Published in final edited form as:

Immunol Rev. 2018 November ; 286(1): 148–159. doi:10.1111/imr.12705.

JAK/STAT signaling in regulation of innate lymphoid cells: the gods before the guardians

Helena Stabile¹, Gianluca Scarno¹, Cinzia Fionda¹, Angela Gismondi¹, Angela Santoni^{1,2}, Massimo Gadina³, and Giuseppe Sciumè^{1,*}

¹Department of Molecular Medicine, Sapienza University of Rome, Laboratory affiliated to Istituto Pasteur Italia – Fondazione Cenci Bolognetti, Rome, Italy.

²IRCCS Neuromed, 86077, Pozzilli (IS), Italy.

³Translational Immunology Section, Office of Science Technology (OST), National Institute of Arthritis and Musculoskeletal and Skin Diseases, NIH, Bethesda, Maryland, USA.

SUMMARY

Immunity to pathogens is ensured through integration of early responses mediated by innate cells and late effector functions taking place after terminal differentiation of adaptive lymphocytes. In this context, innate lymphoid cells (ILCs) and adaptive T cells represent a clear example of how prototypical effector functions, including polarized expression of cytokines and/or cytotoxic activity, can occur with overlapping modalities but different timing. The ability of ILCs to provide early protection relies on their poised epigenetic state, which determines their propensity to quickly respond to cytokines and to activate specific patterns of signal-dependent transcription factors. Cytokines activating the Janus kinases (JAKs) and members of the signal transducer and activator of transcription (STAT) pathway are key regulators of lymphoid development and sustain the processes underlying T cell activation and differentiation. The role of the JAK/STAT pathway has been recently extended to several aspects of ILC biology. Here, we discuss how JAK/STAT signals affect ILC development and effector functions in the context of immune responses, highlighting the molecular mechanisms involved in regulation of gene expression, as well as, the potential of targeting the JAK/STAT pathway in inflammatory pathologies.

Keywords

Innate lymphoid cells; JAK; STAT; cytokine; host defense

1 INTRODUCTION

Innate lymphoid cells (ILCs) provide protection against pathogens using a variety of effector programs, delineated through mechanisms of transcriptional and epigenetic regulation(1–4). The identification and characterization of an increasing number of ILC subsets have brought to light the specular functionality of the innate and adaptive systems(5). This aspect reflects

*Corresponding author: Giuseppe Sciumè, Address: Viale Regina Elena, 291, 00161, Rome, Italy., Telephone number: +39 06 44340632, Fax: +39 06 44340632, giuseppe.sciume@uniroma1.it.

the common requirement of a limited number of lineage defining transcription factors (LDTFs), namely EOMES, T-BET, GATA-3 and ROR γ t, for terminal differentiation and for specification of polarized immune responses(6). On the other hand, the combinatorial expression of a wider array of transcription factors (TFs) ensures the high degree of lymphocyte complexity and functional diversification. As result, the establishment of distinct TF networks instructs the poised phenotype of ILCs, as well as, places a brake on T cell differentiation, which is released only after pathogen entry(7, 8).

Upon infection, signal dependent TFs (SDTFs) enable lymphocytes to detect changes in the microenvironment and to initiate the immune response. In this context, cytokines activating the Janus kinases (JAKs) and members of the signal transducer and activator of transcription (STAT) pathway set the conditions, not only for the development of the entire lymphoid system, but also for specification of different immune responses(9). The role of JAK/STAT signals in the processes of T helper (Th) cell differentiation has been intensively investigated, and our understanding of the molecular actions of STATs in regulation of gene expression has greatly expanded with the progress of chromatin immunoprecipitation followed by parallel massive sequencing (ChIP-seq) technology. While mechanisms of Th cell polarization have been dissected in other outstanding reviews(10–12), here, we discuss the recent advances regarding the JAK/STAT system in the biology of ILCs. In particular, we focus on what we have learned from mouse models and patients carrying mutations on the JAK/STAT pathway, as well as, from the effects observed in ILCs by using JAK inhibitors. Particular attention will be also given to mechanisms of genomic regulation, and to the functions of the JAK/STAT signaling in ILCs during host defense.

2 ILC SUBSETS AND PRECURSORS

For a long time, Natural Killer (NK) cells remained the only known ILC subset, seen as the innate counterpart of cytotoxic CD8⁺ T lymphocytes (CTL)(13). Several studies helped to discriminate distinct developmental stages for NK cells in humans and mice, and to dissect their role in providing protection against viral infections and cancer(14–18). In the late 90s, innate cells having the ability to orchestrate the organization of secondary lymphoid organs were discovered and named, for this reason, lymphoid tissue inducer (LTi) cells(19–24). Other ILC subsets, instead, were neglected until 10 years ago, when potent cytokine responses were observed in non-T lymphoid cells(25–34).

A rapid burst in the field of mucosal immunology led to the identification of a large number of ILC subsets, and to reconsider several aspects of immune responses, previously seen only by a T cell centric point of view. Based on the similarity between ILCs and Th cells, a new nomenclature was defined, which divides ILCs in three groups, according to the profile of cytokine production(35). Due to the ability to release high levels of IFN- γ , NK cells are included in the group of type 1 ILCs. This group also comprises a heterogeneous population of IFN- γ -producing cells that shares features with adaptive Th1 cells(36). Helper ILCs producing IL-13, IL-5 and in certain conditions IL-4 are included in the type 2 group and named ILC2; while IL-22/IL-17-producers belong to the ILC3 group(37, 38). Several surface markers can be used to further discriminate the different ILC subsets, both in humans and mouse. These include natural cytotoxicity triggering receptors (NCRs),

chemokine receptors (CCR6), integrins (CD49a, CD49b, CD103) and other adhesion molecules (CD56, KLRG1).

A further layer for determining ILC identities involves the expression of LDTFs previously associated with Th differentiation. This is the case for T-BET (encoded by *TBX21*), which drives differentiation of ILC1; GATA-3, associated with ILC2 development; and ROR γ t (*RORC*) required by ILC3(39). Although T-BET is expressed by NK cells and required for migration, turnover and optimal IFN- γ production, NK cell differentiation is regulated by another T-box TF, EOMES(40–42). The differential usage/requirement of T-BET and EOMES represents one major difference between NK cells and other ILC1(43). However, expression of LDTFs is not as “strict” as initially thought. For instance, T-BET is required not only for ILC1 but also for development of ILC3 expressing NCRs(44–46); while GATA-3 is expressed at different levels among all subsets, and considered as a multilineage TF (MLTF) required before specification of ILC lineages(47–50). Thus, the linear maturation model or one-to-one instructive maturation model involving one LDTF and one fate has become an oversimplification for ILC development, especially, given the requirement of several other MLTFs.

In the current model of development, ILCs originate downstream of a common lymphoid progenitor (CLP) which loses T and B cell potential and gives rise to an array of multipotent ILC precursors. The α -lymphoid progenitor (α -LP) and early innate lymphoid progenitor (EILP) represent the first precursors having a differentiation potential restricted to ILC only(51–54). Differentiation towards the common helper lymphoid progenitor (CHILP) occurs along with loss of NK cell potential, next, ILC development moves from a multipotent innate lymphoid precursor (ILCP) to unipotent ILCPs endowed with a more restricted cell fate potential(55–57). During this process, committed ILCPs undergo specific epigenetic changes, consisting of acquisition of accessible DNA regulatory elements (DREs) for signature genes expressed only in mature cells(58, 59). This feature makes ILCP prone for further differentiation. A dedicated network of TFs regulates precursor development toward distinct ILC lineages, including the inhibitor of DNA binding-2 (*ID2*), which suppresses the adaptive cell fate, and early MLTFs, such as NFIL3, TCF1 (encoded by *TCF7*), PLZF (*ZBTB16*) and TOX(60, 61). The heterogeneity of ILC progenitors has been recently analyzed by using single-cell RNA-seq, which has helped to resolve developmental trajectories and to unravel the role of *BCL11B* in ILC2 development(62). The functional role of these TFs in ILC development has been validated mainly by using mouse models with conditional genetic deletion. However, the expression of *ID2*, *TCF7*, *GATA3* and *TOX* on a recently identified human ILC precursor implies for similar transcriptional developmental requirements in mouse and human(58).

3 JAK/STAT PATHWAY IN ILC BIOLOGY

3.1 Overview

More than 50 cytokines, growth factors and hormones act upstream of the JAK/STAT pathway, mediating a wide range of functions which extend beyond the hematopoietic system(63, 64). The pleiotropic effects related to this pathway are, in part, due to the extensive genomic rearrangement and selective mutagenesis, which led mammals to

ultimately have in their genomes four genes encoding JAK homologues (*JAK1*, *JAK2*, *JAK3* and *TYK2*) and seven for STATs (*STAT1–4*, *STAT5A*, *STAT5B*, and *STAT6*)(65, 66). Activation of the JAK tyrosine kinases is the first and critical step occurring upon receptor engagement. Sequentially, JAKs phosphorylate each other, the intracellular tail of the receptor subunits and several downstream signalling molecules, including STATs. According to the canonical way of signaling, the juxtaposition of JAKs and STATs allows, after phosphorylation, STAT dimers to dissociate from the membrane complex and to migrate to the nucleus, where they bind specific DNA-motifs modulating gene expression(67). As TFs, STATs can directly bind DREs to activate or inhibit gene transcription(9). These DREs typically consist of proximal or distal GAS (IFN- γ activation site: TTCN3GAA) consensus sequences which can be enhancers as well as other non-coding loci located at promoters or intragenic and intergenic regions; however, STATs can also bind degenerated/not canonical motifs. Activated STATs bind DNA as dimers or tetramers, but they can also interact with other TFs to assembly multi-factorial complexes(68). Moreover, STATs can elicit chromatin remodeling by promoting the deposition of permissive or repressive epigenetic modifications, even if the mechanisms responsible for these changes remain largely unknown(9).

