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REVIEW

STAT3 in immune responses and inflammatory bowel diseases

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STAT3 has been known as a mediator for gene expression induced by many important cytokines. Recent studies have suggested that STAT3 has important functions in regulation of both innate and adaptive immunity. Loss of STAT3 in immune cells caused severe inflammation in response to pathogens. This review discusses the recent progress and suggests directions for the future research on this interesting molecule.

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The STAT signaling pathway

It is known that growth factors or cytokines can activate signaling cascades [1]. For example, many growth factor receptors are associated with, or have intrinsic tyrosine kinase activity. Through some adapters, activation of tyrosine kinases can further signal to the Ras protein, then to downstream serine/threonine kinases, such as the members of MAP kinase family. These serine/threonine kinases in turn can stimulate effectors such as transcription factors in the cytoplasm and nucleus. A vast amount of evidence indicates that both the second messenger and kinase cascade pathways play important roles in mediating intracellular signal transduction.

The STAT proteins initially recognized in the interferon system [2-6]. JAK belongs to a family protein tyrosine kinases that are activators of STAT proteins [7-9]. STAT proteins can interact with the receptor/tyrosine kinase complex through its SH2 domain [10-12]. Many cytokines beside IFNs can activate STAT proteins to regulate gene expression [13-15]. In addition to JAK kinase, a variety of tyrosine kinases, such as Src, and EGF receptor tyrosine kinase have been found to activate STAT proteins [16-19]. Therefore, STAT proteins mediate downstream signal transduction of a variety of protein tyrosine kinases (PTKs) [14]. Studies from our laboratory showed that STAT proteins are regulators of cell growth and apoptosis through regulation of p21 and caspases [19-21] . Many other laboratories have further shown that cyclins and the Bcl-2 family of proteins may also be regulated by STAT proteins (reviewed in [14, 22]). STAT proteins are also known to be involved in tumorigenesis [15, 18, 23].

STATs are major mediators of signaling during immune responses, and also have roles in development and cell differentiation [14, 19, 22, 24-26]. It is well established that many cytokines and STATs are involved in multiple steps in adaptive immune responses [15]. For example, STAT4 knockout mice have normal hematopoiesis, but are unable to respond to IL-12: IL-12-induced mitogenesis and enhancement of natural killer cytolytic function are defective. Most interestingly, T cell differentiation is affected by STAT4 mediated IL-12 signaling: Th1 differentiation is abrogated in STAT4-deficient mice [27, 28]. Additionally, STAT1 is essential for the function of IFN-γ as well as Th1 cell differentiation [15, 29, 30]. It was also reported recently that STAT1 is involved in Inflammatory bowel disease (IBD) [31].

Inflammatory bowel disease (IBD): Breakdown of immune tolerance

The innate immune system initiates immediate host responses to microbial antigens and acts as an effector

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by stimulating adaptive immune responses [32]. The mucosal immune system in the digestive tract is among the first lines of defense against microbial pathogens, which involves both innate and adaptive immune responses [33]. The digestive tract is not normally affected by inflammation despite its exposure to many diverse foreign antigens, indicating that there must be an essential mechanism that can suppress initiation of inflammation by enabling the mucosal immune system to tolerate foreign antigens. One important question is how the mucosal immune system in the gastrointestinal tract responds to a variety of antigens and how the decision is made between tolerance and antigen clearance. Disruption of this balance can lead to disorders in the mucosal immune system that cause severe human diseases [34].

