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# CONFERENCE REPORT

# Novel Insights in the Regulation of the Immune System:

## A Report on the FASEB Summer Research Conference on Autoimmunity

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## ■ Abstract

The bi-annual FASEB autoimmunity conference organized last year by Betty Diamond and Stephen Miller brought together some 150 delegates studying various aspects of autoimmune diseases such as lupus, rheumatoid arthritis and autoimmune diabetes. The conference provided numerous insights into the latest research on autoimmunity and answered many basic research type questions that are important for understanding the complex nature of these diseases. Because some time has elapsed since the conference, data from a number of talks has already been published [1-11]. Thus, I will present an overview of some of the most interesting and at the same time, still unpublished data on T cells presented at the conference. The balance between tolerance and immunity is controlled through a variety of mechanisms such as the presence or absence of co-stimulation or negative regulation of a T cell response. $CD4^+CD25^+$ regulatory T cells were also a focus of interest. Talks that I will discuss focused on the role of molecules such as GITR, Foxp3 and B7 for the development and function of regulatory T cells and the importance of these molecules in the prevention of autoimmunity. As well, a novel form of CTLA-4 and the use of 4-1BB co-stimulation blockade for the control of autoimmunity will be discussed.

**Keywords**: immune system · autoimmunity · T cells · regulation · co-stimulation

## Regulatory T cells

In the past several years, regulatory T cells (Treg) have come to the forefront of immunology research, as they have been phenotypically characterized, isolated and their function demonstrated in vivo and in vitro. This was evident during the FASEB autoimmunity conference where they were the focus of many presentations. It was thus perhaps appropriate that the conference was opened with a keynote address by Ethan Shevach (NIH/NIAID).

Although the existence of Treg has become well accepted, their function in the immune system is still controversial. Dr. Shevach thinks that their main purpose is not only to control autoimmunity (as is generally believed) but rather to control immune responses during infections and to limit hyperinflammation. In a published study, he demonstrated that depletion of $CD4^+CD25^+$ T cells was not sufficient for the induction of autoimmune gastritis, rather secondary events such as nonspecific (lymphopenia induced) proliferation, infection, or inflammation are also required [12]. How do Treg control responses to pathogens? E. Shevach presented data on the molecule GITR, a member of the tumor necrosis receptor family that is expressed on the surface of Treg and functions to control their suppressive effects. GITR is triggered by its ligand, GITR-L that is expressed on B1 lymphocytes. In a steady state, binding of the GITR-L on B1 cells to GITR on Treg blocks their function perhaps by keeping them away from other T cells that they could regulate or by sending an inhibitory signal to the Treg. "Danger signals" associated with an infection or autoimmune response such as LPS, CpGs and/or anti-CD40 result in the down-regulation of GITR-L on B1 cells, allowing the $CD4^+CD25^+$ Treg to perform their

regulatory functions and limit hyperinflammation during an immune response.

One of the key recent discoveries in the field of Treg has been the characterization of the forkhead transcription factor Foxp3 presented at the conference by Jason Fontenot from Alexander Rudensky's lab. Foxp3 is the first molecule to be identified that is specifically expressed in $CD4^+CD25^+$ Treg and is crucial for their development [13-15]. Mutations in this molecule result in a lethal autoimmune syndrome in mice that could be prevented by an adoptive transfer of $CD4^+CD25^+$ T cells. Furthermore, ectopic expression of Foxp3 conferred regulatory functions on $CD4^+CD25^-$ T cells. It is believed that the development of Treg results from high affinity TCR engagement in the thymus that is lower than for negative selection, but higher than for positive selection. The relatively high affinity interactions lead to the expression of Foxp3. Foxp3 may be important to rescue T cells from negative selection that would otherwise occur as a result of a strong selecting signal.

The importance of B7-1 and B7-2 for the proper functioning of Treg was an interesting and novel finding presented at the conference. Harvey Cantor's group (Harvard Medical School) used CD80/86 knockout mice to study the role of these molecules on effector T cell suppression. Co-transfer of $CD4^+CD25^+$ Treg with $CD4^+CD25^-$ T cells resulted in prevention of the normally seen autoimmunity (colitis, gastritis and dermatitis) associated with the adoptive transfer of $CD4^+CD25^-$ T cells alone. If however, the $CD4^+CD25^-$ T cells came from CD80/86-deficient animals, the $CD25^+$ Treg could not prevent disease. This was also demonstrated in vitro where Treg could not suppress proliferation of the $CD4^+CD25^-$ T cells. Retroviral transfection of $CD4^+CD25^-$ T cells with the CD80/86 molecules restored the suppressive activity of the Treg. Mice lacking one of the B7 molecules (CD80 or CD86 KO alone) had an intermediate phenotype where suppression was not as efficient as that of wild type $CD25^-$ T cells. Interestingly, the suppressive effect of Treg on $CD25^-$ T cells was not affected by fixing the Treg, suggesting that their function is mediated by an extracellular protein and does not require synthesis of new molecules. It is unclear however, how CD80/86 can have both co-stimulatory and suppressive roles. Clearly, further studies will need to characterize the molecule(s) expressed on Treg that bind CD80/86 on effector T cells. Regulation by CD80/86 could occur either directly through provision of a negative signal to the $CD25^-$ T cells or indirectly by enhancement of adhesion and contact between the $CD25^+$ and $CD25^-$ cells. It should be noted however, that CD80/86 are not expressed on naïve human T cells. Furthermore, although their expression can be induced by long-term stimulation, levels drop again as cells become quiescent [16, 17]. It remains to be seen whether these traditionally co-stimulatory molecules mediate immunosuppressive effects in humans.