3.2 JAK3/STAT5 axis in ILC development

Historically, distinct JAK/STAT signals have been strictly linked with specific functions of the immune system, and this is the case for the role of cytokines using the common IL-2 γ -chain receptor (IL-2R γ , CD132), JAK3 and STAT5 in regulation of lymphoid development(69) (Figure 1). The crucial role of this pathway in guiding development, homeostasis and activation of lymphoid populations has been established through studies on primary immunodeficiencies in humans and a wide range of genetically modified animals(70, 71). Patients affected by severe combined immunodeficiency (SCID) caused by inactivating mutations of *IL2RG* and *JAK3* genes display defects in lymphocyte lineage development, resulting in the absence of T and NK cells, as well as, impaired B cell function(72, 73). Absence of mature NK cells in patients affected by JAK3 mutations was reported before generation of mice lacking the corresponding gene(74), while other ILC populations have been only recently investigated(75). In this regard, both *JAK3* and *IL2RG* SCID patients show absence of circulating and tissue-resident ILCs and their reconstitution, after allogeneic haematopoietic stem cell transplantation (HSCT), can occur only under myeloablative conditions(75). Similar results were obtained by using a mouse model of HSCT, corroborating the notion that ILCs can reconstitute after birth, both in humans and mice, starting from a BM precursor(75). The stage at which JAK3 is required for ILC and NK cell development has been elucidated by using *Jak3*^{-/-} mice, showing reduced numbers of CLP, CHILP, ILC2P and NKP, in spite of an accumulation of ILCP and pre-NKP. This evidence supports the general role of JAK3 for optimal development of innate lymphoid progenitors and its crucial requirement for the pre-NKP to proceed toward subsequent maturation stages(76).

JAK3 is activated downstream of stimulation with several cytokines including IL-2, IL-4, IL-7, IL-9, IL-15 and IL-21. However, defects in NK cells and other ILCs are mainly due to an impaired IL-15 and IL-7 signalling. Mice deficient for *Il15*, or IL-15 receptor subunits

(*Il5ra* and *Il2rb*) show lack, or substantial alteration, of mature NK cell numbers(77–80). These mice retain a normal number of NKPs, indicating that IL-15 signaling becomes fundamental for NK cell generation only after commitment. Indeed, one of the distinct features of NKP is the acquisition of IL-2R β (CD122) expression, which enables responsiveness to IL-15 and to proceed throughout late differentiation stages(81, 82). Up to date, mutations of *IL15* or *IL15RA* genes have not been reported in humans, while a significant reduction of CD122 (encoded by *IL2RB*) expression was described in a patient with a unique NK-deficient SCID phenotype(83). Accordingly, differentiation of human CD34⁺ precursors into mature NK cells can be induced by *in vitro* culture, in presence of IL-15(84). Mice lacking IL-15 or IL-2R γ show also a strong reduction of distinct ILC1 subsets(29, 56, 85–87). However, a subset of intraepithelial ILC1 has a peculiar behaviour and does not depend on IL-15R α for development/homeostasis(85).

Type 2 and type 3 ILCs are minimally perturbed in absence of IL-15, while their numbers are dramatically reduced when IL-7 signalling is impaired(88). However, the phenotype of *Il7ra*^{−/−} mice is more severe as compared to that observed in *Il7*^{−/−} mice, in terms of ILC2 and LTi-like ILC3 numbers(89, 90). A possible explanation for this phenotype relies on a compensatory mechanism involving TSLP signals through IL-7R α , in absence of IL-7. However, persistence of selective ILC2 and ILC3 populations has been also observed in *Il7ra*^{−/−} mice and the presence of these residual cells is partially sustained by IL-15(91). These studies highlight the possibility that beyond the “canonical” ILC development, compensatory cytokine switches may occur in selective microenvironments.

STAT5 also plays a role in the context of ILC development. There are two genes encoding for this SDTF, *STAT5A* and *STAT5B*, having shared and distinct physiological functions(92). STAT5B plays a major role over STAT5A in regulating development and activation of NK cells and other ILCs(93, 94). Both germline (after which only few mice survive) and conditional deletion of the entire *Stat5a/b* locus have a high impact on the total number of NK cells(95, 96). In addition, the residual NK cells found in the BM of *Stat5^{fl/fl}Ncr1-iCreTg* mice display an immature phenotype characterized by an altered receptor profile and a major developmental block at the NKP stage(96). Due to the profound effects of STAT5 in regulation of lymphoid development, the molecular functions of this SDTF have remained hard to understand. Thus, the use of mice bearing only one allele, *Stat5a*^{−/−}*Stat5b*^{+/-} and *Stat5a*^{+/-}*Stat5b*^{−/−}, represents a “quasi-ideal” strategy for comprehension of STAT5 roles, with the obvious caveat that some of its essential functions are still preserved(94, 97). Deep dissection of the ILC features in these mice has revealed a differential requirement of STAT5 signals among precursors and differentiated ILCs, which show compromised functionalities. At molecular level, STAT5 can act as an upstream regulator of T-BET, explaining why the ILC subsets mainly affected by deprivation of STAT5 signals are NK, ILC1 and NCR⁺ ILC3, all depending on this LDTFs(94). Despite the growing progress made in understanding the specific roles of JAK3/STAT5 axis in ILC development, the details of this process have just started to be addressed, both in humans and mouse.

3.3 New paradigms for STAT4 and STAT1 in ILCs

The effects of STAT4 downstream of IL-12 have represented over the years a cardinal point in the elicitation of cytotoxic response and production of IFN- γ in NK cells(98, 99). On the other hand, the roles of type I IFNs (IFN- α/β) and STAT1 during viral infections have been more controversial and, in some ways, paradoxical, in respect of NK cell functions(100, 101). One of the major confounding elements in evaluating the role of STAT1 has been the discrimination of what is intrinsic to NK cells from what is extrinsic. Just to mention a few examples, the effects of STAT1 on regulation of MHC class I expression(102) and the effects of type I IFNs on IL-15-production/trans-presentation on accessory cells(103–105) have both a high impact on NK cells. A further confounding element is the ability of type I IFNs to access STAT4, in presence of low STAT1 levels. Indeed, although type I IFNs preferentially access STAT1 and STAT2, the high STAT4 expression levels in NK cells, allow IFN- α/β to activate STAT4 in the early phase of viral infection, enhancing IFN- γ production(106). At later stages STAT1 expression levels increase and, by displacement of STAT4 from type I IFN receptors, it leads to a decreased ability of NK cells to produce IFN- γ (106). Thus, based on these observations dissecting early from late responses remains a key aspect to consider for the evaluation of STAT1 role in NK cells.

One of the most intriguing aspects is the ability of NK cells to provide secondary immune responses during cytomegalovirus (CMV) infection. This process leads to generation of long-lived cells, named “memory” or “adaptive” NK cells(107, 108). Experiments in mice have shown that both IL-12 and STAT4 are fundamental for generation of adaptive NK cells by affecting clonal expansion and differentiation(109). At molecular level, the activation of STAT4 downstream of IL-12 induces up-regulation of T-BET expression, which is important for the maintenance of virus-specific memory NK cells(110). Similarly, induction of *Runx1* and *Runx3* is required for memory formation and clonal expansion(111). Although STAT4 is dispensable for NK cell development, during mouse CMV infection, this TF can induce an alternative developmental pathway capable to rescue the absence of *Nfil3*, and to sustain expansion and formation of adaptive NK cells(112). Type I IFNs and STAT1 are also important for generation of adaptive NK cells, as shown in *Ifnar1*^{-/-} (encoding for type I IFN receptor) or *Stat1*^{-/-} mice. Expression of STAT1 in adaptive NK cells is fundamental for their survival, mainly by preventing a mechanism of NK cell mediated fratricide, dependent on NKG2D and perforin expression(113). It is interesting to note how the defects observed in *Stat1*^{-/-} NK cells are not rescued by the expression of STAT4, indicating that the two TFs can have non-redundant functions. Nonetheless, IL-12 and type I IFNs have also redundant roles, since they are both required for induction of the TF ZBTB32, necessary for proliferation of virus-specific NK cells(114).

Recently, mechanisms of host protection against viral infections have been also extended to other type 1 ILCs in a mechanism dependent upon the activity of IL-12 and STAT4. The key role of tissue resident ILC1 in protection against mouse CMV emerges at the very early phase of infection and consists in a rapid and potent production of IFN- γ at sites of infection, induced downstream of the IL-12 produced by XCR1⁺ conventional dendritic cells(115). The relevance of liver-resident ILC1 has been demonstrated through their

selective ablation using *Zfp683*^{-/-} mice, leading to an increased viral load and a lethal phenotype(115, 116).

