Title
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Journal
Immunity, 42(5)

ISSN
1074-7613

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Publication Date
2015-05-01

DOI
10.1016/j.immuni.2015.05.002

Peer reviewed

CellPress

IL-2: Change Structure … Change Function

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http://dx.doi.org/10.1016/j.immuni.2015.05.002

In this issue of *Immunity*, Spangler et al. and Mitra et al. demonstrate how structural changes in the IL-2 molecule alter interactions with the IL-2 receptor, leading to differential cellular targeting and biochemical responses and selective immune consequences.

Interleukin-2 (IL-2) is a four-helix bundle, type I cytokine that functions as a growth factor for a wide range of leukocytes. IL-2 was originally used therapeutically as an immune stimulatory agent due to its ability to enhance T effector (Teff) and NK cell function (Smith 1988). Recombinant human IL-2 (Proleukin) was initially used at high doses to treat metastatic melanoma and renal cell carcinoma. However, only a small subset of patients (5%–10%) respond to such treatment, and adverse effects of high-dose IL-2 therapy limit its use (Rosenberg, 2014). In recent years, it has become clear that IL-2 is a critical cytokine for regulatory T (Treg) cell differentiation, function, and survival. In fact, the combination of genetic disruption and IL-2 therapy studies led to the surprising conclusion that IL-2 is more important in the control than in the promotion of immune responses (Malek, 2008). IL-2 signaling and downstream gene activation of Treg cells occurs at lower IL-2 concentrations as compared to Teff or natural killer (NK) cells. Capitalizing on these observations, an increasing number of animal and human studies have demonstrated that low doses of IL-2 can preferentially expand Treg cells in vivo and suppress graft-versus-host disease (GVHD) and autoimmunity. As such, low-dose IL-2 therapy represents a novel approach to immune modulation for the treatment of disease (Yu et al., 2015).

In 2006, Boyman and Sprent first reported that IL-2 binding antibodies (JES6-1 and S4B6 mAbs) could promote the expansion of opposing cell types in vivo. Much like low-dose IL-2 therapy, JES6-1 promotes the expansion of Treg cells, whereas a distinct antibody, S4B6, preferentially expands effector T cells, akin to high-dose IL-2 therapy (Boyman et al., 2006). Subsequently, JES6-1 in complex with IL-2 has been efficacious in treating many mouse models of autoimmune disease and inflammation (Tang et al., 2008). A major hypothesis put forward to explain these results was that the different mAb affinity and binding sites lead to distinct half-lives of the IL-2, resulting in selective engagement and activation of distinct T cell subsets. However, it remained possible that the antibody binding to IL-2 could induce a conformational change that altered IL-2 binding to the IL-2 receptor. In this regard, in 2012, the Garcia lab identified mutations in human IL-2 that stabilized certain IL-2 conformations with higher binding affinity for IL-2Rβ by locking a flexible helix within IL-2's binding site for IL-2Rβ. This IL-2 mutant, termed "super-2" or H9, proved to be 1–2 logs more potent in inducing pSTAT5 activation of cells lacking IL-2Rα than wild-type IL-2 (Levin et al., 2012). In this issue of *Immunity*, two groups have interrogated the consequences of alterations of IL-2 structure on T cell activation. In the paper by Spangler et al. (2015), the investigators demonstrated the key structural changes induced by mAb binding to IL-2 that leads to selective Treg or Teff cell induction, and in Mitra et al. (2015), the investigators used mutational analysis of IL-2 to develop a molecule that can act as a partial agonist and/or antagonist to blunt immune responses. These two papers extend our understanding of IL-2 signaling and the potential to develop novel IL-2-based therapeutics to treat diseases.

Central to understanding the seemingly divergent activity of IL-2 is an understanding of its interaction with the IL-2 receptor. After antigen stimulation, surface expression of interleukin-2 receptor α chain (IL-2Rα) sensitizes T cells to low concentrations of IL-2 by capturing the cytokine and presenting it first to IL-2Rβ chain, and then the γc chain to form the IL-2R trimeric complex. Treg cells express high amounts of IL-2Rα constitutively. However, for some cell types, such as NK cells and memory $CD8^+$ T cells, IL-2Rα is virtually absent and signaling occurs predominantly through the low-affinity IL-2Rβ-γ_c dimer; thus, stimulation requires much higher levels of IL-2. The paper by Spangler et al. (2015) describes the detailed molecular mechanism of action by which two IL-2 binding antibodies, JES6-1 and S4B6, exert their cell-targeted activity. Spangler et al. (2015) solved the crystal structure of JES6-1 in complex with mouse IL-2 and observed that JES6-1 binds to IL-2 in a way that sterically blocks binding of IL-2Rβ and IL-2R-γ_c. Unexpectedly, binding of JES6-1 also induces an allosteric change in the portion of IL-2 that binds IL-2Rα, resulting in an overall reduced binding affinity for IL-2Rα. Thus, only cells with high levels of IL-2Rα, such as Treg cells, can bind the JES6-1:IL-2 complex. The binding of IL-2:JES6 to IL-2Rα then prompts the dissociation of JES6-1 from IL-2, permitting normal signaling to occur. Cells stimulated with JES6-1:IL-2 complexes also upregulate IL-2Rα, lending an additional layer of transcriptional regulation. In contrast, the binding of S4B6 to IL-2 completely blocks its interaction with IL-2Rα. However, S4B6 binding induces a slight change in the conformation of IL-2, resulting in increased affinity for binding to IL-2Rβ and increased stability of the complex bound to IL-2Rβ. Effector cells with high IL-2Rβ expression are therefore favored with S4B6:IL-2 complexes. The implications of these studies are significant: it goes beyond previous hypotheses to explain the divergent activities of the two prototypic antibodies, by demonstrating that conformation changes in the IL-2

