-2 synthesis resulting in a helper-independent response. CD8⁺ T cells with low avidity may induce IL-2R but produce little or no IL-2 and depend on IL-2 production by CD4⁺ T cells. Heath et al. (3), using transgenic mice expressing a specific TCR for H-2K^b, showed that TCR^{high}/CD8^{high} cells tested against splenocytes expressing different densities of H-2K^b were competent IL-2 producers, whereas TCR^{low}/CD8^{low} presented marginal or no levels of IL-2 in the same assay. Deeths et al. (4) showed that *in vitro* a helper-independent phase of the CD8⁺ T cell response is consistent with the ability of these cells to support their own expansion by producing IL-2 in response to co-stimulation provided by CD28 binding to B7 ligands, leukocyte function accessory-1 molecule (LFA-1) binding to intercellular adhesion

molecule-1 (ICAM-1), and possibly other co-stimulatory receptors binding to their ligands.

In transplantation it has been shown that CD4⁺ T cell effector function is sufficient to mediate allograft failure, and it has been suggested that CD8⁺ T cell-mediated effects are dependent on CD4⁺ T cell help. Thus, whether CD8⁺ T cells are sufficient to reject allografts or play an additive role in the progression of the rejection process has not been settled as yet. In heart-transplanted patients, the presence of CD69⁺/CD8⁺ cells in peripheral blood exceeding 15% of all T cells is correlated with vigorous rejection (5). CD8⁺ T cells infiltrating the myocardium of patients in heart allograft rejection (6) displayed an activated CD69⁺ phenotype with perforin activity. Moreover, Delfs et al. (7) showed in RAG^{-/-} mice that, in the absence of T and B cells, a cardiac allograft survives indefinitely whereas the adoptive transfer of reactive Tc cells caused alterations compatible with rejection. Recipients injected with Tc1 cells (IFN- γ ^{high}) showed graft vasculitis and arteriopathy and recipients injected with Tc2 cells (IL-4^{high}/IL-5^{high}) presented extensive eosinophil infiltration. Gilot et al. (8) showed that during a heart rejection episode in mice the graft-infiltrating antigen-specific CD8⁺ T cell population expanded with modulation of surface markers such as CD62L and CD69 besides production of IFN- γ . Jones et al. (9) showed that in mice depleted of T cells (thymectomy, anti-CD4, anti-CD8), the injection of 6×10^6 TCR antigen-specific CD8⁺ T cells was sufficient to promote rejection of a fully mismatched cardiac allograft. CD8⁺ T cells appeared in the spleen and lymph nodes 7 days after transplantation. These cells divided, were blastic and up-regulated CD44/CD69 and down-regulated CD45RB/CD62L. Bishop et al. (10) showed that IFN- γ -deficient mice treated with anti-CD4 rejected a cardiac allograft through an unusual CD8-mediated, CD4-independent mechanism of allograft rejection. Fur-

thermore, rejection was resistant to treatment with anti-CD154 and was associated with IL-4 production and eosinophil influx into the graft. Therefore, although it has been accepted that CD4⁺ T cells may play a crucial role in allograft rejection there is evidence in certain situations that CD4 help is not always required to generate CD8⁺ CTL.

Results from mouse models of transplantation indicate that intrinsic features of the transplanted tissue primarily dictate the contribution of CD4⁺ and CD8⁺ T cell subsets to graft rejection. For instance, it has been shown that islets are more susceptible to CD8-mediated rejection. CD8 absence either in donors (11,12), i.e., by using islets from CD8-deficient mice (β 2-microglobulin^{-/-}) or using monoclonal antibody directed against MHC class I, or recipients (13) possessing no CD8⁺ T cells (β 2-microglobulin^{-/-}) improved islet allograft survival. Haskova et al. (14) used CD4- or CD8-deficient knockout mice to investigate the role of T cell subsets in allograft rejection. The results showed that CD4⁺ T cells play a critical role in the rejection of corneal allografts, whereas CD8⁺ T cells appear to be involved in the rejection of skin allografts. Boisgérault et al. (15) showed that both CD4⁺ and CD8⁺ T cells play a role during corneal allograft rejection. Mice rejecting corneal allografts mount a potent T cell response associated with the activation of IL-2-producing CD4⁺ and IFN- γ -producing CD8⁺ alloreactive T cells. Cardiac allografts are rejected by both CD4⁺ and CD8⁺ T cells but it seems that CD4 is mandatory to initiate the rejection of this tissue. Wiseman et al. (16) used the adoptive transfer of CD4⁺ T cells into recipients deficient in B and CD8⁺ T cells to investigate the role of CD4⁺ T cells in cardiac allograft. Rejection occurred in a normal fashion in hearts from wild-type donors, demonstrating that CD4⁺ T cells are sufficient to promote cardiac allograft rejection in the absence of CD8 and B cells.

Fischbein et al. (17) showed that intimal lesions were absent in hearts transplanted into nude and CD4^{-/-} knockout mice. In contrast, donor hearts in CD8^{-/-} knockout mice developed cardiac allograft vasculopathy, although significantly less than in wild-type mice. Adoptive transfer of T lymphocyte subset populations into nude recipients confirmed that cardiac allograft vasculopathy was absolutely contingent on CD4⁺ lymphocytes, and that CD8⁺ lymphocytes played an additive role in intimal progression. Although CD8⁺ lymphocytes alone did not cause cardiac allograft vasculopathy, the results suggested that both CD4⁺ and CD8⁺ lymphocytes contribute to the progression of intimal lesion development via secretion of IFN- γ . Ogura et al. (18) used a monoclonal antibody directed against CD8⁺ T cells to investigate the immune response after a liver transplantation in the absence of the CD8 population. Histologic findings indicated that severe acute rejection and cell-mediated toxicity factors such as granzyme B and Fas ligand (FasL) were still evident, albeit at lower levels. These results indicate that cells other than CD8⁺ T cells express cytotoxic mediators in rejecting allograft.