For example, in Crohn's disease (CD) and other IBD such as colitis, chronic inflammation can occur in parts of the gastrointestinal tract. Molecular etiopathology of Crohn's disease and other IBDs have been extensively studied, and abnormalities in both innate and adaptive immune responses have been suggested [33, 35, 36]. It is likely that the loss of tolerance and uncontrolled responses to microbial antigens in the mucosal immune system is a major cause of Crohn's disease and other IBDs [37]. Recently, several groups have shown that the NOD2 gene on chromosome 16 is implicated in Crohn's disease [38-40]. NOD2 is a member of a new gene family that may mediate response to bacterial LPS and other innate signals [41-43]. Thus disruption of the innate immune response is a key factor in Crohn's disease. NOD2 is expressed in monocytes and in intestinal epithelial cells (IECs) but is most strongly expressed in Paneth cells in the terminal ileum. The NOD2 gene is expressed in monocytes, but not in lymphocytes [39], suggesting specific abnormalities of myeloid cells have decisive functions in the development of Crohn's disease. Furthermore, Crohn's disease is a complex disorder of the gut that appears to involve several associated genetic susceptibility factors, priming by the enteric microflora, and immune-mediated tissue injury [37, 44, 45]. The disease is distinguished from ulcerative colitis, by its earlier onset (average age of diagnosis is 18 years), and by the non-colon-specific granulomatous inflammation affecting any part of the gastrointestinal tract but particularly the ileocecal area. The two diseases also differ in their response to therapy: TNF-α blockade usually yields an excellent response in Crohn's disease [46, 47].

It is well established that cytokines such as TNF-α and IFN-γ are of crucial importance in the normal homeostasis of the mucosal immune system and pathogenesis of CD/IBDs [33, 48]. In particular, cytokines involved in Th1 differentiation may have a very critical role. A typical phenotype in IBDs in either human or mouse, is an exaggerated Th1 type response with excess generation of IFN-γ and TNF-α, which may result in tissue injury [33, 49-54]. Th1 cells are induced by the differentiation of activated T-cells in the presence of IL-12. During a balanced response, these effector T-cells are inhibited by IL-10-dependent regulatory T-cells [54]. Th1 cells are a major source of IFN-γ and activate cytotoxic T-cells. Thus, changes affecting the interplay between effector and regulatory T-cells could cause tissue-damage during IBD, and progression and chronic inflammation of Crohn's disease requires the adaptive immune system [45, 49]. The major questions in IBDs include how antigen/pathogen is presented by APCs, such as by dendritic cells, and whether there is a co-ordinate mechanism regulating signals from DCs to T regs and T reactive/effector cells. These two questions are important not only for understanding mechanisms of IBDs, but also for most inflammatory disorders. The major focus of our current research is to address these two questions about how mucosal immune tolerance is systematically regulated by STAT3, possibly through dendritic cells and T regulatory cells.

It is interesting to note that STAT3 was also found to be highly tyrosine phosphorylated in epithelial and lamina propria cells of human ulcerative colitis and Crohn's disease patients as well as in dextran sulfate sodium (DSS)-induced colitis in mice [55], possibly indicating a potential role of STAT3 in epithelial cells during colitis generation.

STAT3, initiation of inflammation and mammalian disorders involving cytokines

STAT3 was originally discovered as a transcription factor activated by IL-6 family cytokines through gp130 [56, 57]. The IL-6 receptor system consists of two chains: the 80 kD IL-6 receptor for ligand binding and a 130 kD co-receptor (gp130) for signal transduction. It is clear that this gp130 signaling chain is used in signal transduction by several other members of this cytokine family including IL-6, LIF, CT-1, oncostatin M (OSM), ciliary neurotrophic factor (CNTF) and IL-11 [58]. These cytokines are believed to play roles in development and normal functions of some important organs and tissues, such as heart, liver and lymphocyte differentiation. For example, disruption of gp130 leads to disorders in these systems and causes embryonic lethality [59]. Interestingly, more and more evidence have implicated STAT3 in myeloid differentiation. A number of cytokines, such as G-CSF and GM-CSF, which act in part through activation of the STAT3, are involved in myeloid differentiation. Thus STAT3 may have many important functions for hematopoiesis and immune responses.