The role of STAT4 in ILC1 is not restricted to protection against pathogens, as this molecule is able to regulate homeostasis of adipose tissues. In particular, diet-induced obesity is associated to both an early production of IL-12 in adipose tissue depots and a subsequent increase in the number of adipose ILC1, which is abrogated in absence of STAT4. By producing IFN- γ , ILC1 contribute to promote obesity-associated insulin resistance via the regulation of macrophage polarization(117). The effects of STAT1 in ILC1, or other ILCs, have been poorly characterized. However, in a model of respiratory syncytial virus (RSV) infection, *Stat1*^{-/-} mice show an unbalanced ILC response associated with decreased IFN- γ -expressing ILC1 and increased IL-5/IL-13-expressing ILC2 and IL-17-expressing ILC3. This phenotype is explained by an ILC extrinsic STAT1 function associated with an increased production of IL-33 and IL-23(118).

Further insights on the role of STAT1 in NK cells have been obtained from studies on patients with STAT1 gain of function (GOF) mutations. NK cells from these patients show an impairment of both cytotoxicity and IL-15-induced IFN- γ production, along with a lower level of STAT5 activation in response of IL-15 stimulation(119). The reduced binding of STAT5 to the *IL2RA* promoter and the decreased ability of NK cells to proliferate in response to IL-2 and IL-15 stimulation are evidence for the high impact of an altered STAT1 signaling on STAT5-dependent responses. Beyond its canonical role of activator of transcription, an unusual role of STAT1 was also described in NK cells, consisting in its ability to localize at cell-cell interface and promote cytotoxicity(120).

3.4 STAT6 and STAT3 in regulation of ILC functions

The symmetric transcriptional regulation existing between ILCs and T cells finds one of its few exceptions in the role of STAT6 in ILC2. This is due to the limited effects of IL-4 in ILC2 homeostasis and activation, which appears secondary when compared to the main role played by IL-25 and IL-33. These two cytokines belong to the IL-17 and IL-1 family, respectively, and regulate most of the ILC2 functions, including proliferation and induction of type 2 signature cytokines(121, 122). However, in specific inflammatory settings a substantial role for STAT6 and IL-4 in regulation of ILC2 proliferation becomes also evident. In particular, during *Nippostrongylus brasiliensis* infection, STAT6 deficient mice have an impaired number of IL-13-producing ILC2, resembling the phenotype observed in *Il4ra*^{-/-} mice(123). A similar function for STAT6 is also observed using a mouse model of lung inflammation induced by the fungal allergen *Alternaria*(124).

In contrast to STAT6, activation of STAT3 downstream of IL-23 is a key event in promoting ILC3 functions. Absence of STAT3 is associated with a decreased IL-17 production in LTi-like ILC3 in spleen(33). Additionally, conditional deletion of STAT3 in ROR γ t expressing cells, by using *Stat3*^{fl/fl}*Rorc-cre* mice, leads to a dramatic reduction of IL-17 and IL-22 production in the gut(125). The importance of this aspect is testified by the evidence indicating that the IL-22 produced by non-T cells is key for early host protection against *Citrobacter rodentium*. Among type 3 ILCs, NCR⁺ ILC3 have been recognized for their hybrid NK/ILC3 phenotype and for IL-22 production. When STAT3 is removed in NKp46

expressing cells, using *Stat3^{fl/fl}Ncr1-icre* mice, a strong reduction of IL-22 expression occurs(126). However, the IL-22 produced by these cells is redundant during *Citrobacter rodentium* infection, possibly because NCR⁺ ILC3 are only a fraction (10–30%) of the total ILC3 compartment. A possible specific role for NCR⁺ ILC3 in this model is supported by the evidence showing that *Stat3^{fl/fl}Ncr1-icre* mice display increased inflammation in the cecum, associated with cecal ulceration, bleeding and crypt hyperplasia. In addition, other specific roles for NCR⁺ ILC3 in regulating inflammation emerge in absence of adaptive immunity(126, 127).

In humans, dominant negative STAT3 mutations in the DNA-binding domain can cause the autosomal dominant form of hyperimmunoglobulin E syndrome (HIES, also known as Job syndrome), a rare disease which is associated with several clinical signs including recurrent bacterial infections and defects in Th17(128–130). An HIES mouse model has been generated, recapitulating several aspects of this disease, including defects in Th17 differentiation(131). Along with impairment in adaptive responses, HIES patients show a decreased ILC number and relative defects in IL-17 production(132).

The role of STAT3 is not restricted to ILC3, but it also extends to NK cells. Indeed, NK cells from HIES-patients and *Stat3^{fl/fl}Tie2-cre* mice show decreased NKG2D expression and impaired effector functions(133). In contrast, conditional deletion of STAT3 in NK cells by using *Stat3^{fl/fl}Ncr1-icre* mice is not associated with developmental or functional defects in NK cells. Instead, loss of STAT3 in these mice leads to an increased NK cell antitumor activity due to the STAT3-dependent repression of DNAM-1, Perforin and Granzyme B, normally occurring in WT cells(134). The usage of different cre-systems inducing deletion of STAT3 before (*Stat3^{fl/fl}Tie2-cre*) or after (*Stat3^{fl/fl}Ncr1-icre*) NK cell differentiation could explain the conflicting results observed in NK cell functions(135).

In a scenario where immune responses can be considered highly dynamic, the combinatorial expression of multiple STATs allows distinct ILC subsets to provide versatile and, in the long run, more plastic effector functions. Thus, it is not surprising that the prolonged exposure to inflammatory cytokines, as IL-23 and IL-12, can skew the ILC phenotypes toward alternative fates, in a process resembling the functional plasticity of Th subsets(45, 136–140). In this regard, IL-12 and IL-1 β can drive transition of ILC2(137, 138, 140) toward the ILC1 phenotype in humans and mice, while IL-23 along with IL-12 are involved in the ILC1-ILC3 transitions(45, 136, 141). Our group recently characterized the genes induced by IL-23 in murine NCR⁺ ILC3 by transcriptomic analyses. Thanks to the co-expression of ROR γ t and T-BET, NCR⁺ ILC3 are able to activate both genes typical of the type 3 response and genes associated with the NK cell response, even if production of IFN- γ requires a longer kinetics than IL-22. We also found that selective expression of STAT4 in NCR⁺ ILC3 is involved in amplification of IFN- γ production in a IL-23-dependent manner(142). Thus, the combinatorial expression of LDTFs and SDTFs can modulate the outcome of cytokine signaling in ILCs. If, on one hand, inflammation is the main driver of ILC plasticity, peculiar polarizing environments, such as the mucosal sites, are the main sites where ILC conversion spontaneously can take place. This is the case for NK/ILC1 and ILC3/ILC1 transition. In this context, the role for SDTFs, including not only STATs, but also

AHRs, RORs, NOTCHs and SMADs has now been consolidated by a large number of evidence(143–146).

4 MOLECULAR MECHANISMS DRIVING STAT-DEPENDENT GENE EXPRESSION IN ILCs

The use of next generation sequencing approaches in combination with mice deficient for STATs represents a useful tool to elucidate the molecular mechanisms underlying regulation of gene expression by these TFs. Due to technical limitations, our knowledge on the *modus operandi* of STATs (Figure 2) in ILCs has been mainly obtained from studies on NK cells, the most abundant subset. However, it is highly probable that thanks to the rapid advances of “single cell” technology this knowledge gap will be soon filled. In comparison to other STATs, the mechanisms of gene regulation by STAT5 have been more extensively studied and better delineated(94, 147–149). Beyond the identification of genes undergoing positive and negative regulation, the analysis of genomic distribution of STAT5 has revealed a widespread DNA binding both in unstimulated and IL-15-stimulated NK cells(94). The activation process induces significant differences in STAT5 localization, with canonical GAS binding sites being occupied mainly in IL-15-treated NK cells. These observations indicate that in NK cells, STAT5 can constitutively bind DNA in homeostatic conditions, while acute stimulation with γ_c cytokines can induce its redistribution and recruitment to specific DREs(94).

STAT5 proteins can form dimers or tetramers endowed with distinct functional properties and ability to interact with different DREs. More specifically, STAT5 dimers bind to high-affinity canonical GAS motifs, while STAT5 tetramers localize at divergent motifs with an optimal spacing of 2–27 base pairs between GAS and GAS-like sequences. Studies on murine NK cells unable to form STAT5-tetramers (*Stat5a-Stat5b* tetramer-deficient double knockin, DKI) have demonstrated their relative contribution in regulation of gene expression(148, 149). In *Stat5* DKI mice, the number of total and terminally differentiated NK cells in the BM is decreased, due to a specific role of STAT5 tetramers in the regulation of normal expression of the anti-apoptotic gene *Bcl2*(148). Although upstream mechanisms promoting tetramerization versus dimerization are not well understood, specificity of timing and cell type of these molecular interactions denotes the high selectivity of STAT5 actions. Further investigations are needed to better understand the orchestration of these events and if this kind of regulation can be extended to other STATs.

Along with genes involved in NK cell differentiation and survival, analyses of the genome-wide distribution of STATs has helped defining their targets in NK cells in a more comprehensive manner. Among STAT5 target genes, regulation of T-BET and ID2 provide further mechanistic insights of how STATs can operate in ILCs. Beyond a direct regulation of T-BET expression, STAT5 can also cooperate with this LDTF to control similar gene patterns in NK cells(94). ChIP-seq and transcriptomic analyses have revealed that, in NK cells, T-BET co-localizes with STAT5 throughout the genome and promotes the expression of genes that are directly regulated by this SDTF. The interaction with distinct TFs to assembly multifactorial complexes may account for STAT5 binding to not canonical motifs

but also for the regulation of cell-type specific genes. T-BET is also an example of how a target gene can be regulated by different STATs. Indeed, it has been demonstrated that in IL-12 plus IL-18 activated NK cells and adaptive NK cells, T-BET expression can be induced by STAT4. Accordingly, a substantial enrichment of STAT4 at a distal enhancer site occurs along with an increase in the permissive histone modification H3K4me3 at the promoter region in activated WT but not *Stat4*^{-/-} NK cells(110).