CD8⁺ T cells and cytokines/chemokines

A review by Hamann et al. (19) reports that up- and down-regulation of surface markers, mediators of cytotoxicity and cytokine production occurs in humans during the differentiation of CD8⁺ T cells, which permits to classify them as naive, memory and effector cells. However, most of the studies were performed during viral infection and could not reflect a transplant situation. During an infection, human effector CD8⁺ T cells express markers of cytotoxicity and cell death such as perforin, granzyme B, Fas and FasL. IFN- γ and TNF- α are the growth factors produced by these cells after activation. Memory CD8⁺ T cells express higher levels

of Fas but lower levels of FasL. Perforin and granzyme B are also expressed at lower levels than in effector CD8⁺ T cells. IL-2 and IL-4 are produced by memory cells and the levels of IFN- γ and TNF- α are similar to those seen in the effector subpopulation.

Antigen-triggered T cell activation and the subsequent infiltration of activated CD4⁺, CD8⁺, macrophages, and natural killer (NK) cells into the graft are key events in acute allograft rejection. CTLs include CD4⁺ and CD8⁺ cells with the ability to secrete cytotoxic cytokines such as TNF- α and IFN- γ in the region of their targets. The potential mechanism of CTL involvement in acute allograft rejection is mediated by cytotoxic granule-based killing by perforin and granzyme B- or FasL-induced programmed cell death. It has been suggested that CD8⁺ cytotoxic lymphocytes depend primarily on the perforin/granzyme system to kill their targets, whereas CD4⁺ T cells utilize the FasL to induce cell death. The presence of activated CTLs and expression of kill mediators have been described in acute rejection of human hearts, lungs and kidneys (20,21).

In the allograft response, several studies have pointed out CD8⁺ T cell as the major effector cells and in addition to their cytotoxic effector function, activated CD8⁺ T cells also have the ability to produce high levels of proinflammatory cytokines including IFN- γ . This cytokine has been shown to up-regulate the expression of MHC molecules and to enhance alloantigen presentation on target tissues; IFN- γ also serves to enhance inflammation and stimulate non-specific effector cells such as macrophages and NK cells (22,23). There is considerable evidence of increased intragraft expression of IFN- γ during rejection of experimental cardiac, renal and islet transplants. Diamond and Gill (22) showed that diabetes induction in SCID mice, which lack functional B and T cells, is reversible by islet transplant. However, the transfer of *in vitro*-primed alloreactive CD8⁺ T cells caused islet allograft rejection.

tion without requiring perforin, being only partially dependent on FasL production but completely dependent of IFN- γ production, suggesting that in the absence of CD4⁺ T cells, IFN- γ contributes to differentiation and/or expansion of effector CD8⁺ T cells. Expression of cytotoxic attack molecules (granzyme B and perforin) has been identified in human renal allograft biopsies by RT-PCR. In addition, granzyme B, IL-2 and IFN- γ mRNA expression has being correlated with acute rejection (24).

CD8⁺ T cells and IFN- γ could have other functions during allograft rejection, as was shown by Braun et al. (25) through a cardiac transplant in mice where synthesis of IFN- γ by CD8⁺ T cells inhibits IL-5 production and consequently intragraft eosinophilia. Depletion of anti-CD8 cells in recipient mice prior to cardiac transplantation induced intragraft IL-5-dependent eosinophilia.

Not only cytokines but also chemokines play a role in transplantation due to their ability to attract cells to the inflammatory site. These factors are stored in the cytolytic granules of CD8⁺ CTLs and can be secreted by these cells as preformed and prepacked chemokines (26). Acute allograft rejection is characterized by an intense cellular immune response marked by influx of circulating leukocytes into the transplant. The accumulation of activated immune cells in the allograft is essential for the pathogenesis of tissue injury. Recruitment of leukocytes into sites of inflammation involves a tightly regulated series of molecular interactions that includes the initial capture and rolling of cells mediated by selectins, followed by firm arrest on the endothelium, a process mediated by integrins. In the course of these events, the leukocyte becomes activated through stimulation of G protein-coupled chemokine receptors, resulting in enhanced integrin adhesiveness and activation-dependent stable arrest. In addition, specific chemokines may amplify tissue inflammation by stimulating neutrophil degranulation and

monocyte superoxide production.

Kapoor et al. (27) showed that CD8⁺ T cells were responsible for the early expression of IP-10 and Mig chemokines in mouse cardiac allografts. These chemokines are dependent on the presence of IFN- γ since in IFN- γ ^{-/-} recipients IP-10 and Mig were completely absent. They are likely to mediate the recruitment of neutrophils, macrophages and NK cells into the graft during the transplant-induced wound healing. After stimulation, CD8⁺ T cells would produce IFN- γ and induce expression of these chemokines in the allograft (28,29).