Since classical knock-out (KO) of the STAT3 gene in mice resulted in early embryonic lethality [59], it was

difficult to study physiological roles of STAT3 in mouse models. Various cell type specific STAT3 knockouts have been established including T-cells [60] and keratinocytes [61]. Loss of STAT3 has been shown to increase sensitivities to apoptosis and certain defect in wound healing. Takeda *et al.* [62] also deleted the STAT3 gene from macrophages and neutrophils by using the macrophage-specific lysozyme promoter. STAT3 ablation in these mice results in an increased sensitivity to endotoxin/LPS shock, chronic inflammation and chronic enterocolitis with age. These mice have an over-abundance of pro-inflammatory cytokines. Th1 activity was enhanced. The phenotypes of this enterocolitis have been attributed to a block in the IL-10 signaling pathway: Only in the presence of STAT3 did IL-10 down regulate inflammatory cytokine TNF-α in macrophages [63]. Th1 activity was over-activated, although IL-10-dependent regulatory T-cells are expected to be normal in these mice, implying that the possible limitations of this system. It is likely that other cells such as dendritic cells, are also involved in directing regulatory T-cells in this regulation of Th1 cells.

In my laboratory, we have obtained conditional and tissue specific STAT3 null mice. Our evidence has shown that one strain of mice with bone marrow cell specific STAT3 KO have developed IBD/Crohn's disease (CD)-like pathogenesis [64]. Importantly, STAT3 deletion in dendritic cells in our mice may also have contributed in the development of these syndromes of inflammation. I believe that these mice provide a useful *in vivo* model system for studies of pathogenesis of inflammatory disorders and the molecular mechanism of regulation of both innate and adaptive immunity, which involve macrophages as well as dendritic cells.

The NOD2 and IL-10 genes and inflammatory bowel diseases

A number of animal models dealing with IBDs have been previously established (recently reviewed in [65]). As mentioned above, mutations in the gene encoding NOD2 result in susceptibility to Crohn's disease, particularly small intestinal disease [38, 39]. Recently, three groups have generated mice with deletions or mutations in the NOD2 gene (reviewed in [65]). Intriguingly, none of these mouse strains developed spontaneous intestinal inflammation. Even more puzzling, some of these mutant mice exhibit increased NF- B activation in response to MDP, a NOD2 ligand, which appeared to be an opposite result from what had been reported in the mice with an absence of NOD2. Recent data derived from one of the NOD2 strains have implicated an interaction between TLR2 and NOD2 as a potential explanation for the association of NOD2 and IBD.

A more interesting model of IBD is IL-10-deficient mice [66]. IL-10 is believed to be generated by regulatory T cells and plays a major role in immune suppression. IL-10 is also expressed in B cells, dendritic cells, and macrophages [67]. IL-10-deficient mice develop anemia, growth retardation, and chronic IBD with transmural leukocyte infiltration [66]. It was shown that over-produced IL-12, IFN and polarized Th1 cell activation are major mediators of inflammation in IL-10 deficient mice. When IL-10-deficient mice are raised under germ-free conditions, IBD does not occur, indicating the importance of innate interactions. A seminal finding by Akira's group revealed that STAT3 is the mediator of IL-10 for immune suppression [62]. However, the detailed mechanism is unclear. Thus, to further study mechanisms of immune suppression by IL-10, we have to address the question about how STAT3 is functioning in immune tolerance. I expect that this line of important investigations will be a direction for the future research.

The roles of T regulatory cells and dendritic cells in IBDs and immune tolerance

As discussed above, IBDs, including Crohn's disease and ulcerative colitis, are resulted from breakdown of mucosal immune tolerance [33, 54, 65]. Loss of immune tolerance may occur when self-reactive lymphocytes are not eliminated or inactivated during their development. In particular, a deregulated CD4 T cell response to a variety of antigens in the lumen may be responsible for chronic inflammation [33, 68]. Activation and expansion of self-reactive T lymphocytes can be suppressed in the periphery by CD4+CD25+ T regulatory cells (T regs). The majority of T regs constitutively expresses CD25 (IL-2 receptor α-chain) [69]. CD25+CD4+ T regs can be produced by the thymus as a functionally mature T cell subpopulation, and their defects could lead to the development of various organ-specific autoimmune diseases. T regs are also believed to maintain a balanced response to environmental antigens, thereby, preventing IBD and allergy in rodents [54]. A striking example of T regs in IBD was shown when adoptive transfer of T regs can "cure" a kind of induced colitis [70]. Further understanding of the biology of T regs and the mechanisms that control T regs differentiation may be of key importance for the development of new therapeutic strategies.