Regulation of *Id2* gene by STAT5 occurs in mice with a peculiar mechanism involving a cis-DRE associated with a long non-coding RNA (lncRNA), named *Rroid* (RNA-demarcated regulatory region of ID2)(147). While *Rroid* is specifically expressed in type 1 ILCs, only the *Rroid* locus, but not the lncRNA itself, is required for the adequate STAT5 occupancy of the *Id2* promoter and transcription of this gene in ILC1. In contrast to type 1 ILCs, the *Rroid* locus does not control *Id2* expression in ILC2 and ILC3 and it is not required for early ILC development, providing evidence for how epigenetic elements can contribute to determine and maintain the identity of individual differentiated ILC subsets(147). Long-range interactions between promoters and enhancers play a critical role in the regulation of gene expression(150). Thus, improving the genomic tools for dissecting the chromatin interactions underlying lineage specification will represent a key element for future studies.

Altogether, these examples have highlighted the complexity and specificity of the mechanisms by which STATs operate at molecular and genome level in NK cells. Further “omics” studies focused on the role of different STATs in ILC subsets will be helpful to gain a more complete comprehension of these mechanisms.

5 TARGETING JAKS

Led by the development of drugs used in the treatment of cancer, targeting of phosphotransferases like the JAKs has become a reality and JAK inhibitors are now used to treat immune-mediated disorders(151). First-generation JAK inhibitors block the enzymatic activity of all the JAKs with various degrees of specificity (Figure 2). So far, four JAK inhibitors have been approved for clinical use. Ruxolitinib is a JAK2/JAK1 inhibitor currently utilized for the treatment of myeloproliferative disorders. Tofacitinib, a JAK3/JAK1 (and to a lesser extent, JAK2) inhibitor was approved in 2012 for the treatment of patients with methotrexate-resistant rheumatoid arthritis (RA) and more recently for the treatment of psoriatic arthritis. Baricitinib is also a JAK2 and JAK1 inhibitor, that has been approved by the European regulatory agency for the treatment of RA. Finally, oclacitinib is a pan-JAK inhibitor which is approved for the treatment of canine allergic dermatitis. Moreover, we have recently shown that tofacitinib is efficacious in ameliorating several aspects of the pathology associated with a murine model of Systemic Lupus Erythematosus(152). A group of genetically inherited diseases characterized by overproduction of Interferons (also known as Interferonopathies) have also been treated with JAK inhibitors with positive results(153). Moreover, JAK inhibitors are being investigated in late phase clinical trials for several other pathologies including alopecia, vitiligo, psoriasis, inflammatory bowel disease and atopic dermatitis(151).

First-generation JAK inhibitors target several cytokines, including interferons, hormones and growth factors, and their activity could contribute to increased rates of infection. Patients treated with tofacitinib and baricitinib are also prone to reactivation of varicella zoster virus. Moreover, inhibition of JAK2-dependent erythropoietin results in lower hemoglobin levels. Other changes in laboratory parameters include increase in circulating lipoproteins, creatinine and transaminases. So far, the risk of cancer in JAK inhibitors-treated patients seems to be equivalent to other biological therapies although long-term follow-up studies are needed(154). Blocking a single JAK could potentially reduce their toxicity, especially in the setting of long-term use as it is in the case of autoimmune diseases. Therefore, more selective JAK inhibitors have been developed and are currently being tested. However, it is also possible that selectivity may also reduce their efficacy.

JAK inhibitors have been used *ex vivo* and *in vivo* to evaluate their effects on homeostasis and functions of several adaptive and innate immune cells(154). In CD4⁺ T cells, *ex vivo* treatment with tofacitinib affects differentiation towards the different Th subsets and inhibits the expression of genes associated with rheumatoid arthritis risk, through a mechanism affecting their super enhancer structure(155, 156). Similarly, production of signature cytokines and proliferation of human ILCs are inhibited after *ex vivo* stimulation with cytokines(76, 157, 158). Transcriptomic analysis of IL-2 activated NK cells has shown that specific JAK3 and JAK1 inhibitors have similar effects on gene expression. However, from a therapeutic point of view, it is interesting to note that a JAK1-selective inhibitor is more effective in inhibiting secondary autocrine responses induced by IFN- γ in NK cells(159). *In vivo* treatment with JAK inhibitors in animal models has been associated with a strong reduction of the NK cell frequency(159, 160). The same effect has been also observed in patients undergoing tofacitinib treatment(161, 162). In comparison to adaptive cells which remain mainly unaltered, NK cells are highly sensitive to both first-generation of JAK inhibitors (tofacitinib and baricitinib), as well as JAK1- or JAK3- specific inhibitors (159). Based on these observations, further studies are clearly needed to establish more appropriate dosing of these drugs in order to maximize their efficacy on specific immune responses while minimizing toxicity.

6 CONCLUSION

ILCs are considered potent amplifiers of inflammation and their role in providing host protection has been shown in several mouse models of infection and cancer. Cytokines activating the JAK/STAT pathway control several aspects of ILC biology, including development, early effector functions, plasticity and secondary responses. However, given the broad expression of STATs and their pleiotropic effects, it will be key to understand what is specific to ILCs and what is redundant. This aspect is particularly relevant when targeting ILC functions which has the potential to be used as therapeutic strategy for human diseases. In this regard, primary immunodeficiencies have revealed specific roles for NK cells in controlling viral infections(163, 164), while specific ILC functions in humans remain to be defined(75). What is becoming clear is that manipulation of cytokine signaling in NK cells and other ILCs can have profound implications in protecting against inflammation and cancer, as it has been shown by interfering with IL-18, TGF- β and IL-15 signalling(165–167). A class of modulators of the JAK/STAT pathway exists in nature, namely suppressor

of cytokine signaling (SOCS) protein(168). As a consequence of deletion of *Cish*, a SOCS protein which negatively regulate IL-15 signaling, NK cell dependent tumor immunity is enhanced in mouse models(167). On the other hand, pharmacological inhibition of JAK enzymatic activity has proved successful for several immune-mediated pathologies, and represents both a strategy to modulate ILC effector functions during diseases and a great opportunity to study and better understand ILC biology(169). However, a lot remains to be learned regarding the optimal degree of JAK inhibition required for a specific disease and/or phase of the illness, as well as the definition of biomarkers that could predict patient responsiveness. Thus, several questions regarding this new class of drugs remain to be answered, and this represents fantastic opportunities for future research.

Acknowledgments

The final manuscript was a result of the joint efforts of all the authors. G.Sci. and A.S. are supported by Institut Pasteur, PTR 113–17; A.S. is supported by the Italian Association for Cancer Research (AIRC) Special Program Molecular and Clinical Oncology-5 per Mille; M.G. is supported by the NIAMS Intramural Research Programs. The authors declare no commercial or financial conflict of interest.

References

1. Serafini N, Vosshehrich CAJ, Di Santo JP. Transcriptional regulation of innate lymphoid cell fate. *Nat. Rev. Immunol* 2015; 15: 415–28. [PubMed: 26065585]
2. Klose CSN, Diefenbach A Transcription factors controlling innate lymphoid cell fate decisions. *Curr. Top. Microbiol. Immunol* 2014; 381: 215–55. [PubMed: 25038936]
3. Mjösberg J, Spits H Human innate lymphoid cells. *J. Allergy Clin. Immunol* 2016; 138: 1265–1276. [PubMed: 27677386]
4. Sciumè G, Shih H-Y, Mikami Y, O'Shea JJ. Epigenomic Views of Innate Lymphoid Cells. *Front. Immunol* 2017; 8: 1579. [PubMed: 29250060]
5. Robinette ML, Colonna M Immune modules shared by innate lymphoid cells and T cells. *J. Allergy Clin. Immunol* 2016; 138: 1243–1251. [PubMed: 27817796]
6. De Obaldia ME, Bhandoola A. Transcriptional regulation of innate and adaptive lymphocyte lineages. *Annu. Rev. Immunol* 2015; 33: 607–42. [PubMed: 25665079]
7. Shih H-Y, Sciumè G, Poholek AC, et al. Transcriptional and epigenetic networks of helper T and innate lymphoid cells. *Immunol. Rev* 2014; 261: 23–49. [PubMed: 25123275]
8. Christie D, Zhu J Transcriptional regulatory networks for CD4 T cell differentiation. *Curr. Top. Microbiol. Immunol* 2014; 381: 125–72. [PubMed: 24839135]
9. Villarino AV, Kanno Y, O'Shea JJ. Mechanisms and consequences of Jak-STAT signaling in the immune system. *Nat. Immunol* 2017; 18: 374–384. [PubMed: 28323260]
10. O'Shea JJ, Paul WE. Mechanisms underlying lineage commitment and plasticity of helper CD4+ T cells. *Science* 2010; 327: 1098–102. [PubMed: 20185720]
11. Zhu J, Paul WE. Peripheral CD4+ T-cell differentiation regulated by networks of cytokines and transcription factors. *Immunol. Rev* 2010; 238: 247–62. [PubMed: 20969597]
12. Rochman Y, Spolski R, Leonard WJ. New insights into the regulation of T cells by gamma(c) family cytokines. *Nat. Rev. Immunol* 2009; 9: 480–90. [PubMed: 19543225]
13. Sun JC, Lanier LL. NK cell development, homeostasis and function: parallels with CD8⁺ T cells. *Nat. Rev. Immunol* 2011; 11: 645–57. [PubMed: 21869816]
14. Huntington ND, Vosshehrich CAJ, Di Santo JP. Developmental pathways that generate natural-killer-cell diversity in mice and humans. *Nat. Rev. Immunol* 2007; 7: 703–14. [PubMed: 17717540]
15. Marçais A, Viel S, Grau M, Henry T, Marvel J, Walzer T Regulation of mouse NK cell development and function by cytokines. *Front. Immunol* 2013; 4: 450. [PubMed: 24376448]