Skin transplantation in mice (29) presented an early IP-10 expression (iso- and allografts) which decreased by day 7, with high levels occurring in the allografts only at 9 days post-transplant. Expression of Mig reached high levels in allografts only 9 days after transplantation. Intragraft expression of RANTES was also undetectable until day 9 when low levels were detected. In CD8-mediated rejection, anti-CD4 monoclonal antibody-treated mice presented a delay in skin rejection (20 days), with an intragraft expression of Mig and IP-10 undetectable until day 9 but expressed at high levels on day 18. RANTES expression was detectable at high levels during rejection (starting on day 18).

Fractalkine and CXCR3 (induced by IFN- γ) are chemokines that interact with CX₃CR1 to affect firm adhesion of resting and activated CD8⁺ T lymphocytes, monocytes and NK cells. CX₃CR1 is predominantly expressed by activated Th1, CD8⁺ and NK cells, and its expression is regulated by cytokines like IL-2. In a cardiac allograft model, Robinson et al. (30) showed that recipient treatment with anti-CX₃CR1 caused an increase in graft survival.

Fahy et al. (31) used a model of SCID mice reconstituted with human peripheral blood mononuclear cells, submitted to human skin transplant and injected with several chemokines to address the migration of

human leukocytes *in vivo*. Monocyte-derived chemokine had a predominant effect on recruitment of CD8 T⁺ cells, inducing a moderate increase in the number of CCR4⁺ cells and a slight increase in CCR3⁺ cells besides IL-5-secreting cells.

CD8⁺ T cells and endothelium

Chronic rejection of cardiac allografts is responsible for 23 to 36% of deaths after the first year of transplant and is characterized by a diffuse, concentric intimal proliferative response within the arteries of transplanted organs. To date, chronic rejection seems to initiate after the allorecognition of graft endothelium with subsequent leukocyte infiltration and production of cytokines, chemokines, and growth factors. In response, vascular smooth muscle cells are thought to transmigrate to the intimal compartment resulting in occlusive lesion formation.

CTLs (32) reactive with endothelium have been proposed to be the effectors of cell-mediated vascular rejection, a potential precursor lesion of chronic graft rejection. Endothelial cell-selective CTLs have been isolated from endomyocardial biopsies of acutely rejected heart transplants. It has been shown that endothelial cell-stimulated CTL clones present low production of IFN- γ and constitutive expression of CD40L, a molecule that is not usually seen on CD8⁺ T cells. Endothelial cells have been proposed to be a semiprofessional APC of intermediate stimulatory capacity. This suggests a predominantly modulatory role for the endothelium that might be inhibitory for immune-mediated injury. Endothelial cell-selective CTLs could develop a specific endothelial rejection independently of widespread parenchymal rejection. Using flow cytometry, Dengler et al. (33) showed that in endothelial cell-stimulated CTL cultures there was a lower frequency of reactive precursors and clonal expansion than in conventional CTL cultures in spite of perforin and IFN- γ detection.

Légaré et al. (34) showed that in an aortic transplant model, the loss of medial smooth muscle cells was associated with CD8⁺ T cells. The use of anti-CD8 monoclonal antibody reduced CD8⁺ T cell counts in peripheral blood, reduced medial smooth muscle cell apoptosis at 20 days and increased smooth muscle cell counts at 60 days. Using PCR, the group showed an up-regulation of CD8⁺ T cell mediators of apoptosis (perforin, granzyme B, FasL).

CD8⁺ T cells and immunosuppressive therapy

CD8⁺ T cells seem to be less affected by most of the immunosuppressive therapies used to prevent rejection than CD4⁺ T cells. For instance, it has been proposed that CD8⁺ T cells are not dependent on co-stimulation provided by the CD40L-CD40 pathway (35-38). In addition, only some of these cells express CD40L. In models of skin and small bowel allografts the rejection seems to occur despite the CD28 and/or CD40L pathways and is mediated by alloreactive CD8⁺ T cells (35,39).

In a model of aorta transplant in mice (36) it was shown that anti-CD40L and anti-CD4 therapy could delay but not prevent the graft from developing transplant arteriosclerosis. Anti-CD8 antibody caused a decrease in IL-12 and IFN- γ expression and a decrease in macrophage infiltration and inducible nitric oxide synthase. However, increased expression of IL-4 was observed within the graft, which in turn may be responsible for the development of transplant arteriosclerosis in the long term. Moreover, treatment with both anti-CD8 and MR1 (anti-CD40L) resulted in a significant reduction of intimal proliferation in sections of the cardiac allograft at day 30 but by day 50 the allograft began to exhibit progressive intimal proliferation (37). Thus, subsets of CD8⁺ T cells present in the repertoire may be differentially susceptible to targeting via CD40-

CD40L interaction. Using a cardiac model in mice and the adoptive transfer of a TCR antigen-specific CD8⁺ T cell population, Jones et al. (38) showed that CD4⁺ T cell-mediated rejection is prevented by anti-CD40L monoclonal antibody but that CD8⁺ T cells remain fully functional. Blocking CD40L interaction had no effect on CD8⁺ T cell activation, proliferation, differentiation, homing to the target allograft or cytokine production. Rothstein et al. (35) showed that rejection of islets or skin in mice was prevented or delayed by the combined blockade of CD45RB and CD40L molecules, respectively, whereas either agent used alone was not efficient. Combined blockade inhibited CD8 infiltration and caused a decrease in CD8 cells in lymph nodes, suggesting that anti-CD45RB may affect CD8 homing to lymph nodes or induce partial depletion of this T cell subset.

