

SCIENCE @LERT

Suppressor T cells, rebranded as regulatory T cells, emerge from the wilderness bearing surface markers

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Regulatory T cells express the glucocorticoid-induced tumour necrosis factor receptor family related gene (GITR) and antibodies against this receptor block regulatory cell activity. Signaling through this receptor may be involved in diseases where normal immune tolerance is broken, such as inflammatory bowel disease or autoimmune gastritis.

The 1970s and early 1980s were the heyday of cellular immunology. Complicated circuits of T cells were constructed in which helper cells were inhibited by suppressor cells which in turn were inhibited by contrasuppressor cells, and so on. By and large the actual experiments involved mixing different populations of T cells and B cells together in vitro and measuring the antibody response to the major human pathogens, sheep erythrocytes, or haptenated synthetic polymers. If the response went down, suppressor cell activity was evident. Suppressor cells were considered to be a lineage of CD8+ T cells, so that CD8 cells were either cytotoxic cells or suppressor cells. In the early to mid 1980s however, suppressor T cell research fell dramatically out of fashion. As molecular immunology took over, cellular immunology was seen as unsophisticated black magic. CD4+ helper T cells were cloned, as were cytotoxic CD8 T cell clones, but no suppressor T cell clones were made. In fact cellular immunology became a term of abuse. For those of us who teach immunology to medical students the existence of suppressor T cells was an embarrassment, dealt with by the phrase “nobody believes in these cells anymore”.

Fortunately, rodents do not read editorials in eminent journals and persistently gave results in which a particular manipulation resulted in a response which went down. The best example of this is orally induced systemic tolerance, where there are hundreds of papers which show that feeding antigens induces specific unresponsiveness when the same antigen is given parenterally and you can transfer unresponsiveness to normal mice with T cells.¹ To avoid intruding into the private grief of some immunologists, these cells were termed “regulatory” T cells.

One of the most important aspects of regulatory cell activity is that it is largely based on in vivo experiments (reviewed in Maloy and Powrie²). In the example of oral tolerance, feeding animals myelin

Shimizu J, Yamazaki S, Takahashi T, et al. Stimulation of CD25(+)CD4(+) regulatory T cells through GITR breaks immunological self-tolerance. *Nat Immunol* 2002;**3**:135–42.

CD25(+)CD4(+) regulatory T cells in normal animals are engaged in the maintenance of immunological self-tolerance. We show here that glucocorticoid-induced tumor necrosis factor receptor family-related gene (GITR, also known as TNFRSF18)—a member of the tumor necrosis factor-nerve growth factor (TNF-NGF) receptor gene superfamily—is predominantly expressed on CD25(+)CD4(+) T cells and on CD25(+)CD4(+)CD8(−) thymocytes in normal naive mice. We found that stimulation of GITR abrogated CD25(+)CD4(+) T cell-mediated suppression. In addition, removal of GITR-expressing T cells or administration of a monoclonal antibody to GITR produced organ-specific autoimmune disease in otherwise normal mice. Thus, GITR plays a key role in dominant immunological self-tolerance maintained by CD25(+)CD4(+) regulatory T cells and could be a suitable molecular target for preventing or treating autoimmune disease.

McHugh RS, Whitters MJ, Piccirillo CA, et al. CD4(+)CD25(+) immunoregulatory T cells: gene expression analysis reveals a functional role for the glucocorticoid-induced TNF receptor. *Immunity* 2002;**16**:311–23.

CD4(+)CD25(+) immunoregulatory T cells represent a unique lineage of thymic-derived cells that potently suppress both in vitro and in vivo effector T cell function. We analyzed CD4(+)CD25(+) and CD4(+)CD25(−) T cells by DNA microarray, identifying 29 genes differentially expressed in the resting subpopulations, and 77 that were differentially expressed following activation. Most of these genes were elevated in the CD4(+)CD25(+) population, suggesting a previously activated phenotype. Among these were a number of genes that antagonize signaling, including members of the SOCS family, which may contribute to their anergic phenotype. Multiple cell surface receptors also had increased expression in CD4(+)CD25(+) cells, including GITR, a member of the TNF receptor superfamily. Importantly, antibodies to GITR abrogated suppression, demonstrating a functional role for this receptor in regulating the CD4(+)CD25(+) T cell subset.

