A Novel Means of Favorably Tipping the Balance between Cytopathic and Regulatory T Cells

In this issue of *Immunity*, Zheng and coworkers present a strategy to create transplantation tolerance with three critical elements, the combined administration of rapamycin, an agonist IL-2/Fc, and an antagonist mutant IL-15/Fc cytolytic-fusion protein to inhibit cytopathic effects but retain negative regulatory T cells, to permit allograft retention in animal models.

Harnessing the innate and adaptive immune systems to provide long-lasting therapy for autoimmune diseases and to prevent allograft rejection are major goals of immunotherapy. There have been significant advances in these efforts that involve the use of monoclonal antibodies, receptor-Fc fusion proteins, cytokines, and small molecules that interrupt cytokine receptor signaling pathways. These efforts have traditionally focused on abrogating the immune-effector responses mediated predominantly by T lymphocytes including cytotoxic-effector cells. More recently, novel approaches have also addressed a series of immunological checkpoints that represent negative immunoregulatory safety mechanisms that are normally dedicated to preventing the self-reactive, destructive immune responses that lead to autoimmune diseases. Among the best studied of these brakes on the immune system is the cytotoxic T lymphocyte antigen-4 (CTLA-4), a negative costimulatory molecule. Another negative regulatory control on the immune system is mediated by the phenomenon of activation-induced cell death (AICD) (Lenardo, 1996), a process that leads to the elimination of self-reactive T cells. A third checkpoint is mediated by $CD4^+CD25^+$ negative regulatory T cells (Tregs or suppressor cells) that impede effective immune responses to antigens including transplantation antigens (Shevach, 2001).

Targeting the IL-2 and IL-15 cytokine systems that regulate the life and death of lymphocytes represents one of the emerging strategies to treat autoimmune diseases and lymphoid malignancies and to facilitate allograft retention (Waldmann and O'Shea, 1998; Waldmann et al., 2001). The goal of this particular strategy is to reduce the cytotoxic action mediated by activated immune effector T cells involved in the rejection process while maintaining the negative immunoregulatory checkpoints. IL-15 and IL-2 share two receptor subunits, IL-2Rβ as well as the γ-c subunit that is also shared by IL-4, IL-7, IL-9, and IL-21 (Waldmann and Tagaya, 1999; Leonard, 2001). In addition, each cytokine receptor system includes a private cytokine specific α subunit, IL-15Rα and IL-2Rα. As might be anticipated from their sharing of two subunits, IL-15 and IL-2 also share a number of biological activities, especially those involved in the activation of T and- NK cells. However, especially in adaptive immune responses, they also provide distinct and, at times, contrasting contributions to the survival of lymphocytes that are involved in the immune response (Waldmann et al., 2001; Zhang et al., 1998; Ku et al., 2000). In particular, IL-2 is pivotally involved in the AICD process whereas IL-15 inhibits IL-2-induced AICD. Furthermore, IL-15 stimulates the maintenance of memory phenotype T cells whereas IL-2 inhibits their persistence in vivo (Waldmann et al., 2001; Zhang et al., 1998; Ku et al., 2000). Finally IL-2 is required for the maintenance of the $CD4^+CD25^+$ negative regulatory cells (Shevach, 2001). Thus IL-15 appears to be dedicated toward the maintenance of a long-term memory and effector-immune response to antigens, an effect that impairs allograft retention. IL-2 is involved in both the initial activation of cytotoxic T cells that interfere with allograft rejection and the generation of the peripheral tolerance that is crucial for the retention of the allograft and for the prevention of autoimmune diseases. Thus, in the immunotherapy directed toward the retention of allografts, one wishes to interrupt some IL-2-mediated actions while retaining others. Previously, the individual or simultaneous administration of monoclonal antibodies directed toward IL-2Rα and IL-2Rβ has been used to modulate the actions of IL-2 and IL-15 in the treatment of autoimmune diseases and in the prevention of allograft rejection (Waldmann and O'Shea, 1998; Guex-Crosier et al., 1997).

In this issue, Zheng and coworkers propose a strategy targeting the IL-2 and IL-15 systems to inhibit the cytopathic effects that are mediated by both cytokines but also to retain the IL-2-dependent negative regulatory T cells to create transplantation tolerance to yield long-term allograft retention. These authors propose a strategy with three critical elements, which include the combined administration of rapamycin, an agonist IL-2/Fc, as well as an antagonist mutant IL-15/Fc cytolytic fusion protein to permit allograft retention in diverse animal models. The rapamycin interrupts the cytokine receptor signals mediated by IL-2 and IL-15, thereby limiting the early expansion of activated T cells, while permitting the antigen-induced induction of IL-2 and its private IL-2Rα receptor that are involved in lymphocyte apoptotic clearance, while preserving $CD4^+CD25^+$ negative immunoregulatory networks. The mutant IL-15/Fc element does not manifest IL-15 agonist functions, but rather interferes with the interaction of native IL-15 with its receptor thereby preventing the unwanted effects of IL-15, including its actions in the maintenance of effector and memory CD8 cells as well as the role of IL-15 as an inhibitor of AICD. Inclusion of the native IgG-Fc element in this cytolytic fusion protein facilitates the deletion by antibody-dependent cellular cytotoxicity (ADCC) of those activated T cells that express antigen-induced IL-15 receptors. The IL-2/Fc fusion protein is used to provide a survival factor for $CD4^+CD25^+$ regulatory T cells and to generate the IL-2-mediated agonist signals involved in apoptotic clearance of self-reactive T cells. This agent also acts to eliminate by ADCC the activated T cells that express the private IL-2Rα receptor subunit, a subunit that is present on the surface of antigen activated T cells, but not on resting T cells. IL-2Rα is also expressed by the $CD4^+CD25^+$ negative-regulatory T cells. Interestingly, neither the administration of rapamycin nor IL-2/Fc leads to the cytotoxic elimination of those negative regulatory cells that are of importance in the maintenance of the transplant. This discordance in the effects of IL-2/Fc on activated effector cells, as con-