In a skin model, Trambley et al. (40) showed that co-stimulation blockade (CD40 and CD28) combined with anti-asialo GM1 antibodies delayed allograft rejection by inhibition of CD8-dependent rejection since 20% of CD8⁺ T cells express asialo GM1. Recipients treated with co-stimulation blockade and CD8⁺ T cell depletion had markedly prolonged allograft survival. In addition, RAG^{-/-} mice reconstituted with CD8⁺ T cells and treated with anti-asialo GM1 showed increased allograft survival with very few CD8⁺ T cells undergoing division.

Concerning immunosuppressive drugs (41), it has been proposed that in mice CD8⁺ T cells require a higher dose of cyclosporin A or FK506 than CD4⁺ T cells to become susceptible. van Hoffen et al. (42), using heart biopsies from transplanted patients on an immunosuppressive regimen showed that macrophages, CD4 and CD8 (2:1 ratio) cells were infiltrating the graft. Co-stimulatory molecules such as CD28, CD40 or CTLA4 were expressed at low levels, possibly representing chronic activation of T cells instead of anergy. CD4⁺ T cells presented a higher

percentage of apoptosis (65%) than CD8⁺ T cells (26%), suggesting that the presence of CD8 could contribute to the ongoing rejection process.

Chen et al. (43), using a cardiac transplant in mice and the injection of TCR antigen-specific CD8⁺ T cells, showed that the use of rapamycin for immunosuppressive therapy, although promoting indefinite graft survival, had no effect on the up-regulation of CD44/CD25 surface markers. Using human blood lymphocytes, Slavik et al. (44) showed that CD8⁺ T cells could proliferate even in the presence of rapamycin when activated by TCR cross-linking *ex vivo* or of some CD8⁺ T cell clones. The results suggested that both the strength of the signal delivered through the TCR and secondary co-stimulatory signals determine whether rapamycin inhibits or enhances the clonal expansion of CD8⁺ T cells.

CD8⁺ T cells and suppression

In the 1970's Gershon proposed the possibility of a suppressor CD8⁺ T cell population based on an *in vitro* assay (45). The inability to clone such cells with an antigen or to show evidence for CD8⁺ T cell immunoregulation *in vivo* created new questions about tolerance.

Cobbold and Waldmann (46), using an anti-CD4 monoclonal antibody in mice submitted to transplant, showed that it is possible to develop a powerful form of immune regulation that acts to suppress any naive or primed CD4⁺ or CD8⁺ T cells against the same antigen. Thus, a tolerant immune system is maintained by a population of CD4⁺ T cells that act to suppress the generation of any effector cell in response to the same antigen or to different antigens expressed on the same APC. Although CD4⁺ T cells seem to be unique in the process of tolerance induction, it has been possible to generate a regulatory population of CD8⁺CD28⁻ T cells *in vitro* (47). Moreover, recently a popula-

tion of CD8⁺CD28⁻ T cells (Ts) was isolated from peripheral blood of renal, cardiac and liver transplanted patients and its ability to inhibit the up-regulation of CD80 and CD86 expression by donor APC in culture was shown by Ciubotariu et al. (48). During rejection episodes, patients did not present the suppressor (Ts) population, whereas rejection-free patients presented Ts with the suppressor activity specific for the donor's HLA class I antigens.

Zhang et al. (49) identified a new subset of antigen-specific regulatory double negative T cells able to suppress *in vitro* responses and enhance donor-specific skin allograft survival. This suppression required direct contact with activated CD8⁺ T cells, promoting apoptosis of these cells through the Fas-FasL pathway.

Vukmanovic-Stejic et al. (50) reported in their review the possibility of CD8⁺ T cell-inducing modulation as these cells can secrete potent immunoregulatory cytokines such as IL-4, IL-10 and transforming growth factor β (TGF- β). They suggested that the immunoregulatory effects of this cell population could be mediated by direct lysis or apoptosis induction in specific CD4⁺ T cell targets or by modifying the behavior of APC. Moreover, Vignes et al. (51) reported that an immunoregulatory population of CD8⁺ T cells arose in rats submitted to cardiac transplantation due to a donor-specific transfusion before transplantation. Increased graft survival was associated with the production of the suppressive cytokine TGF- β 1 and the inhibition of Th1- and Th2-related cytokine expression.

In a model of kidney transplant in rats, Zhou et al. (52) showed that oral administration of donor splenocytes increased allograft survival in a fully mismatched combination. Graft infiltrating cells in non-rejected kidneys presented a reduction of the CD4⁺ T cell population, whereas the percentage of CD8⁺ T cells did not change. Although it was possible to detect mRNA for granzyme B,

perforin and FasL besides IFN- γ and TGF- β , confirming that CD8⁺ T cells in the graft infiltrating cells were alloresponsive CTLs, adoptive transfer of CD8⁺ T cells (graft infiltrating cells) to naive rats significantly improved allograft survival, whereas CD4⁺ T cells did not. Moreover, detection of IL-4 mRNA suggested that these cells were Tc2 deviated and potentially regulatory.

In conclusion, these controversial results suggest that CD8⁺ regulatory cells might be sufficient for, but not essential to, the development of tolerance.

