Selectively Expanding Subsets of T Cells in Mice by Injection of Interleukin-2/Antibody Complexes: Implications for Transplantation Tolerance

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ABSTRACT

The biological activity of interleukin (IL)-2 and other cytokines in vivo can be augmented by binding to certain anti-cytokine monoclonal antibodies (mAb). Here, we review evidence on how IL-2/anti–IL-2 mAb complexes can be used to cause selective stimulation and expansion of certain T-cell subsets. With some anti–IL-2 mAbs, injection of IL-2/mAb complexes leads to expansion of CD8 T effector cells but not CD4 T regulatory cells (Tregs); these complexes exert less adverse side effects than soluble IL-2 and display powerful antitumor activity. Other IL-2/mAb complexes have minimal effects on CD8 T cells but cause marked expansion of Tregs. Preconditioning mice with these complexes leads to permanent acceptance of MHC-disparate pancreatic islets in the absence of immunosuppression.

INTERLEUKIN (IL)-2 is known to control the growth and differentiation of a number of lymphocyte subsets, notably CD8 T cells, natural killer (NK) cells, and CD4 T regulatory cells (Tregs).[1] CD8 cells play a decisive role in eliminating tumor cells, and injection of IL-2 has emerged as a valuable clinical tool for cancer immunotherapy.[2] However, a problem with this approach is that IL-2 can cause toxic adverse effects, including severe pulmonary edema and liver cell damage. Another problem is that IL-2 causes parallel expansion of Tregs able to blunt antitumor immune responses, thereby limiting the beneficial effects of expanding tumor-reactive CD8 cells.[3] In addition to cancer immunotherapy, IL-2 injection is potentially useful for organ transplantation, ie, by augmenting the suppressive activity of Tregs. Here again the problem is that the beneficial effect of expanding Tregs is countered by stimulation of CD8 effector cells. In this article we provide an update on how these problems can be largely overcome by injecting IL-2 complexed with certain anti-IL-2 monoclonal antibodies (mAb). This work has been described in detail elsewhere.[4–8]

SELECTIVE EXPANSION OF CD8 CELLS VERSUS TREGS

Initial studies with S4B6 anti–IL-2 mAb demonstrated that the surprising capacity of this mAb to cause expansion of memory-phenotype (MP) CD8 cells in vivo after injection required the presence of endogenous IL-2.[4] This finding led to the discovery that injecting mice with preformed complexes of IL-2 and S4B6 mAb caused much greater proliferation and expansion of CD8 cells than injection of either reagent alone.[4] Although injection of such IL-2/S4B6 complexes also causes expansion of NK cells, there was little or no expansion of Tregs, which contrasted with prominent Treg stimulation after injection of soluble IL-2 alone. The results observed with S4B6 were observed with several other anti-IL-2 mAbs. However, quite different findings applied with another anti–IL-2 mAb, JES6-1. Here, the intriguing finding was that injection of IL-2/JES6-1 complexes had the opposite effect, ie, strong stimulation of Tregs but minimal stimulation of CD8 cells. These contrasting results appear to reflect that the IL-2 receptors (IL-2Rs)

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on CD8 cells and Tregs are different. Because of their activated state, Tregs express typical high-affinity $\alpha\beta\gamma$ IL-2Rs. By contrast, normal resting MP CD8 cells (and also naïve CD8 cells) express low-affinity $\beta\gamma$ IL-2Rs. Our working hypothesis is that binding of S4B6 to IL-2 occludes IL-2 interaction with IL-2Rα but allows interaction with IL-2R$\beta\gamma$, thereby enabling IL-2/S4B6 complexes to preferentially stimulate CD8 cells but not Tregs. For JES6-1, however, we envisage that this mAb has considerable, although incomplete IL-2–neutralizing activity, thereby restricting stimulation by IL-2/JES6-1 complexes to cells expressing high-affinity IL-2Rs (Tregs); stimulation of cells expressing low-affinity IL-2Rs (CD8 cells) is negligible.

MECHANISMS CONTROLLING THE STRONG POTENCY OF IL-2/MAB COMPLEXES

The capacity of anticytokine mAbs to boost the biological activity of cytokines in vivo applies not only to IL-2 but also to certain other cytokines, notably IL-7 and IL-4.[4,5] Anticytokine mAbs are known to act in part by increasing the half-life of cytokines.[9] In the case of IL-2 and S4B6 mAb, however, recent work has shown that the conspicuous potency of IL-2/S4B6 complexes cannot be explained solely by a prolongation of IL-2 half-life. Thus, increasing the half-life of IL-2 by injecting IL-2-Fc fusion protein (IL-2 FP) had only a mild potentiating effect, the activity of IL-2 FP in vivo being much weaker than that of IL-2/S4B6 complexes.[6] Hence, the marked augmenting effect of S4B6 mAb on IL-2 function appeared to involve an additional mechanism. Probing this issue revealed that IL-2 function in vivo was limited by rapid removal of IL-2 by cells expressing IL-2Rα (CD25), including not only activated T cells but also nonimmune cells such as endothelial cells in the lung.[6,7] In support of this notion, the limited potency of soluble IL-2 after injection was mildly enhanced by co-injection of anti-CD25 mAb. The notable finding, however, was that the activity of IL-2 FP was greatly enhanced by co-injection of anti-CD25 mAb. Indeed, the activity of injections of IL-2 FP plus anti-CD25 mAb approached the potency of IL-2/S4B6 complexes. Hence, the boosting effect of S4B6 reflected a combination of increasing IL-2 half-life plus blocking the absorption of IL-2 by CD25+ cells. For JES6-1 mAb, by contrast, this mAb seemed to act mainly by increasing IL-2 half-life.