16. Vidal SM, Khakoo SI, Biron CA. Natural killer cell responses during viral infections: flexibility and conditioning of innate immunity by experience. *Curr. Opin. Virol* 2011; 1: 497–512. [PubMed: 22180766]
17. Stabile H, Fionda C, Gismondi A, Santoni A Role of Distinct Natural Killer Cell Subsets in Anticancer Response. *Front. Immunol* 2017; 8: 293. [PubMed: 28360915]
18. Marcus A, Gowen BG, Thompson TW, et al. Recognition of tumors by the innate immune system and natural killer cells. *Adv. Immunol* 2014; 122: 91–128. [PubMed: 24507156]
19. Mebius RE, Streeter PR, Michie S, Butcher EC, Weissman IL. A developmental switch in lymphocyte homing receptor and endothelial vascular addressin expression regulates lymphocyte homing and permits CD4+ CD3- cells to colonize lymph nodes. *Proc. Natl. Acad. Sci. U. S. A* 1996; 93: 11019–24. [PubMed: 8855301]
20. Mebius RE, Rennert P, Weissman IL. Developing lymph nodes collect CD4+CD3- LTbeta+ cells that can differentiate to APC, NK cells, and follicular cells but not T or B cells. *Immunity* 1997; 7: 493–504. [PubMed: 9354470]
21. Adachi S, Yoshida H, Kataoka H, Nishikawa S Three distinctive steps in Peyer's patch formation of murine embryo. *Int. Immunol* 1997; 9: 507–14. [PubMed: 9138010]
22. Yoshida H, Honda K, Shinkura R, et al. IL-7 receptor alpha+ CD3(-) cells in the embryonic intestine induces the organizing center of Peyer's patches. *Int. Immunol* 1999; 11: 643–55. [PubMed: 10330270]
23. Cupedo T, Kraal G, Mebius RE. The role of CD45+CD4+CD3- cells in lymphoid organ development. *Immunol. Rev* 2002; 189: 41–50. [PubMed: 12445264]
24. Cherrier M, Eberl G The development of LT α cells. *Curr. Opin. Immunol* 2012; 24: 178–83. [PubMed: 22386930]
25. Neill DR, Wong SH, Bellosi A, et al. Nuocytes represent a new innate effector leukocyte that mediates type-2 immunity. *Nature* 2010; 464: 1367–70. [PubMed: 20200518]
26. Moro K, Yamada T, Tanabe M, et al. Innate production of T(H)2 cytokines by adipose tissue-associated c-Kit(+)Sca-1(+) lymphoid cells. *Nature* 2010; 463: 540–4. [PubMed: 20023630]
27. Price AE, Liang H-E, Sullivan BM, et al. Systemically dispersed innate IL-13-expressing cells in type 2 immunity. *Proc. Natl. Acad. Sci. U. S. A* 2010; 107: 11489–94. [PubMed: 20534524]
28. Saenz SA, Siracusa MC, Perrigoue JG, et al. IL25 elicits a multipotent progenitor cell population that promotes T(H)2 cytokine responses. *Nature* 2010; 464: 1362–6. [PubMed: 20200520]
29. Satoh-Takayama N, Vosschenrich CAJ, Lesjean-Pottier S, et al. Microbial flora drives interleukin 22 production in intestinal NKp46+ cells that provide innate mucosal immune defense. *Immunity* 2008; 29: 958–70. [PubMed: 19084435]
30. Cella M, Fuchs A, Vermi W, et al. A human natural killer cell subset provides an innate source of IL-22 for mucosal immunity. *Nature* 2009; 457: 722–5. [PubMed: 18978771]
31. Luci C, Reynders A, Ivanov II, et al. Influence of the transcription factor ROR γ on the development of NKp46+ cell populations in gut and skin. *Nat. Immunol* 2009; 10: 75–82. [PubMed: 19029904]
32. Sanos SL, Bui VL, Mortha A, et al. ROR γ and commensal microflora are required for the differentiation of mucosal interleukin 22-producing NKp46+ cells. *Nat. Immunol* 2009; 10: 83–91. [PubMed: 19029903]
33. Takatori H, Kanno Y, Watford WT, et al. Lymphoid tissue inducer-like cells are an innate source of IL-17 and IL-22. *J. Exp. Med* 2009; 206: 35–41. [PubMed: 19114665]
34. Cupedo T, Crellin NK, Papazian N, et al. Human fetal lymphoid tissue-inducer cells are interleukin 17-producing precursors to ROR γ + CD127+ natural killer-like cells. *Nat. Immunol* 2009; 10: 66–74. [PubMed: 19029905]
35. Spits H, Artis D, Colonna M, et al. Innate lymphoid cells--a proposal for uniform nomenclature. *Nat. Rev. Immunol* 2013; 13: 145–9. [PubMed: 23348417]
36. Spits H, Bernink JH, Lanier L NK cells and type 1 innate lymphoid cells: partners in host defense. *Nat. Immunol* 2016; 17: 758–64. [PubMed: 27328005]
37. McKenzie ANJ. Type-2 innate lymphoid cells in asthma and allergy. *Ann. Am. Thorac. Soc* 2014; 11 Suppl 5: S263–70. [PubMed: 25525730]

38. van de Pavert SA, Vivier E. Differentiation and function of group 3 innate lymphoid cells, from embryo to adult. *Int. Immunol* 2016; 28: 35–42. [PubMed: 26374472]
39. Diefenbach A, Colonna M, Koyasu S Development, differentiation, and diversity of innate lymphoid cells. *Immunity* 2014; 41: 354–65. [PubMed: 25238093]
40. Gordon SM, Chaix J, Rupp LJ, et al. The transcription factors T-bet and Eomes control key checkpoints of natural killer cell maturation. *Immunity* 2012; 36: 55–67. [PubMed: 22261438]
41. Daussy C, Faure F, Mayol K, et al. T-bet and Eomes instruct the development of two distinct natural killer cell lineages in the liver and in the bone marrow. *J. Exp. Med* 2014; 211: 563–77. [PubMed: 24516120]
42. Townsend MJ, Weinmann AS, Matsuda JL, et al. T-bet regulates the terminal maturation and homeostasis of NK and Valpha14i NKT cells. *Immunity* 2004; 20: 477–94. [PubMed: 15084276]
43. Zhang J, Marotel M, Fauteux-Daniel S, et al. T-bet and Eomes govern differentiation and function of mouse and human NK cells and ILC1. *Eur. J. Immunol* 2018
44. Sciumé G, Hirahara K, Takahashi H, et al. Distinct requirements for T-bet in gut innate lymphoid cells. *J. Exp. Med* 2012; 209: 2331–8. [PubMed: 23209316]
45. Klose CSN, Kiss EA, Schwierzeck V, et al. A T-bet gradient controls the fate and function of CCR6-ROR γ t+ innate lymphoid cells. *Nature* 2013; 494: 261–5. [PubMed: 23334414]
46. Rankin LC, Groom JR, Chopin M, et al. The transcription factor T-bet is essential for the development of NKp46+ innate lymphocytes via the Notch pathway. *Nat. Immunol* 2013; 14: 389–95. [PubMed: 23455676]
47. Yagi R, Zhong C, Northrup DL, et al. The transcription factor GATA3 is critical for the development of all IL-7R α -expressing innate lymphoid cells. *Immunity* 2014; 40: 378–88. [PubMed: 24631153]
48. Serafini N, Klein Wolterink RGJ, Satoh-Takayama N, et al. Gata3 drives development of ROR γ t+ group 3 innate lymphoid cells. *J. Exp. Med* 2014; 211: 199–208. [PubMed: 24419270]
49. Zhong C, Cui K, Wilhelm C, et al. Group 3 innate lymphoid cells continuously require the transcription factor GATA-3 after commitment. *Nat. Immunol* 2016; 17: 169–78. [PubMed: 26595886]
50. Zhu J GATA3 Regulates the Development and Functions of Innate Lymphoid Cell Subsets at Multiple Stages. *Front. Immunol* 2017; 8: 1571. [PubMed: 29184556]
51. Yu X, Wang Y, Deng M, et al. The basic leucine zipper transcription factor NFIL3 directs the development of a common innate lymphoid cell precursor. *Elife* 2014; 3:
52. Xu W, Domingues RG, Fonseca-Pereira D, et al. NFIL3 orchestrates the emergence of common helper innate lymphoid cell precursors. *Cell Rep* 2015; 10: 2043–54. [PubMed: 25801035]
53. Geiger TL, Abt MC, Gasteiger G, et al. Nfil3 is crucial for development of innate lymphoid cells and host protection against intestinal pathogens. *J. Exp. Med* 2014; 211: 1723–31. [PubMed: 25113970]
54. Yang Q, Li F, Harly C, et al. TCF-1 upregulation identifies early innate lymphoid progenitors in the bone marrow. *Nat. Immunol* 2015; 16: 1044–50. [PubMed: 26280998]
55. Constantinides MG, McDonald BD, Verhoef PA, Bendelac A A committed precursor to innate lymphoid cells. *Nature* 2014; 508: 397–401. [PubMed: 24509713]
56. Klose CSN, Flach M, Möhle L, et al. Differentiation of type 1 ILCs from a common progenitor to all helper-like innate lymphoid cell lineages. *Cell* 2014; 157: 340–356. [PubMed: 24725403]
57. Lim AI, Verrier T, Vosshenrich CA, Di Santo JP. Developmental options and functional plasticity of innate lymphoid cells. *Curr. Opin. Immunol* 2017; 44: 61–68. [PubMed: 28359987]
58. Lim AI, Li Y, Lopez-Lastra S, et al. Systemic Human ILC Precursors Provide a Substrate for Tissue ILC Differentiation. *Cell* 2017; 168: 1086–1100.e10. [PubMed: 28283063]
59. Shih HY, Sciumè G, Mikami Y, et al. Developmental Acquisition of Regulomes Underlies Innate Lymphoid Cell Functionality. *Cell* 2016; 165: 1120–1133. [PubMed: 27156451]
60. Gronke K, Kofoed-Nielsen M, Diefenbach A Innate lymphoid cells, precursors and plasticity. *Immunol. Lett* 2016; 179: 9–18. [PubMed: 27394700]
61. Juelke K, Romagnani C Differentiation of human innate lymphoid cells (ILCs). *Curr. Opin. Immunol* 2016; 38: 75–85. [PubMed: 26707651]