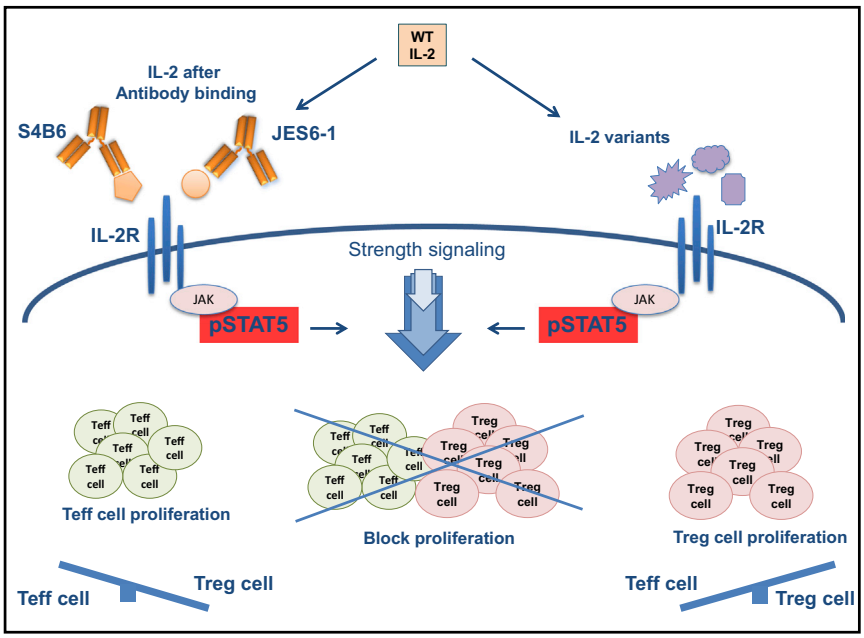

Figure 1. Different Approaches to Change IL-2 Conformation to Alter Function

On the left side, binding of JES6-1 to IL-2 changes the conformation of the molecule resulting in reduced IL-2Rα binding affinity. Only cells with high levels of IL-2Rα (such as Treg cells) can bind the JES6-1:IL-2 complex efficiently. The opposite function is elicited by S4B6, which increases IL-2 affinity for IL-2Rβ, resulting in effector T cell activation. The right side illustrates a different, mutational approach to generate distinct IL-2 variants, which alters IL-2R-γ_c binding. The affinity of the interaction between these mutants and the γ_c regulates signal intensity, generating IL-2 molecules that can more potently activate pSTAT5 or completely block Treg cell differentiation and induce proliferation of pre-activated $CD8^+$ T cells. Both of these approaches generate IL-2 molecules that can function as agonists or antagonists depending on cell type.

molecule alter its binding kinetics and signaling properties favoring one cell type over another (Spangler et al., 2015).

In the second paper in this issue, Mitra et al. (2015) have exploited recent structural analyses of IL-2 to develop new IL-2 variants to alter IL-2R signaling. Built on the super-2 backbone, the investigators made additional mutations that alter binding to IL-2R-γ_c, leading to a heterodimerization defect. By modulating the severity of the defect, a series of IL-2 molecules was developed that function as partial agonists, and even a functional antagonist. Because of their increased binding to IL-2Rβ, these molecules engage cells dominantly over endogenous IL-2, and their levels of interaction with the γ_c regulate signal intensity. As proof of principle, Mitra et al. (2015) showed that freshly isolated $CD8^+$ T cells and pre-activated $CD8^+$ T cells have distinct activation thresholds for IL-2 signaling, demonstrated by the differential effects of two muteins, H9-T and H9-RET. The H9-T mutein more potently activated STAT5 phosphorylation on activated T cells than on naive cells. On the other hand, H9-RET almost completely blocked Treg cell differentiation and could induce proliferation of pre-activated $CD8^+$ T cells. Conversely, the mutein H9-RETR, which is an extremely weak partial agonist, was capable of inhibiting the activity of wild-type IL-2. Mitra et al. (2015) also showed that the "null" H9-RETR mutein blocked IL-2Rα induction, prolonged GVHD survival, and strongly inhibited the spontaneous proliferation of adult T cell leukemia cells. H9-RETR, which is specific for IL-2Rβ but independent of IL-2Rα, might offer a novel approach for treatment of autoimmune diseases and organ rejection, potentially even in combination with antibodies that block IL-2Rα to disrupt the signaling of the high-affinity IL-2 receptor (Mitra et al., 2015).