CD8⁺ T cells and indirect/direct antigen presentation

Recipient T cell recognition of donor intact allo-MHC molecules (+peptide) presented on both the allograft itself and on donor passenger leukocytes has been termed direct pathway. Peptides derived from allogeneic MHC molecules and presented by recipient APC to recipient T cells are termed indirect pathway.

In animal models, the direct pathway has been estimated to represent >90% of the T cell repertoire participating in the process of acute rejection, whereas the indirect pathway would include only 1-10% (53). T cell responses occurring via direct allorecognition play a critical role during the early phase of acute graft rejection by sensitizing the host to graft antigens. However, it has been suggested (54) that the indirect pathway plays a critical role in the development and progression of chronic rejection. In addition, while the direct alloresponse is highly sensitive to treatment with immunosuppressive drugs including cyclosporin A, indirect allorecognition is thought to be poorly sensitive to blockade with cyclosporin A.

After transplantation, over time the donor passenger leukocytes are washed out from allografts, whereas recipient APC continually infiltrate the allograft and process/present shed donor allopeptides. This pro-

cess results in the diminishing importance of the alloresponse mediated by directly primed T cells and suggests that the indirect pathway represents the driving force in the actual destruction of transplanted tissues. Moreover, T cells from renal, cardiac and lung transplant recipients with chronic rejection show evidence of reactivity to donor HLA allopeptides and patients with cardiac allograft vasculopathy show evidence of donor-specific hyporesponsiveness to directly presented but not indirectly presented donor HLA antigens.

Lee et al. (54), using mice submitted to heart transplantation, showed that indirect allorecognition plays a major role in the pathogenesis of chronic cardiac allograft vasculopathy mainly through IFN- γ production by CD4⁺ T cells. CD4⁺ T cells reactive via the indirect pathway could initiate chronic rejection by facilitating either alloantibody production or CD8⁺ T cell effector functions. Valujskikh et al. (55), using a model of mouse skin transplantation, showed that indirect recognition by CD4⁺ and CD8⁺ T cells occurs when donor and recipient are fully mismatched for MHC. IFN- γ was the cytokine most frequently identified by ELISPOT both in indirect and direct priming models. Indirectly primed CD8⁺ T cells were the prominent component of the indirect response, comprising up to 3-5% of the total alloreactive repertoire. Benichou (56) showed that, during mouse skin allograft rejection, donor MHC molecules are processed and presented as peptides by the recipient's APC *in vivo*, eliciting CD4⁺ and CD8⁺ T cell responses which are restricted to the recipient's own MHC molecules.

Future prospects

Although controversy still remains concerning the relative contribution of CD4⁺ and CD8⁺ T cells to allograft rejection, the important role played by CD8 as effector cells in transplantation has been well estab-

lished. In addition, it has been shown that CD8⁺ T cells can escape from the immunosuppressive effects of drugs such as cyclosporine and rapamycin. This suggests that these CD8⁺ T cells may be involved in the development of chronic rejection. Moreover, Wang et al. (57) showed in an experimental model that in high numbers, primed CD8⁺ T cells can provide help to naive CD8⁺ T cells and promote activation of the latter.

Our group has performed kidney transplants since 1976 (554 transplants only in 2001) and, as also reported by other groups, 2-3% of our allografts are lost during the first year to irreversible acute rejection, whereas a larger number is lost during each subsequent year to chronic rejection. We are now participating in a multicenter study using drugs such as FTY720, FK506, and RAD amongst others and a flow cytometry assay to evaluate the contribution of each T cell population to allograft rejection.

Monitoring the recipient's immune response after transplantation has been pointed out as crucial for the adequacy of immunosuppressive therapy. Also the individualization of maintenance immunosuppressive therapy could provide a balance between the absence of an allogeneic specific response and an adequate immune response against pathogens. For both monitoring of immune response and an individualized approach to antirejection therapy, conditions are needed to monitor T cell behavior after transplantation. The development of new techniques to identify the CD8⁺ T cell population, its fate and action during rejection is crucial for the design of rational immunosuppressive therapy. However, few attempts have been made to focus on new strategies to identify T cell subsets during allograft rejection. Kusaka et al. (58) developed a T cell clonotype analysis (RT-PCR) of peripheral blood and graft biopsies in kidney-transplanted patients with voluntary immunosuppression withdrawal and stable graft function for 9 years to detect antigen-specific T cells. A high level of do-

nor-specific CD4⁺-CD8⁺ T cell clonotypes was found during the late tolerance pre-rejection stage, indicating that changes in the alloreactive T cell repertoire may be leading indicators of chronic rejection. Benlagha et al. (59) reported the generation and use of tetramers to identify mouse or human T cells restricted to MHC-like molecules (CD1d). Altman et al. (60) showed that the use of multimeric pMHC complexes provides a general, rapid, and direct method for analysis of the phenotypic state of antigen-specific T cells. The study was done on HIV-infected patients and was consistent with previous estimates for anti-HIV CTL populations by limiting dilution analysis.

Using the knowledge about the CD8 MHC

class I crystal structure, cytotoxic T cell inhibitors have been developed and used in infected mice (61), showing dose-dependent inhibition of a primary allogeneic CTL assay while having no effect on the CD4-dependent mixed lymphocyte reaction. Moreover, Choksi et al. (62) reported that the SC4 analogue of the CD8 α molecule was found to be inhibitory during both the generation and effector stages of CTLs, and also to significantly prolong skin allograft survival across an MHC class I barrier. As described above, some strategies are being developed to identify or to deplete specific T cell populations, but although they appear promising their potential use in transplantation still remains to be elucidated.