basic protein (MBP), a component of the nerve sheath, induces regulatory T cells with that specificity.³ If rats are injected with MBP in Freund’s adjuvant (dead *Mycobacterium tuberculosis* mixed in thick oil) to break self tolerance and induce a massive proinflammatory Th1 response which is specific for MBP in the brain, animals develop encephalomyelitis and may die. Cotransfer of MBP specific regulatory T cells abrogates this response. Similarly, if an immunodeficient mouse is injected with small numbers of virgin CD4+ T cells, within 6–8 weeks the mouse will die of autoimmune

disease, including severe colitis.⁴ The disease can be abrogated by the cotransfer of small numbers of memory T cells. In the final model worth mentioning, injection of CD4+, CD25− cells into immunodeficient mice results in autoimmunity, including a nasty gastritis.⁵ CD4+CD25+ cells injected at the same time can prevent disease. CD25 (the alpha chain of the interleukin 2 receptor) was traditionally thought to be involved in T cell growth, but it is present on approximately 10% of virgin CD4 T cells. The biology of these cells is remarkable. In mice and humans, if CD4+, CD25−

cells are stimulated to divide in vitro with anti-CD3 antibody, the response can be ablated by the addition of a small number of CD4+, CD25+ cells.⁶ Inhibition requires activation of the CD4+,CD25+ cells and is contact dependent. One might however quite reasonably ask, is this different from the 1980s? Again, cells are being mixed together and although the readouts may be more robust, it all seems too complicated.

Two recent papers, highlighted above, now show that regulatory T cell activity is not just phenomenology but that specific receptors control their activity.^{7,8} In the first paper by Shimizu and colleagues,⁷ the authors made a monoclonal antibody against a surface molecule on regulatory CD4+CD25+ cells. They selected a particular antibody because it functionally inhibited the ability of CD4+25+ cells to inhibit the response of CD4+25- cells; in other words it reversed suppression. When they cloned the molecule recognised by this antibody, they found that it was a known molecule, namely glucocorticoid induced tumour necrosis factor receptor family related gene (GITR). GITR is highly expressed on regulatory cells and even though it does increase on other CD4 cells when they are activated, these cells do not have regulatory function. On regulatory cells, when the antibody binds to the GITR, it delivers a signal which functionally prevents the cell from downregulating the activity of its neighbours. Importantly, if you inject normal mice with the antibody, they develop autoimmune gastritis and antiparietal cell antibodies. GITR has not been well studied but its structure is similar to other members of the tumour necrosis factor receptor family such as OX40 and 1BB, which function as costimulatory molecules on T cells to maintain and regulate ongoing cell mediated immune responses.

The second paper by McHugh and colleagues⁸ reaches the same conclusion, albeit by a different route. These investigators were interested in the different

genes expressed by CD4+ CD25+ cells and CD4+CD25- cells. Taking advantage of gene microarray technology, they made RNA from each of these cell types and then screened against 11 000 oligonucleotide cDNAs on a gene chip. Twenty nine genes were differentially expressed on CD4+25+ cells, and one of them was GITR. Antibody against GITR also inhibited the function of the CD4+25+ cells. Interestingly, although they express CD25, regulatory T cells do not respond to interleukin 2. However, after antibody binding to GITR, they do respond. At the moment, the signaling events which occur after GITR ligation are not known but this is being pursued actively by many groups across the world.

CD4+CD25+ cells with regulatory activity are found in the thymus medulla and express high levels of GITR, and therefore there is evidence that they represent a real cell lineage. Taken together with other data, it now appears that this population controls the function of potentially self reactive T cells in the periphery. There are very good reasons why we need a mechanism to censor self reactive T cells in the periphery. Clonal deletion in the thymus eliminates T cells with receptors which have a high affinity for self-MHC and allows those with moderate affinity to leave, but these are still potentially self reactive. T cell activation needs not only T cell receptor activation but costimulation through accessory molecules such as CD4, LFA1, CD28, LFA-3, OX40, and ICOS. It is thus possible to envisage that a high degree of costimulation might allow even a low affinity self reactive T cell receptor to pass the threshold for activation, and this needs to be prevented. In mice it is not clear why this should result in an autoimmune gastritis, and not a colitis.

It is still too early to say what this means for gastroenterology. The lesson from mouse models of colitis is that the normal flora can clearly drive chronic inflammation when the immune system is dysregulated.² There is the possibility that some degree of tolerance involving regulatory T cells is involved in prevent-

ing tissue damaging responses to the flora.⁹ However, the normal human intestinal mucosa is packed with activated CD4 Th1 cells in the lamina propria and cytolytic CD8+ effectors in the epithelium, and yet remains disease free,¹⁰ so any regulation would have to be at the effector phase and not the induction phase of the mucosal immune response.

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