A novel role for STAT5 in DC

Controlling the Th2 response

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Dendritic cells (DC) play a key role in immunity by recognizing and presenting antigens. Cytokines and cytokine-activated transcription factors are fundamental in the regulation of the DC differentiation and their functions. While the role of STAT3 in DC development is well established, the function of STAT5 in DC has yet to be fully elucidated. A recent study published in *Nature Immunology* by Bell et al., using the DC-specific deletion of *Stat5*, demonstrated the importance of STAT5 in the induction of a Th2 response in DC. As the activation of this transcription factor is not required for the induction of a Th1 response, the authors further investigated the role STAT5 in the signaling to thymic stromal lymphopoietin (TSLP), a cytokine known to be important for type 2 inflammatory responses. Their results demonstrate the importance of STAT5 activation during TSLP-induced Th2 responses and suggest that DC are a key TSLP target.

Keywords: STAT5, dendritic cells, Th2 induced response, TSLP, CD11c-Cre *Stat5^{fl/fl}* mice

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differentiation and maturation is well documented. For example studies in mice deficient in Fms-related tyrosine kinase 3 ligand (Flt3L) indicate that this cytokine plays nonredundant roles in DC development.⁷ Signal transducer and activator of transcription 3 (STAT3) is known to be crucial for DC progenitor expansion and DC differentiation.⁸ The reduction in DC numbers observed in Flt3L-deficient mice has been attributed to the absence of STAT3 activation.⁸

In contrast to the established role of STAT3, the function of STAT5 in DC development remains unclear. Investigating the latter has been rendered difficult by the redundancy between STAT5A and STAT5B as well as the lethality of *Stat5a* and *Stat5b* double inactivation in mice.⁹ To circumvent this obstacle, *Stat5^{-/-}* chimeric mice have been used by Esashi et al. to demonstrate that GM-CSF-induced STAT5 activation inhibits pDC development.¹⁰ This inhibition was shown to be due to the blockade of *Irf8*, an essential factor that plays a role in pDC maturation by modulating the expression of key regulatory transcription factors such as Spi-B, IRF7, and receptors like Toll-like receptor 9 and Flt3.¹¹

Like GM-CSF,¹² thymic stromal lymphopoietin (TSLP) induces STAT5 activation in DC. TSLP is a four-helix bundle cytokine, mostly expressed in epithelial cells.¹³ It is an important regulator of mucosal immunity.¹⁴ TSLP activates a receptor formed by the association of the TSLP and IL-7R α receptor (R) chains.¹⁵ IL-7R α and TSLPR recruit, respectively, the Janus kinases JAK1 and JAK2.¹⁶ Activation of its receptor by TSLP was

The dendritic cell compartment (DC) is a key cell population within the immune system. DC antigen-presenting and regulatory capacities are necessary for both an immune response and self-tolerance maintenance.¹ DC can be classified in two main types: plasmacytoid (pDC) and conventional (cDC) based on their phenotypes, locations, and functional properties.^{2,3} While pDC are involved in immune tolerance and T cell activation, cDC are required for adaptive immune responses.⁴⁻⁶

The role of cytokines and cytokine-responsive transcription factors in DC

shown to induce STAT5 phosphorylation and expression of the STAT5-inducible gene *Cis* encoding the cytokine inducible SH2-containing protein.¹⁷ Activation of DC by TLSP triggers inflammatory Th2 responses. The Th2-microenvironment created by TLSP-stimulated DC is similar to that observed in allergic inflammation, suggesting that it could contribute to pathologies involving Th2 immune responses such as allergies, atopy, and asthma. In line with this hypothesis, TLSP-transgenic mice develop Th2-type inflammation at the sites of transgene expression.¹⁷

To investigate the role of STAT5 in TLSP-induced Th2 responses, Bell et al. used an elegant DC-specific *Stat5* deletion mouse model in which mice with a “floxed” *Stat5a-Stat5b* locus were crossed with CD11c-Cre transgenic mice.¹⁸ Bell et al. observed that CD11c-Cre *Stat5*^{fl/fl} mice (i.e., with a DC-specific deletion of *Stat5*) had a normal immune cellularity when compared with control littermates and no changes in specific immune cell type or DC subset (pDC, CD8⁺ DC, and CD4⁺ DC) frequencies. This led the authors to conclude that STAT5 is not required for the development of any spleen, lung, lymph node, or skin DC subtype. It is important to note that the efficiency of STAT5 depletion in the model used by Bell et al. was only 50%, thus limiting the conclusion that can be drawn regarding the roles of STAT5 in this DC subtype.

