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Changing the STATus quo in T helper cells

Gretta L. Stritesky[1] and Mark H. Kaplan[2,*]

[1]Department of Lab Medicine and Pathology; Center for Immunology; University of Minnesota; Minneapolis, MN USA; [2]Department of Pediatrics and Department of Microbiology and Immunology; Indiana University; Indianapolis, IN USA

STAT proteins are obligate promoters of T helper cell differentiation and initial studies suggested that activation of a single STAT protein resulted in a particular phenotype. More recent work has supported a more complex paradigm wherein the activation of several STAT proteins is required for differentiation to a single effector lineage.

Key words: STAT, differentiation, CD4 T helper cell, cytokine, gene regulation

Abbreviations: IL, interleukin; STAT, signal transducer and activator of transcription; Th, T helper

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*Correspondence to: Mark H. Kaplan; Email: mkaplan2@iupui.edu

Signal Transducer and Activator of Transcription (STAT) proteins are phosphorylated by Janus (JAK) tyrosine kinases following stimulation of a cell with cytokines or growth factors. Phosphorylation of specific tyrosine residues converts latent cytoplasmic STATs into dimers that move to the nucleus, bind DNA and activate transcription. Cytokines or growth factors activate specific members of the seven member STAT family generating one component of specificity in responses to extracellular signals.

The differentiation of $CD4^+$ T helper (Th) cells into effector subsets that secrete specific cytokines is largely dependent upon the cytokine environment present when naïve T cells are activated by antigen from pathogens, foreign proteins such as allergens, or self-antigens in the case of autoimmunity (**Fig. 1**). An environment containing IL-12 promotes the STAT4-dependent development of IFNγ-secreting Th1 cells.[1,2] IL-4 induces the STAT6-dependent differentiation of Th2 cells.[3-5] Similarly, several cytokines including IL-6, IL-21 and IL-23 promote the STAT3-dependent development of Th17 cells that secrete IL-17 and other cytokines.[6-8] STAT5, activated in response to IL-2, contributes to inducible regulatory T cell development.[9,10] Each of the STATs binds to hundreds of loci that contribute to the effector phenotype.[11-14] All of these data supported a paradigm wherein activation of a particular STAT protein in T cells would result in the activation of a specific effector phenotype. However, continuing investigations revealed that a one STAT-one phenotype paradigm was too simple to define the complex responses of differentiating T cells to a diverse cytokine milieu.

The first evidence that more than one STAT was involved in a particular differentiation pathway was observed following the identification of T-bet, a T-box transcription factor that promotes the Th1 phenotype.[15] Although both STAT4 and T-bet promote Th1 development, efforts to place these factors in a linear pathway failed because IFNγ-activated STAT1, rather than IL-12-activated STAT4, resulted in increased T-bet expression.[16,17] More recent work suggested that IFNγ activates T-bet expression early, and IL-12 activates it later in differentiation, supporting parallel pathways where both STAT4 and T-bet are required for separate and overlapping aspects of the Th1 genetic program.[18,19]

A similar parallel requirement was observed for STAT5 in Th2 development (**Fig. 2**). Based on the observations that IL-2 increased the development of Th2 cells, more detailed studies demonstrated that STAT5 promoted Th2 cytokine production through a parallel pathway that involved accessibility of Th2 cytokine loci.[20] STAT5 also contributed to allergic inflammation in vivo, demonstrating that both STAT5 and STAT6 proteins collaborated on the induction of Th2-mediated inflammation.[21] These examples added