References

- Gao GF & Jakobsen BK (2000). Molecular interaction of coreceptor CD8 and MHC class I: the molecular basis for functional coordination with the T-cell receptor. *Immunology Today*, 21: 630-636.
- Smith PA & Potter TA (1998). Alloreactive T cells that do not require TCR and CD8 coengagement are present in naïve mice and contribute to graft rejection. *Journal of Immunology*, 160: 5382-5389.
- Heath WR, Kjer-Nielsen L & Hoffmann MW (1993). Avidity for antigen can influence the helper dependence of CD8⁺ T lymphocytes. *Journal of Immunology*, 151: 5993-6001.
- Deeths MJ, Kedl RM & Mescher MF (1999). CD8⁺ T cells become nonresponsive (anergic) following activation in the presence of costimulation. *Journal of Immunology*, 163: 102-110.
- Schowengerdt KO, Fricker FJ, Bahjat KS & Kuntz ST (2000). Increased expression of the lymphocyte early activation marker CD69 in peripheral blood correlates with histologic evidence of cardiac allograft rejection. *Transplantation*, 69: 2102-2107.
- Rutella S, Rumi C, Lucia MB, Barberi T, Puggioni PL, Lai M, Romano A, Cauda R & Leone G (1999). Induction of CD69 antigen on normal CD4⁺ and CD8⁺ lymphocyte subsets and its relationship with the phenotype of responding T cells. *Cytometry*, 38: 95-101.
- Delfs MW, Furukawa Y, Mitchell RN & Lichtman AH (2001). CD8⁺ T cell subsets TC1 and TC2 cause different histopathologic forms of murine cardiac allograft rejection. *Transplantation*, 71: 606-610.
- Gilot BJ, Hara M, Jones ND, van Maurik A, Niimi M, Hadjianastassiou V, Morris PJ & Wood KJ (2000). Visualization of the *in vivo* generation of donor antigen-specific effector CD8⁺ T cells during mouse cardiac allograft rejection. *Transplantation*, 69: 639-648.
- Jones ND, van Maurik A, Hara M, Gilot BJ, Morris PJ & Wood KJ (1999). T-cell activation, proliferation, and memory after cardiac transplantation *in vivo*. *Annals of Surgery*, 229: 570-578.
- Bishop DK, Wood SC, Eichwald EJ & Oroz CG (2001). Immunobiology of allograft rejection in the absence of IFN- γ : CD8⁺ effector cells develop independently of CD4⁺ cells and CD40-CD40 ligand interactions. *Journal of Immunology*, 166: 3248-3255.
- Osorio RW, Ascher NL & Stock PG (1994). Prolongation of *in vivo* mouse islet allograft survival by modulation of MHC class I antigen. *Transplantation*, 57: 783-788.
- Osorio RW, Ascher NL, Jaenisch R, Freise CE, Roberts JP & Stock PG (1993). Major histocompatibility complex class I deficiency prolongs islet allograft survival. *Diabetes*, 42: 1520-1527.
- Desai NM, Bassiri H, Kim J, Koller BH, Smithies O, Barker CF, Naji A & Markmann JF (1993). Islet allograft, islet xenograft, and skin allograft survival in CD8⁺ T lymphocyte-deficient mice. *Transplantation*, 55: 718-722.
- Haskova Z, Usiu N, Pepose JS, Ferguson TA & Stuart PM (2000). CD4⁺ T cells are critical for corneal, but not skin, allograft rejection. *Transplantation*, 69: 483-487.
- Boisgérault F, Liu Y, Anosova N, Ehrlich E, Dana MR & Benichou G (2001). Role of CD4⁺ and CD8⁺ T cells in allorecognition: lessons from corneal transplantation. *Journal of Immunology*, 167: 1891-1899.
- Wiseman AC, Pietra BA, Kelly BP, Rayat GR, Rizeq M & Gill RG (2001). Donor IFN- γ receptors are critical for acute CD4⁺ cell-mediated cardiac allograft rejection. *Journal of Immunology*, 167: 5457-5463.
- Fischbein MP, Yin J, Laks H, Irie Y, Fishbein MC, Espejo M, Bonavida B & Ardehali A (2001). CD8⁺ lymphocytes augment chronic rejection in a MHC class II mismatched model. *Transplantation*, 71: 1146-1153.
- Ogura Y, Martinez OM, Villanueva JC, Tait JF, Strauss HW, Higgins JPT, Tanaka K, Esquivel CO, Blankenberg FG & Krams SM (2001). Apoptosis and allograft rejection in the absence of CD8 T cells. *Transplantation*, 71: 1827-1834.
- Hamann D, Roos MTL & van Lier RAW (1999). Faces and phases of human CD8⁺ T cell development. *Immunology Today*,