Are Treg really important for prevention of human autoimmune diseases? David Hafler (Harvard Medical School) presented data from human Multiple Sclerosis patients showing that they had fewer $CD4^+CD25^+$ Treg and these had less suppressive activity than controls. Although no data on diabetic patients was shown, it is possible that people who succumb to autoimmune diabetes also have a defect in the function or number of their Treg cells. This has been shown in NOD mice that develop spontaneous diabetes, as well as other mouse strains that are susceptible to autoimmunity such as SJL [18].

## Co-stimulation

Besides the evidence for a role for B7 on Treg, there was other interesting data in the field of co-stimulation. Linda Wicker (JDRF) described interesting findings in the genetics of type 1 diabetes. Different autoimmune diseases such as rheumatoid arthritis, multiple sclerosis, type 1 diabetes and Grave's disease cluster in the same families and thus probably share some susceptibility genes. The susceptibility genes have in fact been localized to the same area of the genome, a region that contains T cell regulatory genes *CD28*, *CTLA4* and *ICOS* (inducible co-stimulator). Susceptibility genes in NOD mice have been mapped to an equivalent region of the mouse genome. Dr. Wicker and colleagues were able to further localize susceptibility to CTLA4 and this data has now been published in Nature [19]. Surprisingly, there were no polymorphisms in the coding sequence between NOD and C57Bl/6 form of CTLA4. There was however, a single nucleotide polymorphism (SNP) in exon 2 of CTLA4, which resulted in differential splicing of the molecule. The splice variant of CTLA4 (called ligand independent or liCTLA4) lacked exon 2, which is important for binding to B7. Vijay Kuchroo (Harvard Medical School) presented further work on this ligand independent form of CTLA4. In contrast to the full length (fl) CTLA4 that is expressed only after stimulation, liCTLA4 is expressed in unstimulated T cells. After T cell activation the levels go down, returning

 

back to normal about 48 hr after stimulation. liCTLA4 is a very potent inhibitor of T cell proliferation and IFN-γ production. liCTLA4 appears to be expressed at lower levels in autoimmune susceptible strains of mice such as NOD than in resistant strains. There was also preferential expression of the molecule in the regulatory $RB^{lo}$ versus $RB^{hi}$ T cell subset. liCTLA4 appears to work by associating and dephosphorylating CD3ζ since T cells retrovirally transfected with liCTLA4 had no CD3ζ phosphorylation. liCTLA4 also inhibits phosphorylation of ERK (extracellular signal-regulated kinase), MAPK (mitogen-activated protein kinase) and Jun, although the effect is not as dramatic. Thus, liCTLA4 controls the threshold of T cell activation and its disruption may contribute to autoimmunity. It will be interesting to see if human diabetic patients have a similar mutation in CTLA4 that changes its processing, perhaps also resulting in decreased levels of the liCTLA4.

Other co-stimulatory molecules besides B7 may play a role in autoimmunity, as well. Humans and mice deficient in Fas progressively develop a lymphoproliferative disease characterized by autoreactive antibodies and lupus like syndromes. Yang-Xin Fu and colleagues explored the use of agonistic anti 4-1BB (CD137) antibodies to induce activated T cells to undergo AICD (activation induced cell death) [8]. Treatment with the anti-CD137 antibody led to prolonged survival of Fas-deficient mice and a block in lymphadenopathy and autoimmunity. Antibody treatment led to increased IFN-γ production, depletion of autoreactive B cells and the double negative $CD4^{-}CD8^{-}$ T cells that are characteristic of Fas-deficient mice. It is still unclear how B cells can be deleted by the anti-CD137 antibody treatment since they do not express this molecule. This effect may be indirect with the antibody inducing IFN-γ production by T cells and activating macrophages that would then affect B cells. Therefore, antibodies to co-stimulatory molecules such as CD137 may be of potential therapeutic benefit by resulting in the deletion of autoreactive lymphocytes and blocking progression of autoimmunity.

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