APPLICATIONS

For cancer immunotherapy, it was mentioned earlier that using IL-2 to boost antitumor responses has 2 serious drawbacks, namely, induction of pulmonary edema and stimulation of Tregs. Injecting mice with IL-2 bound to an S4B6-like anti-human IL-2 mAb solves both problems.[7] Thus, the capacity of this mAb to prevent IL-2 binding to CD25 has the joint benefit of preventing stimulation of Tregs and decreasing activation of CD25+ lung endothelial cells. Injection of such IL-2/S4B6-like mAb complexes thereby targets IL-2 selectively to CD8 effector cells with minimal induction of pulmonary edema. With these properties, IL-2/S4B6 complexes have proved highly effective for tumor immunotherapy in preclinical animal models of melanoma.[7]

With regard to organ transplantation, recent work has shown that a course of 3 daily injections of IL-2/JES6-1 complexes in mice is sufficient to cause numbers of Tregs to increase by 10- to 20 fold.[8] This marked increase in Treg counts induces transient immunosuppression and allows long-term acceptance of pancreatic islets in the strong

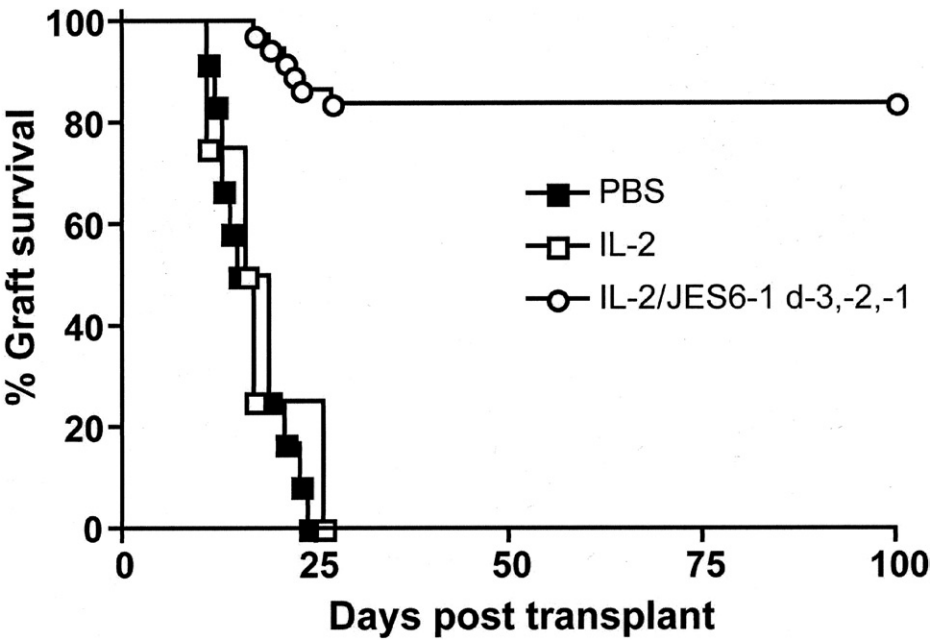

Fig 1. IL-2/JES6-1 treatment induces long-term allograft survival. Streptozotocin-induced diabetic C57BL/6 ($H2^b$) mice were treated with PBS (n = 10), IL-2 (n = 4), or IL-2/JES6-1 (1 mg/5 mg, n = 34) complexes on days -3, -2, and -1 (1 mg/injection). On day 0, mice were transplanted with BALB/c ($H2^d$) islets under the renal capsule. Blood glucose levels (BGL) were monitored as a measure of graft function and survival. Grafts were considered rejected after 2 consecutive BGL >16 mmol/L following a period of normoglycemia. Data adapted from Reference [8].

MHC-different BALB/c → B6 combination. Although around 15% of the grafts are rejected within the first month, the remainder of the grafts are accepted indefinitely (Fig 1). This finding is unexpected because Treg numbers return to normal within 2 weeks; moreover, the hosts are not treated with immunosuppressive drugs. Why the grafts are not rejected is still a mystery because T cells from the host display strong reactivity to the donor in vitro and are able to reject donor islets after transfer to secondary hosts. One possibility is that tolerance is maintained by residual Tregs in or near the grafts. Another idea is that, after healing, the grafts eventually become part of "self" and cannot be sensed by host T cells unless there is some local inflammation. We are currently testing these ideas. Whatever the explanation, the results would seem to have considerable potential for human organ transplantation. We caution, however, that some mice (15%) did show early graft rejection. This problem might be avoided by supplementing IL-2/JES6-1 injection with rapamycin; the latter treatment proved highly effective in preventing the onset of disease in an experimental autoimmune encephalomyelitis (EAE) model.[8] It should also be noted that acceptance of allografts after IL-2/JES6-1 injection is much less effective for skin than pancreatic islets. Whether the results for skin can be improved by addition of rapamycin is under investigation.

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