Having concluded that STAT5 deletion had no detectable effect on DC development, Bell et al. next investigated the role of STAT5 in Th2-induced responses using a mouse model of allergic contact dermatitis.¹⁹ In this model, the Th2 response is induced by a combination of the hapten FITC and the phthalate ester DBP.²⁰ The mice with a CD11c-CRE induced *Stat5* deletion had lower IL-4 mRNA levels, reduced ear swelling, and less DC present in the FITC/DBP exposed skin-draining lymph nodes. These results unambiguously demonstrate the role of DC STAT5 in activating antigen-specific T cells during a Th2 skin response.

The specificity of these observations for the Th2 response was established using a Th1 contact hypersensitivity model,

induced by sensitization, and challenged with the hapten 2,4-dinitrofluorobenzene DNFB,¹⁹ in which no difference between WT and Cre⁺ *Stat5*^{fl/fl} mice were observed.

Bell et al. further demonstrated the role of DC STAT5 in Th2 inflammation using two well established Th1 and Th2 mouse models of airway inflammation. Using ovalbumin-induced airway inflammation, they showed that DC require STAT5 for the development and promotion of Th2 responses in the lungs. Alternatively, when airway inflammation was induced by infection with influenza A virus, which induces a Th1 immune response, no difference could be observed between Cre⁻ *Stat5*^{fl/fl} and Cre⁺ *Stat5*^{fl/fl} mice, indicating that the role of STAT5 was Th2-specific and that *Stat5* deletion did not simply lead to overall lung inflammation reduction. No difference between control and STAT5-deficient mice were observed in the total number of lung cells, mediastinal lymph nodes, or bronchoalveolar lavage fluids or in the immunodominant CD8 T cell response. In conclusion, STAT5 activation in DC is required for mounting an anti-ovalbumin Th2 response but unnecessary for Th1 and cytotoxic T cell response to influenza A virus.

TLSP is a cytokine known to activate STAT5; mice deficient in the TLSP receptor, like the CD11c-Cre *Stat5*^{fl/fl} mice, were resistant to inflammation induced by FITC-DBC.²¹ This led Bell et al. to hypothesize that TLSP is the cytokine that activates STAT5 in the DC of the skin and lung to promote Th2 immunity.

Previous studies have shown that Flt3L-generated bone marrow-derived DC (FL-DC) in vitro respond to TLSP.²² By infecting FL-DC generated from *Jak2*^{fl/fl} bone marrow infected with a retrovirus expressing the Cre recombinase and green fluorescent protein (GFP), Bell et al. generated *Jak2*⁻ GFP⁺ and *Jak2*⁺ GFP⁻ FL-DC. These control and *Jak2*⁻ FL-DC were used to demonstrate that the TLSP sensitivity of DC also requires JAK2 as well as STAT5 activation.

To complete this study, the authors analyzed the production of a direct target of TLSP, the Th2 type chemokine CCL17, as an indicator of TLSP signaling. They demonstrated, using a mouse strain with the sequence encoding GFP inserted in

the *Ccl17* locus, that TLSP induces less CCL17 production in DC lacking STAT5 compared with control DC. As CCL17 attracts CCR4-expressing Th2 cells,²³ Bell et al. finally demonstrated using CD4 T cells transgenic for an OVA-specific TCR that the ability of TLSP to promote a Th2 response in vitro and in vivo was compromised in STAT5-deficient DC (Fig. 1).

In conclusion, this study that utilized CD11c-Cre *Stat5*^{fl/fl} mice demonstrate that the activation of DC STAT5 is required for the induction of Th2-type CD4 T cell response but dispensable for its Th1 counterpart. STAT5 is required for TLSP signaling in DC and for DC-mediated TLSP stimulation of Th2 responses. Altogether, the results further underline the importance of TLSP and TLSP-induction of STAT5 activation in allergic diseases and suggests that STAT5 might represent a key therapeutic target for Th2 airway and skin inflammation.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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Figure 1. Importance of STAT5 in TSLP-activated DC. The study by Bell et al. demonstrates the role of STAT5 activation by TSLP in DC. This STAT5 activation induces an upregulation of costimulation molecules like CD80, CD86, or OX40L; associated to a CCL17 production. TSLP-induced STAT5 activation mediates Th2 responses by CD4 T cells.

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