Cytokines and cytokine receptors have long been recognized as intriguing targets of therapeutic intervention. The therapeutic conundrum lies in the fact that seemingly opposing effects can be achieved with the same molecule, as with IL-2 therapy described above. Cytokine receptor sub-unit expression varies on different cell types, and receptor sub-units can be shared by different cytokines, adding to the complexity. To date, the relatively blunt therapeutic approach of a neutralizing antibody or soluble receptor fusion protein has left little room for finesse. The approaches discussed here, of using antibodies to bias cytokines to specific cells or of manipulating the cytokine itself to fine-tune the signaling outcome, provide new tools, which will allow for a more nuanced understanding of cytokine biology (Figure 1). However, it should be noted that there might be additional implications of these alterations that could lead to unexpected consequences. For instance, recent studies have shown that innate lymphoid cells 2 (ILC2s) express high amounts of IL-2Rα and are activated by low-dose IL-2 in mouse and humans, which can lead to eosinophilia (Van Gool et al., 2014). Also, it remains possible, as suggested by Bayer et al. (2013), that IL-2 signaling is distinct in different T cell subsets, and therefore, simple alterations of IL-2 binding to the IL-2R might not lead to predictable changes of IL-2 function in vivo. Finally, the studies discussed here suggest that appropriate antibodies or muteins could lead to partial IL-2R agonists that "tune" IL-2 signaling to induce desired functional properties while avoiding thresholds for undesired responses. Moreover, the results provide a road map to the development of anti-human IL-2 antibodies that can be developed to selectively promote regulation or effector function depending on the clinical setting. These efforts could be extremely powerful in deepening our understanding of the biological functions of this pleiotropic cytokine, lead to distinctive therapeutic benefits depending on the context, and provide a blueprint from which to engineer analogous molecules in other cytokine/cytokine receptor systems. Most importantly, it is interesting to speculate that should such IL-2 molecules or anti-IL-2:IL-2 complexes be therapeutically viable, the potential clinical applications might be quite broad, perhaps even adaptable to cancer and autoimmunity and also to non-immune diseases such as Alzheimer's, Parkinson's, cardiac diseases, or NASH, where chronic inflammation has been shown to be pathogenic and immune suppression via Treg cells to be

therapeutic. Therefore, these studies validate the adage that appearance is more than skin deep and that structure can influence function.

ACKNOWLEDGMENTS

N.C. is an employee of Pfizer, Inc., and J.B. and E.T. receive funding from Pfizer CTI to study Treg cell promoting therapies.

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New Lamp Posts Allow for New Views of the Immunological Synapse

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http://dx.doi.org/10.1016/j.immuni.2015.05.008

Using new rapid, super-resolution imaging methods, Ritter et al. (2015) define the early events of immunological synapse formation and granule release.

A hallmark of the adaptive immune response is T cell interaction with antigen-presenting cells (APCs). An important concept established over the last 10 years is the discovery that during the process of antigen recognition, membrane and intracellular proteins become rearranged in the contact area. This rearrangement of molecules is now known as the immunological synapse.

Applying fluorescence microscopy to study the process of T cell activation was the critical tool that allowed the synapse to be discovered. Investigators determined the position of molecules in the synapse by using antibodies to stain fixed T-cell-APC conjugates or by imaging the movement of fluorescent molecules embedded in freely mobile lipid bilayers (Bromley et al., 2001). However, these types of approaches were limited by the quality and diversity of available antibodies and the availability of fluorescently labeled purified membrane proteins required for bilayer studies. The application of GFP and its derivatives enabled molecules of interest to be directly labeled and imaged in live cells. Later, the use of total internal reflection fluorescence (TIRF) microscopy, which excites a thin 100- to 200-nm layer of molecules in the plasma membrane, markedly improved the resolution and allowed for single-molecule tracking. However, the small size of the synapse (between 8 and 10 μm in diameter) and the 200- to 300-nm resolution of light microscopy still continue to limit what can be seen. Fortunately, new methods applied to imaging the synapse continue to shed new light on the cell biology of the immunological synapse.

In this issue, Ritter et al. (2015) used a variety of cutting-edge methods, including spinning-disk confocal microscopy and lattice light-sheet microscopy, to get an unparalleled look into the events that underpin synapse formation and granule secretion.

Conventional confocal microscopy uses a laser to illuminate a single point on the sample and rasters across to generate the image. Because it takes seconds to generate each optical section, events that occur in the millisecond range cannot be visualized via conventional confocal microscopy. In addition, while the image is being assembled point by point, the laser strikes the complete thickness of the specimen repeatedly but only detects a single focal plane, leading to cell toxicity and bleaching of the fluorophore. Thus, the ''efficiency'' of excitation to emission detection is quite low. However, in spinning-disk confocal microscopy, the excitation light is split through a disk with multiple pinholes, allowing for several points on the sample to be imaged at the same time. This allows for high-speed confocal imaging and reduced toxicity because of decreased repeated illumination. In both methods, moving the focal plane up and down allows a 3D image to be obtained but also significantly increases the amount of time required for generating an image.