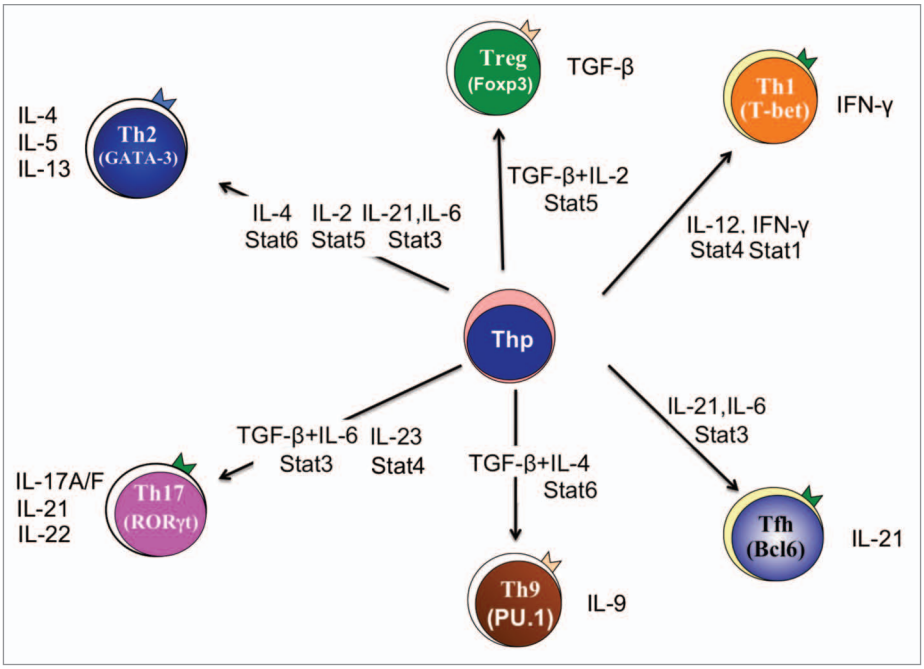

Figure 1. T helper (Th) cell phenotypes. Cytokine environments, and the downstream STAT proteins that are subsequently activated (adjacent to arrows from naïve T helper precursors (Thp) to each effector subset) promote the development of various Th cell effector phenotypes. The hallmark transcription factor for each phenotype is indicated within brackets in each cell type, and the cytokines produced by each subset are shown. Th, T helper; Treg, regulatory T cell; Tfh, T follicular helper cell.

to the complexity of the role of STATs in Th development. They demonstrated that although one cytokine might be the predominant initiator of a differentiation pathway, the developing effector T cell responds to additional cytokines in the environment, and those cytokines activate additional STAT proteins. Thus, the T cell is able to integrate multiple signals in the process of differentiation. Our recent work adds to the more complex paradigm by demonstrating that STAT3 is required for Th2 development.

As mentioned above, a number of cytokines that activate STAT3 are present in the developing Th2 environment from autocrine or paracrine sources, including IL-6, IL-21 and IL-2, all of which might contribute to the activation of specific components of the Th2 genetic program.[22-24] We demonstrated that STAT3 is activated throughout Th2 development, and that neutralization of all of the cytokines listed above was required to decrease phosphorylated STAT3 within developing Th2 cells, suggesting that there is some redundancy in their function.[25] Moreover, STAT3-deficient T cells had diminished production of Th2 cytokines, reduced expression of transcription factors required for Th2 development, and altered histone modification patterns at the loci encoding those transcription factors. STAT3 is bound to the promoters of loci encoding transcription factors expressed in Th2 cells in both naïve and differentiated Th2 cells (**Fig. 2**). Importantly, STAT3 was not required for normal activation of STAT6, but was required for the development of Th2 cells in vivo, as well as the development of Th2-mediated allergic inflammation.

How the cell integrates signals from multiple STATs is still unclear, and several mechanisms might be involved. Data suggest that the STAT1-STAT4 interplay has a temporal basis, each STAT acting on common loci at different times,[18] likely due to changes in the expression of receptors for the requisite activating cytokines. STAT5 also works with STAT4 by binding to gene loci important for Th1 development.[26,27] In Th2 development, STAT5 and STAT6 appear to work separately, although STAT5 regulates receptors for the STAT6-activating cytokine IL-4.[20,28] The theme of regulating the receptor for another cytokine and altering the activation of downstream STATs recurs in several instances during T helper cell differentiation, including regulation of IL-2R, IL-4R and IL-12R.[27,29]

However, we observed normal expression of IL-4R and normal activation of STAT6 in the absence of STAT3. Despite this, STAT6 was not bound appropriately to target loci.[25] The lack of STAT6 binding suggests either that STAT3 and STAT6 cooperate in binding, or that STAT3 mediates accessibility of the loci to facilitate binding of STAT6. Indeed, we observed decreased accessibility at several Th2 transcription factor loci in the absence of STAT3. It is still possible that STAT3 and STAT6 might physically interact to mediate binding to target loci. Several STAT proteins can form hetero- as well as homodimers. STAT6 is thought to function mostly as a homodimer, but it is possible these factors might heterodimerize. STAT proteins can also "tetramerize" through interactions of N-terminal domains that facilitate cooperative binding to non-consensus DNA elements, and though these interactions are thought to be homotypic, occurring only among dimers of like STATs, it is conceivable that