Besides T regs, dendritic cells (DCs) also play essential roles in both immune responses and tolerance [71, 72]. The primary function of dendritic cells is thought as antigen presentation, and to play a pivotal role at the intersection of innate and adaptive immunity. Recent data have shown that DCs can determine whether non-responsiveness (tol-

erance) or an active immune response occurs. Similarly intestinal DCs are critical for regulation of immune reaction or tolerance in the gut. The balanced actions of DCs in tolerance and active immunity to bacterial flora in the gut may be required for a healthy condition, while loss of DC mediated tolerance results in inflammatory responses, including Crohn's disease and ulcerative colitis [73].

In human peripheral blood, two major DC populations can be classified, namely CD11c+ DCs and CD11c- DCs. CD11c+ DCs are termed DC1 or myeloid DC. They express high levels of the granulocyte macrophage-colony stimulating factor (GM-CSF) receptor and respond to GM-CSF. In contrast, CD11c- DCs are designated as plasmacytoid or lymphoid DC [74]. They express high levels of IL-3 receptor (CD123) but little GM-CSF receptor and require activation before displaying characteristic stimulatory activity *in vitro*. They are sometimes called precursors of DC2 (pDC2) because of their relative immaturity [73]. However, mice are somewhat different: there are three major DC populations, myeloid, plasmacytoid and "lymphoid" DCs. All mouse DCs are CD11c+. DC differentiation from hematopoietic precursors has been extensively examined using *in vitro* systems. DC progeny generated *in vitro* by GM-CSF or Flt3L are now well defined: DC derived from ***GM-CSF***-supplemented cultures express cell surface antigens typically associated with CD11c+ CD11b+, or called "***myeloid***" DC [75], while DC derived from ***Flt3L***-supplemented cultures exhibit characteristics typically associated with plasmacytoid ***CD11c+ CD11b- DC*** (pDC) [76, 77]. In my laboratory we have used this approach and directly compared the activity of Flt3L versus GM-CSF on DC differentiation in the absence of STAT3 and we will continue this line of investigation.

Different DC subsets are thought to have particular functions, such as Th1/Th2 differentiation or tolerance induction. An important cytokine generated by pDC is type1 interferons (IFN-α/β), which become a characteristic of plasmacytoid DC in both humans and mice [78-80]. Mouse lymphoid DC population is characterized by expression of a homodimer of the CD8 molecule. In contrast to pDC, murine CD8+ DC is associated with elevated IL-12 production and the following induction of IFN-γ, leading to differentiation of Th1 cells [81, 82]. Understanding how these DC subsets are differentiated should provide critical information on how immune tolerance is maintained or getting breakdown in mucosal immunity. However, little was known about how these subsets of DCs are generated until recently. It is interesting to point out that we have recently shown that in the mice with STAT3 knock out in bone marrow cells, IL-12 and IFN-γ were all elevated [64], which resembles possible selective activation of CD8+ DCs. or /and absence of pDCs [83]. We found that STAT3 is an essential factor for DC differentiation in response to Flt3L. We have demonstrated that loss of STAT3 in DCs blocked Flt3, but not GM-CSF signaling for DC differentiation, which consequently significantly affects T cell differentiation and activation (unpublished results). In consistency with this idea, a recent report revealed that STAT3 in DCs is involved in production of T regs during tumorigenesis [84].

In the future, it is necessary to continue our studies on roles of STAT3 in DC differentiation and T cell function, and to define mechanisms of STAT3-controlled regulatory events in immune tolerance. The key is to reveal how STAT3 controls the immunity through DCs or T regs. It is warranted that there will be more data supporting the hypothesis and concept that STAT3 mediates function and differentiation of T regs and DCs that are responsible for maintaining intestinal immune homeostasis.

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