62. Yu Y, Tsang JCH, Wang C, et al. Single-cell RNA-seq identifies a PD-1(hi) ILC progenitor and defines its development pathway. *Nature* 2016; 539: 102–106. [PubMed: 27749818]
63. Schindler C, Levy DE, Decker T JAK-STAT signaling: from interferons to cytokines. *J. Biol. Chem* 2007; 282: 20059–63. [PubMed: 17502367]
64. Stark GR, Darnell JE. The JAK-STAT pathway at twenty. *Immunity* 2012; 36: 503–14. [PubMed: 22520844]
65. Liongue C, Ward AC. Evolution of the JAK-STAT pathway. *JAK-STAT* 2013; 2: e22756. [PubMed: 24058787]
66. Wang Y, Levy DE. Comparative evolutionary genomics of the STAT family of transcription factors. *JAK-STAT* 2012; 1: 23–33. [PubMed: 24058748]
67. Levy DE, Darnell JE. Stats: transcriptional control and biological impact. *Nat. Rev. Mol. Cell Biol.* 2002; 3: 651–62. [PubMed: 12209125]
68. Levy DE, Marié IJ. STATus report on tetramers. *Immunity* 2012; 36: 553–5. [PubMed: 22520849]
69. Pesu M, Candotti F, Husa M, Hofmann SR, Notarangelo LD, O'Shea JJ. Jak3, severe combined immunodeficiency, and a new class of immunosuppressive drugs. *Immunol. Rev* 2005; 203: 127–42. [PubMed: 15661026]
70. Di Santo JP, Kühn R, Müller W. Common cytokine receptor gamma chain (gamma c)-dependent cytokines: understanding in vivo functions by gene targeting. *Immunol. Rev* 1995; 148: 19–34. [PubMed: 8825280]
71. Leonard WJ. Cytokines and immunodeficiency diseases. *Nat. Rev. Immunol* 2001; 1: 200–8. [PubMed: 11905829]
72. Casanova J-L, Holland SM, Notarangelo LD. Inborn errors of human JAKs and STATs. *Immunity* 2012; 36: 515–28. [PubMed: 22520845]
73. Sponzilli I, Notarangelo LD. Severe combined immunodeficiency (SCID): from molecular basis to clinical management. *Acta Biomed* 2011; 82: 5–13. [PubMed: 22069950]
74. Macchi P, Villa A, Giliani S, et al. Mutations of Jak-3 gene in patients with autosomal severe combined immune deficiency (SCID). *Nature* 1995; 377: 65–8. [PubMed: 7659163]
75. Vély F, Barlogis V, Vallentin B, et al. Evidence of innate lymphoid cell redundancy in humans. *Nat. Immunol* 2016; 17: 1291–1299. [PubMed: 27618553]
76. Robinette ML, Cella M, Telliez JB, et al. Jak3 deficiency blocks innate lymphoid cell development. *Mucosal Immunol* 2018; 11: 50–60. [PubMed: 28513593]
77. Kennedy MK, Glaccum M, Brown SN, et al. Reversible defects in natural killer and memory CD8 T cell lineages in interleukin 15-deficient mice. *J. Exp. Med* 2000; 191: 771–80. [PubMed: 10704459]
78. Ranson T, Vosshehrich CAJ, Corcuff E, Richard O, Müller W, Di Santo JP. IL-15 is an essential mediator of peripheral NK-cell homeostasis. *Blood* 2003; 101: 4887–93. [PubMed: 12586624]
79. Lodolce JP, Boone DL, Chai S, et al. IL-15 receptor maintains lymphoid homeostasis by supporting lymphocyte homing and proliferation. *Immunity* 1998; 9: 669–76. [PubMed: 9846488]
80. Huntington ND. The unconventional expression of IL-15 and its role in NK cell homeostasis. *Immunol. Cell Biol* 2014; 92: 210–3. [PubMed: 24492800]
81. Stabile H, Fionda C, Santoni A, Gismondi A Impact of bone marrow-derived signals on NK cell development and functional maturation. *Cytokine Growth Factor Rev* 2018
82. Vosshehrich CAJ, Ranson T, Samson SI, et al. Roles for common cytokine receptor gamma-chain-dependent cytokines in the generation, differentiation, and maturation of NK cell precursors and peripheral NK cells in vivo. *J. Immunol* 2005; 174: 1213–21. [PubMed: 15661875]
83. Gilmour KC, Fujii H, Cranston T, Davies EG, Kinnon C, Gaspar HB. Defective expression of the interleukin-2/interleukin-15 receptor beta subunit leads to a natural killer cell-deficient form of severe combined immunodeficiency. *Blood* 2001; 98: 877–9. [PubMed: 11468191]
84. Cavazzana-Calvo M, Hacein-Bey S, de Saint Basile G, et al. Role of interleukin-2 (IL-2), IL-7, and IL-15 in natural killer cell differentiation from cord blood hematopoietic progenitor cells and from gamma c transduced severe combined immunodeficiency X1 bone marrow cells. *Blood* 1996; 88: 3901–9. [PubMed: 8916956]

85. Fuchs A, Vermi W, Lee JS, et al. Intraepithelial type 1 innate lymphoid cells are a unique subset of IL-12- and IL-15-responsive IFN- γ -producing cells. *Immunity* 2013; 38: 769–81. [PubMed: 23453631]
86. Satoh-Takayama N, Lesjean-Pottier S, Vieira P, et al. IL-7 and IL-15 independently program the differentiation of intestinal CD3-NKp46+ cell subsets from Id2-dependent precursors. *J. Exp. Med* 2010; 207: 273–80. [PubMed: 20142427]
87. Cortez VS, Fuchs A, Cella M, Gilfillan S, Colonna M Cutting edge: Salivary gland NK cells develop independently of Nfil3 in steady-state. *J. Immunol* 2014; 192: 4487–91. [PubMed: 24740507]
88. Vonarbourg C, Diefenbach A Multifaceted roles of interleukin-7 signaling for the development and function of innate lymphoid cells. *Semin. Immunol* 2012; 24: 165–74. [PubMed: 22541512]
89. Vonarbourg C, Mortha A, Bui VL, et al. Regulated expression of nuclear receptor ROR γ t confers distinct functional fates to NK cell receptor-expressing ROR γ t(+) innate lymphocytes. *Immunity* 2010; 33: 736–51. [PubMed: 21093318]
90. Hoyler T, Klose CSN, Souabni A, et al. The transcription factor GATA-3 controls cell fate and maintenance of type 2 innate lymphoid cells. *Immunity* 2012; 37: 634–48. [PubMed: 23063333]
91. Robinette ML, Bando JK, Song W, Ulland TK, Gilfillan S, Colonna M IL-15 sustains IL-7R-independent ILC2 and ILC3 development. *Nat. Commun* 2017; 8: 14601. [PubMed: 28361874]
92. Teglund S, McKay C, Schuetz E, et al. Stat5a and Stat5b proteins have essential and nonessential, or redundant, roles in cytokine responses. *Cell* 1998; 93: 841–50. [PubMed: 9630227]
93. Imada K, Bloom ET, Nakajima H, et al. Stat5b is essential for natural killer cell-mediated proliferation and cytolytic activity. *J. Exp. Med* 1998; 188: 2067–74. [PubMed: 9841920]
94. Villarino AV, Sciumè G, Davis FP, et al. Subset- and tissue-defined STAT5 thresholds control homeostasis and function of innate lymphoid cells. *J. Exp. Med* 2017; 214: 2999–3014. [PubMed: 28916644]
95. Yao Z, Cui Y, Watford WT, et al. Stat5a/b are essential for normal lymphoid development and differentiation. *Proc. Natl. Acad. Sci. U. S. A* 2006; 103: 1000–5. [PubMed: 16418296]
96. Eckelhart E, Warsch W, Zebedin E, et al. A novel Ncr1-Cre mouse reveals the essential role of STAT5 for NK-cell survival and development. *Blood* 2011; 117: 1565–73. [PubMed: 21127177]
97. Villarino A, Laurence A, Robinson GW, et al. Signal transducer and activator of transcription 5 (STAT5) paralog dose governs T cell effector and regulatory functions. *Elife* 2016; 5
98. Thierfelder WE, van Deursen JM, Yamamoto K, et al. Requirement for Stat4 in interleukin-12-mediated responses of natural killer and T cells. *Nature* 1996; 382: 171–4. [PubMed: 8700208]
99. Watford WT, Hissong BD, Bream JH, Kanno Y, Muul L, O'Shea JJ. Signaling by IL-12 and IL-23 and the immunoregulatory roles of STAT4. *Immunol. Rev* 2004; 202: 139–56. [PubMed: 15546391]
100. Nguyen KB, Cousens LP, Doughty LA, Pien GC, Durbin JE, Biron CA. Interferon alpha/beta-mediated inhibition and promotion of interferon gamma: STAT1 resolves a paradox. *Nat. Immunol* 2000; 1: 70–6. [PubMed: 10881178]
101. Paolini R, Bernardini G, Molfetta R, Santoni A NK cells and interferons. *Cytokine Growth Factor Rev* 2015; 26: 113–20. [PubMed: 25443799]
102. Robbins SH, Tessmer MS, Van Kaer L, Brossay L. Direct effects of T-bet and MHC class I expression, but not STAT1, on peripheral NK cell maturation. *Eur. J. Immunol* 2005; 35: 757–65. [PubMed: 15719366]
103. Lucas M, Schachterle W, Oberle K, Aichele P, Diefenbach A Dendritic cells prime natural killer cells by trans-presenting interleukin 15. *Immunity* 2007; 26: 503–17. [PubMed: 17398124]
104. Baranek T, Manh T-PV, Alexandre Y, et al. Differential responses of immune cells to type I interferon contribute to host resistance to viral infection. *Cell Host Microbe* 2012; 12: 571–84. [PubMed: 23084923]
105. Beuneu H, Deguine J, Bouvier I, Santo JP Di, Albert, ML, Bousso, P. Cutting Edge: A dual role for type I IFNs during polyinosinic-polycytidylic acid-induced NK cell activation. *J. Immunol* 2011; 187: 2084–8. [PubMed: 21810605]