trasted with the negative regulatory cells both expressing CD25 (IL-2Rα), was not anticipated. The sparing of the negative regulatory cells from the ADCC process may reflect the lower levels of expression of IL-2Rα on these cells when contrasted with that of fully activated effector T cells. The combined IL-2/Fc, mIL-15/Fc, and rapamycin treatment had a number of desirable effects including the hastening of the expression of IL-2Rα among activated T cells. Furthermore, as just noted, the three element combination therapy exclusively acted upon activated T cells but spared the $CD4^+CD25^+$ T cells that were resistant to the lytic effects of IL-2/Fc. The combination strategy involving IL-2/Fc, mutant IL-15/Fc, and rapamycin treatment was successful in the induction of the indefinite engraftment of MHC mismatched skin and heart allografts and also permitted the long-term engraftment of allogenic islets in nonobese diabetic (NOD) mice (Zheng et al., 2003).

The present study was developed to prevent allograft rejection but the approach may also be applicable to the treatment of autoimmune diseases where the elimination of activated cytotoxic T cells and self-reactive memory T cells with the simultaneous retention of $CD4^+CD25^+$ negative regulatory cells would be desirable. In parallel with receptor directed monoclonal antibody-mediated strategies being used to interdict the interactions of IL-2 and IL-15 with their receptors on activated T cells and memory CD8 cells, the therapeutic approach being proposed by Zheng has successfully translated the emerging insights concerning the roles of IL-2 and IL-15 cytokines and their receptors in the balance between cytopathic and regulatory cells to yield a rational approach for the treatment of autoimmune diseases and for the maintenance of allograft retention (Zheng et al., 2003; Guex-Crosier et al., 1997).

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Selected Reading

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It's a Good Year for Blimp-1 (and Plasma Cells)

Immunoglobulin secreting plasma cells are critical mediators of an effective humoral immune response. In this issue of *Immunity,* an article by Shapiro-Shelef et al. defines an essential role for the transcription factor Blimp-1 in plasma cell differentiation and preplasma memory B cell formation.

During a primary humoral response, engagement of antigen-specific receptors on naive B cells initiates a series of temporally and spatially regulated events that lead to the differentiation of both memory B cells and antibody-secreting plasma cells. As terminally differentiated effector cells, plasma cells play an essential role in the humoral immune response by producing large amounts of immunoglobulin (Ig). These antibodies act as protective effector molecules in the elimination of invading pathogens. Conversely, production of autoreactive antibodies is pathogenic in autoimmune diseases such as systemic lupus erythematosus. Therefore a better understanding of the molecular mechanisms involved in plasma cell differentiation and Ig secretion are crucial for effective drug design targeting autoimmune diseases and vaccine development.

Currently only two transcriptional regulators, X box protein-1 (XBP-1) and B lymphocyte induced maturation protein 1 (Blimp-1), have been shown to be involved in plasma cell differentiation. Chimeric mice whose lymphoid system lacks XBP-1 have a severe impairment in the production of immunoglobulin of all isotypes despite the presence of normal numbers of T and B cells. Inspection of the peripheral lymphoid tissue revealed an absence of plasma cells, demonstrating an absolute requirement for XBP-1 in B cell terminal differentiation (Reimold et al., 2001). Now an article by Shapiro-Shelef et al. in this issue of *Immunity* provides definitive proof that Blimp-1 is also essential for plasmacytic differentiation.

Blimp-1 (also called PRD1), isolated by Mark Davis' laboratory almost a decade ago, was the first transcription factor described to drive B cell differentiation to the plasma cell stage (Turner et al., 1994). This zinc finger protein is specifically expressed in a subset of germinal center B cells and plasma cells. Overexpression of Blimp-1 in the BCL1 cell line induces plasmacytic differentiation accompanied by J chain expression and Ig secretion (Calame, 2001). Unequivocal proof that Blimp-1 was required for plasma cell differentiation was missing, however, because of the early embryonic lethality of homozygous mutant embryos. Shapiro-Shelef et al. have now solved this problem. They generated mice lacking Blimp-1 in B cells by crossing mice expressing CD19-driven Cre recombinase with mice in which the