- 20: 177-180.
20. Shresta S, Pham CTN, Thomas DA, Graubert TA & Ley TJ (1998). How do cytotoxic lymphocytes kill their targets? *Current Opinion in Immunology*, 10: 581-587.
 21. Strehlau J, Pavlakis M, Lipman M, Shapiro M, Vasconcellos L, Harmon W & Strom TB (1997). Quantitative detection of immune activation transcripts as a diagnostic tool in kidney transplantation. *Proceedings of the National Academy of Sciences, USA*, 94: 695-700.
 22. Diamond AS & Gill RG (2000). An essential contribution by IFN- γ to CD8⁺ T cell-mediated rejection of pancreatic islet allografts. *Journal of Immunology*, 165: 247-255.
 23. Nagano H, Mitchell RN, Taylor MK, Hasegawa S, Tilney N & Libby P (1997). Interferon- γ deficiency prevents coronary arteriosclerosis but not myocardial rejection in transplanted mouse hearts. *Journal of Clinical Investigation*, 100: 550-557.
 24. Kamoun M (2001). Cellular and molecular parameters in human renal allograft rejection. *Clinical Biochemistry*, 34: 29-34.
 25. Braun MY, Desalle F, Le Moine A, Pretolani M, Matthys P, Kiss R & Goldman M (2000). IL-5 and eosinophils mediate the rejection of fully histoincompatible vascularized cardiac allografts: regulatory role of alloreactive CD8⁺ T lymphocytes and IFN- γ . *European Journal of Immunology*, 30: 1290-1296.
 26. Smyth MJ, Kelly JM, Sutton VR, Davis JE, Browne KA, Sayers TJ & Trapani JA (2001). Unlocking the secrets of cytotoxic granule proteins. *Journal of Leukocyte Biology*, 70: 18-27.
 27. Kapoor A, Morita K, Engeman TM, Koga S, Vapnek EM, Hobart MG & Fairchild RL (2000). Early expression of interferon- γ inducible protein 10 and monokine induced by interferon- γ in cardiac allografts is mediated by CD8⁺ T cells. *Transplantation*, 69: 1147-1155.
 28. Hancock W, Gao W, Faia KL & Csizmadia V (2000). Chemokines and their receptors in allograft rejection. *Current Opinion in Immunology*, 12: 511-516.
 29. Watarai Y, Koga S, Paolone DR, Engeman TM, Tannenbaum C, Hamilton TA & Fairchild RL (2000). Intraallograft chemokine RNA and protein during rejection of MHC-matched/multiple minor histocompatibility-disparate skin grafts. *Journal of Immunology*, 164: 6027-6033.
 30. Robinson LA, Nataraj C, Thomas DW, Howell DN, Griffiths R, Bautch V, Patel DD, Feng L & Coffman TM (2000). A role for fractalkine and its receptor (CX3CR1) in cardiac allograft rejection. *Journal of Immunology*, 165: 6067-6072.
 31. Fahy O, Porte H, Sénéchal S, Vorng H, McEuen AR, Buckley MG, Walls AF, Wallaert B, Tonnel AB & Tsiocopoulos A (2001). Chemokine-induced cutaneous inflammatory cell infiltration in a model of Hu-PBMC-SCID mice grafted with human skin. *American Journal of Pathology*, 158: 1053-1063.
 32. Dengler TJ & Pober JS (2000). Human vascular endothelial cells stimulate memory but not naïve CD8⁺ T cells to differentiate into CTL retaining an early activation phenotype. *Journal of Immunology*, 164: 5146-5155.
 33. Dengler TJ, Johnson DR & Pober JS (2001). Human vascular endothelial cells stimulate a lower frequency of alloreactive CD8⁺ pre-CTL and induce less clonal expansion than matching B lymphoblastoid cells: development of a novel limiting dilution analysis method based on CFSE labelling of lymphocytes. *Journal of Immunology*, 166: 3846-3854.
 34. Légaré JF, Issekutz T, Lee TDG & Hirsch G (2000). CD8⁺ T lymphocytes mediate destruction of the vascular media in a model of chronic rejection. *American Journal of Pathology*, 157: 859-865.
 35. Rothstein DM, Livak MFA, Kishimoto K, Ariyan C, Qian HY, Fecteau S, Sho M, Deng S, Zheng XX, Sayegh MH & Basadonna GP (2001). Targeting signal 1 through CD45RB synergizes with CD40 ligand blockade and promotes long term engraftment and tolerance in stringent transplant models. *Journal of Immunology*, 166: 322-329.
 36. Ensminger SM, Spriewald BM, Witzke O, Morrison K, van Maurik A, Morris PJ, Rose ML & Wood KJ (2000). Intra-graft interleukin-4 mRNA expression after short-term CD154 blockade may trigger delayed development of transplant arteriosclerosis in the absence of CD8⁺ T cells. *Transplantation*, 70: 955-963.
 37. Ensminger SM, Witzke O, Spriewald BM, Morrison K, Morris PJ, Rose ML & Wood KJ (2000). CD8⁺ T cells contribute to the development of transplant arteriosclerosis despite CD154 blockade. *Transplantation*, 69: 2609-2612.
 38. Jones ND, van Maurik A, Hara M, Spriewald BM, Witzke O, Morris PJ & Wood KJ (2000). CD40-CD40 ligand-independent activation of CD8⁺ T cells can trigger allograft rejection. *Journal of Immunology*, 165: 1111-1118.
 39. Honey K, Cobbold SP & Waldmann H (1999). CD40 ligand blockade induces CD4⁺ T tolerance and linked suppression. *Journal of Immunology*, 163: 4805-4810.
 40. Trambley J, Bingaman AW, Lin A, Elwood ET, Waitze SY, Ha J, Durham MM, Corbascio M, Cowan SR, Pearson TC & Larsen CP (1999). Asialo GM1⁺ CD8⁺ T cells play a critical role in costimulation blockade-resistant allograft rejection. *Journal of Clinical Investigation*, 104: 1715-1722.
 41. Bierer BE, Hollander G, Fruman D & Burakoff SJ (1993). Cyclosporin A and FK506: molecular mechanisms of immunosuppression and probes for transplantation biology. *Current Opinion in Immunology*, 5: 763-773.
 42. van Hoffen E, van Wichen DF, Leemans JC, Broekhuizen RA, Bruggink AH, De Boer M, De Jonge N, Kirkels H, Slootweg PJ, Gmelig-Meyling FH & De Weger RA (1998). T cell apoptosis in human heart allografts: association with lack of co-stimulation? *American Journal of Pathology*, 153: 1813-1824.
 43. Chen H, Luo H, Xu D, Loh DY, Daloze PM, Veillette A, Qi S & Wu J (1996). Impaired signaling in alloantigen-specific CD8⁺ T cells tolerized *in vivo*. *Journal of Immunology*, 157: 4297-4308.
 44. Slavik JM, Lim DG, Burakoff SJ & Hafler DA (2001). Uncoupling p70^{S6} kinase activation and proliferation: Rapamycin-resistant proliferation of human CD8⁺ T lymphocytes. *Journal of Immunology*, 166: 3201-3209.
 45. Gershon RK, Cohen P, Hencin R & Libhaber AS (1972). Suppressor T cells. *Journal of Immunology*, 108: 586-590.
 46. Cobbold S & Waldmann H (1998). Infectious tolerance. *Current Opinion in Immunology*, 10: 518-524.
 47. Zhai Y & Kupiec-Weglinski J (1999). What is the role of regulatory T cells in transplantation tolerance? *Current Opinion in Immunology*, 11: 497-503.
 48. Ciubotariu R, Vasilescu R, Ho E, Cinti P, Cancedada C, Poli L, Late M, Liu Z, Berloco P, Cortesini R & Suci-Foca Cortesini N (2001). Detection of T suppressor cells in patients with organ allografts. *Human Immunology*, 62: 15-20.
 49. Zhang ZX, Yang L, Young K, Temple B & Zhang L (2000). Identification of a previously unknown antigen-specific regulatory T cell and its mechanism of suppression. *Nature Medicine*, 6: 782-789.
 50. Vukmanovic-Stejic M, Thomas MJ, Noble TA & Kemeny DM (2001). Specificity, restriction and effector mechanisms of immunoregulatory CD8⁺ T cells. *Immunology*, 102: 115-122.