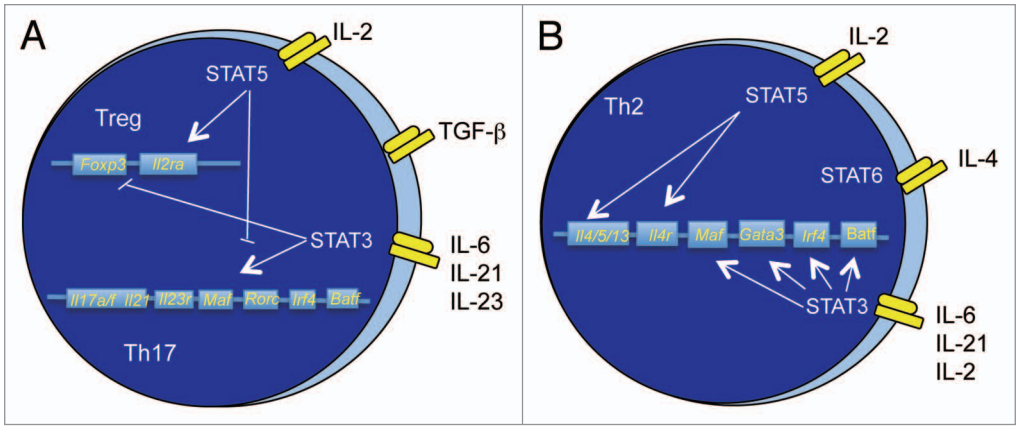

Figure 2. Opposing and cooperative STAT pathways. (A) During Treg development, STAT5 promotes the expression of *Foxp3* and *Il2ra*, while repressing Th17 development by binding to the *Il17a/f* locus, and potentially other loci. Conversely, STAT3 binds to multiple gene loci that comprise a Th17 genetic program, while inhibiting the expression of *Foxp3*. (B) In developing Th2 cells, STAT6 binds to multiple loci associated with the Th2 genetic program. STAT5 works in concert by binding to the *Il4r* locus, and the *Il4* locus. STAT3 binds to loci expressing transcription factors that contribute to the Th2 phenotype. The loci indicated are only a subset of potential binding sites for each factor.

there could be higher-order interactions in an enhanceosome complex. STAT3 might also separately contribute to recruitment of factors to a STAT6-dependent enhanceosome.[30] All of these functions could occur in parallel to the direct effect of STAT3 on additional documented targets including *Maf* and *Socs1*.[31,32]

STAT3 and STAT5 are mutually antagonistic as they respectively promote Th17 and Treg generation (**Fig. 2A**). Th17 cells develop when STAT3 is activated in the presence of TGFβ or IL-1 and a combination of IL-6 and IL-23,[33-36] and a constitutively active STAT3 can promote IL-17 production in several culture conditions.[6,7] In the absence of STAT3, IL-6 does not inhibit iTreg development.[7,10,37] However, STAT3 is required for the IL-10-induced function of Tregs in controlling Th17-mediated inflammatory disease.[38,39] Conversely, an active STAT5 promotes expression of the Treg transcription factor Foxp3, and in the absence of STAT5, IL-2 neither promotes Treg development nor inhibits Th17 development.[40] At the level of cytokine regulation, STAT3 and STAT5 bind to overlapping sites at the *Il17a/f* loci but have opposing effects on transcription from the loci.[41]

Despite opposing functions of STAT3 and STAT5 when they are activated in the presence of TGFβ-induced signals, in the absence of TGFβ, and in the presence of IL-4 and STAT6 activation, STAT3 and STAT5 cooperate with STAT6 in promoting Th2 development (**Fig. 2B**). When STAT6 is activated, STAT3 demonstrates reduced binding to *Il17a/f* genes, and induces the expression of Th2, rather than Th17, transcription factors.[25] Moreover, multiple cytokine signals decrease binding of STAT5 to the Foxp3 locus as they decrease iTreg generation.[37] Thus, signals from multiple STATs may integrate at a genome-wide level of resolution by altering the binding of one STAT when more than one STAT is activated.

Importantly, binding of STAT3 in Th2 cells is not entirely dissimilar to binding in Th17 cells. Although binding to *Il17* genes is decreased, there is overlap in the binding of STAT3 to genes that are required for both Th17 and Th2 cells, including *Irf4*, *Maf* and *Batf*.[25] In support of this concept, we have recently shown that STAT3 is required for IL-21 production in multiple T helper subsets.[42] Similar targeting of STATs among commonly expressed genes might also provide a basis for some of the flexibility of programming in Th subsets.[43] For example, we have shown that polarized Th17 cells will repress IL-17 production and induce IL-4 production when cultured under conditions that promote Th2 development.[44] Thus, plasticity might result from an ability to integrate multiple signals and establish epigenetic modifications at the relevant loci that are poised to respond to additional changes in the cytokine environment.[45]

Conclusions

STAT protein activation is the first response of a differentiating T cell to the cytokine milieu and represents a necessary step in establishing an effector phenotype. Although one STAT protein might be the predominant factor required for the development of each phenotype, additional STAT proteins also impact differentiation. The requirement for multiple signal inputs likely reflects a necessity for a complex inflammatory cytokine environment to allow the development of Th subsets that potently enhance the inflammatory process. The balance of signals, not only pro- versus anti-inflammatory, but also in the integration of multiple potentially opposing pro-inflammatory signals, leads to changes in the phenotype of the differentiating cell. Changes in the balance could result in modest or significant shifts in the type of inflammation that develops to allow the fine-tuning of the immune response required to mediate pathogen immunity, but avoid damaging inflammatory disease.

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