106. Miyagi T, Gil MP, Wang X, Louten J, Chu W-M, Biron, CA. High basal STAT4 balanced by STAT1 induction to control type 1 interferon effects in natural killer cells. *J. Exp. Med* 2007; 204: 2383–96. [PubMed: 17846149]
107. Sun JC, Beilke JN, Lanier LL. Adaptive immune features of natural killer cells. *Nature* 2009; 457: 557–61. [PubMed: 19136945]
108. Holmes TD, Bryceson YT. Natural killer cell memory in context. *Semin. Immunol* 2016; 28: 368–76. [PubMed: 27354353]
109. Sun JC, Madera S, Bezman NA, Beilke JN, Kaplan MH, Lanier LL. Proinflammatory cytokine signaling required for the generation of natural killer cell memory. *J. Exp. Med* 2012; 209: 947–54. [PubMed: 22493516]
110. Madera S, Geary CD, Lau CM, Pikovskaya O, Reiner SL, Sun JC. Cutting Edge: Divergent Requirement of T-Box Transcription Factors in Effector and Memory NK Cells. *J. Immunol* 2018; 200: 1977–1981. [PubMed: 29440505]
111. Rapp M, Lau CM, Adams NM, et al. Core-binding factor β and Runx transcription factors promote adaptive natural killer cell responses. *Sci. Immunol* 2017; 2
112. Firth MA, Madera S, Beaulieu AM, et al. Nfil3-independent lineage maintenance and antiviral response of natural killer cells. *J. Exp. Med* 2013; 210: 2981–90. [PubMed: 24277151]
113. Madera S, Rapp M, Firth MA, Beilke JN, Lanier LL, Sun JC. Type I IFN promotes NK cell expansion during viral infection by protecting NK cells against fratricide. *J. Exp. Med* 2016; 213: 225–33. [PubMed: 26755706]
114. Beaulieu AM, Zawislak CL, Nakayama T, Sun JC. The transcription factor Zbtb32 controls the proliferative burst of virus-specific natural killer cells responding to infection. *Nat. Immunol* 2014; 15: 546–53. [PubMed: 24747678]
115. Weizman O-E, Adams NM, Schuster IS, et al. ILC1 Confer Early Host Protection at Initial Sites of Viral Infection. *Cell* 2017; 171: 795–808.e12. [PubMed: 29056343]
116. Mackay LK, Minnich M, Kragten NAM, et al. Hobit and Blimp1 instruct a universal transcriptional program of tissue residency in lymphocytes. *Science* 2016; 352: 459–63. [PubMed: 27102484]
117. O’Sullivan TE, Rapp M, Fan X, et al. Adipose-Resident Group 1 Innate Lymphoid Cells Promote Obesity-Associated Insulin Resistance. *Immunity* 2016; 45: 428–41. [PubMed: 27496734]
118. Stier MT, Goleniewska K, Cephus JY, et al. STAT1 Represses Cytokine-Producing Group 2 and Group 3 Innate Lymphoid Cells during Viral Infection. *J. Immunol* 2017; 199: 510–519. [PubMed: 28576981]
119. Tabellini G, Vairo D, Scomodoni O, et al. Impaired natural killer cell functions in patients with signal transducer and activator of transcription 1 (STAT1) gain-of-function mutations. *J. Allergy Clin. Immunol* 2017; 140: 553–564.e4. [PubMed: 28069426]
120. Putz EM, Majoros A, Gotthardt D, et al. Novel non-canonical role of STAT1 in Natural Killer cell cytotoxicity. *Oncoimmunology* 2016; 5: e1186314. [PubMed: 27757297]
121. Ealey KN, Moro K, Koyasu S Are ILC2s Jekyll and Hyde in airway inflammation? *Immunol. Rev* 2017; 278: 207–218. [PubMed: 28658554]
122. Kim BS, Artis D Group 2 innate lymphoid cells in health and disease. *Cold Spring Harb. Perspect. Biol* 2015; 7
123. Liang H-E, Reinhardt RL, Bando JK, Sullivan BM, Ho I-C, Locksley RM. Divergent expression patterns of IL-4 and IL-13 define unique functions in allergic immunity. *Nat. Immunol* 2011; 13: 58–66. [PubMed: 22138715]
124. Doherty TA, Khorram N, Chang JE, et al. STAT6 regulates natural helper cell proliferation during lung inflammation initiated by *Alternaria*. *Am. J. Physiol. Lung Cell. Mol. Physiol* 2012; 303: L577–88. [PubMed: 22865552]
125. Guo X, Qiu J, Tu T, et al. Induction of innate lymphoid cell-derived interleukin-22 by the transcription factor STAT3 mediates protection against intestinal infection. *Immunity* 2014; 40: 25–39. [PubMed: 24412612]
126. Rankin LC, Girard-Madoux MJH, Seillet C, et al. Complementarity and redundancy of IL-22-producing innate lymphoid cells. *Nat. Immunol* 2016; 17: 179–86. [PubMed: 26595889]