51. Vignes C, Chiffolleau E, Douillard P, Josien R, Peche H, Heslan JM, Usal C, Souillow JP & Cuturi MC (2000). Anti-TCR-specific DNA vaccination demonstrates a role for a CD8⁺ T cell clone in the induction of allograft tolerance by donor-specific blood transfusion. *Journal of Immunology*, 165: 96-101.
52. Zhou J, Carr RI, Liwski RS, Stadnyk AW & Lee TDG (2001). Oral exposure to alloantigen generates intragraft CD8⁺ regulatory cells. *Journal of Immunology*, 167: 107-113.
53. Braun MY, Grandjean I, Feunou P, Duban L, Kiss R, Goldman M & Lantz O (2001). Acute rejection in the absence of cognate recognition of allograft by T cells. *Journal of Immunology*, 166: 4879-4883.
54. Lee RS, Yamada K, Houser SL, Womer KL, Maloney ME, Rose HS, Sayegh MH & Madsen JC (2001). Indirect recognition of allopeptides promotes the development of cardiac allograft vasculopathy. *Proceedings of the National Academy of Sciences, USA*, 98: 3276-3281.
55. Valujskikh A, Hartig C & Heeger PS (2001). Indirectly primed CD8⁺ T cells are a prominent component of the allogeneic T-cell repertoire after skin graft rejection in mice. *Transplantation*, 71: 418-421.
56. Benichou G (1999). Direct and indirect antigen recognition: the pathways to allograft immune rejection. *Frontiers in Bioscience*, 4: 476-480.
57. Wang B, Norbury CC, Greenwood R, Bennink JR, Yewdell JW & Frelinger JA (2001). Multiple paths for activation of naive CD8⁺ T cells: CD4-independent help. *Journal of Immunology*, 167: 1283-1289.
58. Kusaka S, Grailer AP, Fechner JH, Jankowska-Gan E, Oberley T, Sollinger HW & Burlingham WJ (2000). Clonotype analysis of human alloreactive T cells: a novel approach to studying peripheral tolerance in a transplant recipient. *Journal of Immunology*, 164: 2240-2247.
59. Benlagha K, Weiss A, Beavis A, Teyton L & Bendelac A (2000). *In vivo* identification of glycolipid antigen-specific T cells using fluorescent CD1d tetramers. *Journal of Experimental Medicine*, 191: 1895-1903.
60. Altman JD, Moss PAH, Goulder PJR, Barouch DH, McHeyzer-Williams MG, Bell JL, McMichael AJ & Davis MM (1996). Phenotypic analysis of antigen-specific T lymphocytes. *Science*, 274: 94-96.
61. Tretiakova AP, Little CS, Blank KJ & Jameson BA (2000). Rational design of cytotoxic T-cell inhibitors. *Nature Biotechnology*, 18: 984-988.
62. Choksi S, Jameson BA & Korngold R (1998). A structure-based approach to designing synthetic CD8alpha peptides that can inhibit cytotoxic T-lymphocyte responses. *Nature Medicine*, 4: 309-314.