127. Song C, Lee JS, Gilfillan S, et al. Unique and redundant functions of NKp46+ ILC3s in models of intestinal inflammation. *J. Exp. Med* 2015; 212: 1869–82. [PubMed: 26458769]
128. Holland SM, DeLeo FR, Elloumi HZ, et al. STAT3 mutations in the hyper-IgE syndrome. *N. Engl. J. Med* 2007; 357: 1608–19. [PubMed: 17881745]
129. Heimall J, Freeman A, Holland SM. Pathogenesis of hyper IgE syndrome. *Clin. Rev. Allergy Immunol* 2010; 38: 32–8. [PubMed: 19452285]
130. Minegishi Y, Saito M, Tsuchiya S, et al. Dominant-negative mutations in the DNA-binding domain of STAT3 cause hyper-IgE syndrome. *Nature* 2007; 448: 1058–62. [PubMed: 17676033]
131. Steward-Tharp SM, Laurence A, Kanno Y, et al. A mouse model of HIES reveals pro- And anti-inflammatory functions of STAT3. *Blood* 2014; 123: 2978–2987. [PubMed: 24632714]
132. Chang Y, Kang S-Y, Kim J, Kang H-R, Kim HY. Functional Defects in Type 3 Innate Lymphoid Cells and Classical Monocytes in a Patient with Hyper-IgE Syndrome. *Immune Netw* 2017; 17: 352–364. [PubMed: 29093656]
133. Zhu S, Phatarpekar PV, Denman CJ, et al. Transcription of the activating receptor NKG2D in natural killer cells is regulated by STAT3 tyrosine phosphorylation. *Blood* 2014; 124: 403–11. [PubMed: 24891320]
134. Gotthardt D, Putz EM, Straka E, et al. Loss of STAT3 in murine NK cells enhances NK cell-dependent tumor surveillance. *Blood* 2014; 124: 2370–9. [PubMed: 25185262]
135. Gotthardt D, Sexl V STATs in NK-Cells: The Good, the Bad, and the Ugly. *Front. Immunol* 2016; 7: 694. [PubMed: 28149296]
136. Bernink JH, Krabbendam L, Germar K, et al. Interleukin-12 and -23 Control Plasticity of CD127(+) Group 1 and Group 3 Innate Lymphoid Cells in the Intestinal Lamina Propria. *Immunity* 2015; 43: 146–60. [PubMed: 26187413]
137. Silver JS, Kearley J, Copenhaver AM, et al. Inflammatory triggers associated with exacerbations of COPD orchestrate plasticity of group 2 innate lymphoid cells in the lungs. *Nat. Immunol* 2016; 17: 626–35. [PubMed: 27111143]
138. Ohne Y, Silver JS, Thompson-Snipes L, et al. IL-1 is a critical regulator of group 2 innate lymphoid cell function and plasticity. *Nat. Immunol* 2016; 17: 646–55. [PubMed: 27111142]
139. Huang Y, Guo L, Qiu J, et al. IL-25-responsive, lineage-negative KLRG1(hi) cells are multipotential “inflammatory” type 2 innate lymphoid cells. *Nat. Immunol* 2015; 16: 161–9. [PubMed: 25531830]
140. Lim AI, Menegatti S, Bustamante J, et al. IL-12 drives functional plasticity of human group 2 innate lymphoid cells. *J. Exp. Med* 2016; 213: 569–83. [PubMed: 26976630]
141. Cella M, Otero K, Colonna M Expansion of human NK-22 cells with IL-7, IL-2, and IL-1beta reveals intrinsic functional plasticity. *Proc. Natl. Acad. Sci. U. S. A* 2010; 107: 10961–6. [PubMed: 20534450]
142. Mikami Y, Scarno G, Zitti B, et al. NCR+ ILC3 maintain larger STAT4 reservoir via T-BET to regulate type 1 features upon IL-23 stimulation in mice. *Eur. J. Immunol* 2018
143. Cella M, Colonna M Aryl hydrocarbon receptor: Linking environment to immunity. *Semin. Immunol* 2015; 27: 310–4. [PubMed: 26561251]
144. Lim AWY, McKenzie ANJ. Deciphering the transcriptional switches of innate lymphoid cell programming: the right factors at the right time. *Genes Immun* 16: 177–86. [PubMed: 25611557]
145. Montaldo E, Juelke K, Romagnani C Group 3 innate lymphoid cells (ILC3s): Origin, differentiation, and plasticity in humans and mice. *Eur. J. Immunol* 2015; 45: 2171–82. [PubMed: 26031799]
146. Eberl G ROR γ t, a multitask nuclear receptor at mucosal surfaces. *Mucosal Immunol.* 2017; 10: 27–34. [PubMed: 27706126]
147. Mowel WK, McCright SJ, Kotzin JJ, et al. Group 1 Innate Lymphoid Cell Lineage Identity Is Determined by a cis-Regulatory Element Marked by a Long Non-coding RNA. *Immunity* 2017; 47: 435–449.e8. [PubMed: 28930659]
148. Lin J-X, Du N, Li P, et al. Critical functions for STAT5 tetramers in the maturation and survival of natural killer cells. *Nat. Commun* 2017; 8: 1320. [PubMed: 29105654]

149. Lin J-X, Li P, Liu D, et al. Critical Role of STAT5 transcription factor tetramerization for cytokine responses and normal immune function. *Immunity* 2012; 36: 586–99. [PubMed: 22520852]
150. Bulger M, Groudine M Functional and mechanistic diversity of distal transcription enhancers. *Cell* 2011; 144: 327–39. [PubMed: 21295696]
151. Gadina M, Gazaniga N, Vian L, Furumoto Y Small molecules to the rescue: Inhibition of cytokine signaling in immune-mediated diseases. *J. Autoimmun* 2017; 85: 20–31. [PubMed: 28676205]
152. Furumoto Y, Smith CK, Blanco L, et al. Tofacitinib Ameliorates Murine Lupus and Its Associated Vascular Dysfunction. *Arthritis Rheumatol. (Hoboken, N.J.)* 2017; 69: 148–160.
153. Sanchez GAM, Reinhardt A, Ramsey S, et al. JAK1/2 inhibition with baricitinib in the treatment of autoinflammatory interferonopathies. *J. Clin. Invest* 2018
154. Schwartz DM, Kanno Y, Villarino A, Ward M, Gadina M, O'Shea JJ. JAK inhibition as a therapeutic strategy for immune and inflammatory diseases. *Nat. Rev. Drug Discov* 2017; 17: 78.
155. Ghoreschi K, Jesson MI, Li X, et al. Modulation of innate and adaptive immune responses by tofacitinib (CP-690,550). *J. Immunol* 2011; 186: 4234–43. [PubMed: 21383241]
156. Vahedi G, Kanno Y, Furumoto Y, et al. Super-enhancers delineate disease-associated regulatory nodes in T cells. *Nature* 2015; 520: 558–62. [PubMed: 25686607]
157. Glatzer T, Killig M, Meisig J, et al. ROR γ ⁺ innate lymphoid cells acquire a proinflammatory program upon engagement of the activating receptor NKp44. *Immunity* 2013; 38: 1223–35. [PubMed: 23791642]
158. Crellin NK, Trifari S, Kaplan CD, Satoh-Takayama N, Di Santo JP, Spits H. Regulation of cytokine secretion in human CD127(+) LTi-like innate lymphoid cells by Toll-like receptor 2. *Immunity* 2010; 33: 752–64. [PubMed: 21055975]
159. Moodley D, Yoshida H, Mostafavi S, et al. Network pharmacology of JAK inhibitors. *Proc. Natl. Acad. Sci. U. S. A* 2016; 113: 9852–7. [PubMed: 27516546]
160. Takahashi Y, Byraredy SN, Albrecht C, et al. In vivo administration of a JAK3 inhibitor during acute SIV infection leads to significant increases in viral load during chronic infection. *PLoS Pathog* 2014; 10: e1003929. [PubMed: 24603870]
161. Valenzuela F, Papp KA, Pariser D, et al. Effects of tofacitinib on lymphocyte sub-populations, CMV and EBV viral load in patients with plaque psoriasis. *BMC Dermatol* 2015; 15: 8. [PubMed: 25951857]
162. Schönberg K, Rudolph J, Vonnahme M, et al. JAK Inhibition Impairs NK Cell Function in Myeloproliferative Neoplasms. *Cancer Res* 2015; 75: 2187–99. [PubMed: 25832652]
163. Voss M, Bryceson YT. Natural killer cell biology illuminated by primary immunodeficiency syndromes in humans. *Clin. Immunol* 2017; 177: 29–42. [PubMed: 26592356]
164. Orange JS. Natural killer cell deficiency. *J. Allergy Clin. Immunol* 2013; 132: 515–25; quiz 526. [PubMed: 23993353]
165. Molgora M, Bonavita E, Ponzetta A, et al. IL-1R8 is a checkpoint in NK cells regulating anti-tumour and anti-viral activity. *Nature* 2017; 551: 110–114. [PubMed: 29072292]
166. Gao Y, Souza-Fonseca-Guimaraes F, Bald T, et al. Tumor immunoevasion by the conversion of effector NK cells into type 1 innate lymphoid cells. *Nat. Immunol* 2017; 18: 1004–1015. [PubMed: 28759001]
167. Delconte RB, Kolesnik TB, Dagley LF, et al. CIS is a potent checkpoint in NK cell-mediated tumor immunity. *Nat. Immunol* 2016; 17: 816–24. [PubMed: 27213690]
168. Palmer DC, Restifo NP. Suppressors of cytokine signaling (SOCS) in T cell differentiation, maturation, and function. *Trends Immunol* 2009; 30: 592–602. [PubMed: 19879803]
169. Sciumè G, Le MT, Gadina M HiJAKing Innate Lymphoid Cells? *Front. Immunol* 2017; 8: 438. [PubMed: 28450869]

Figure 1. JAK-STAT signals in ILC development and effector functions.

ILC ontogeny involves generation of an array of multipotent ILC precursors. Starting from common lymphoid progenitor (CLP), development proceeds from multi-potent ILC progenitors (including α-lymphoid progenitor (α-LP), early innate lymphoid progenitor (EILP), common helper lymphoid progenitor (CHILP), innate lymphoid precursor (ILCP)), to unipotent ILC precursors (upper part). The cartoon depicts the relative contribution of IL-15 and IL-7, and downstream players, in this process. Intraepithelial ILC1 (iILC1) present in the intestine are not dependent on IL-15Rα for development/homeostasis. In the lower part, the contribution of STAT1, STAT3, STAT4, STAT5 and STAT6 in regulation of ILC functions is shown.

Figure 2. Molecular features of the JAK-STAT signaling pathway.

Schematic representation of the molecular events downstream cytokines activating the JAK/STAT pathway, involved in ILC development and functions. Specific molecular mechanisms of gene regulation and JAK inhibitors are also illustrated. Briefly, different cytokines bind specific combinations of receptors. Once engaged, cytokine receptors undergo oligomerization, initiating the signal transduction. JAKs bind the intra-cytoplasmic tail of the receptor, dimerize, and transactivate each other. STATs are recruited and associate to JAKs resulting in STAT phosphorylation and activation. Phosphorylated STATs migrate to the nucleus, modulating gene expression through several mechanisms. As illustrated, a STAT dimer can bind DNA without any modulation of gene expression (neutral or opportunistic binding). Transcription is regulated instead through binding of different DREs, including, promoters and enhancers. STAT5 can also act as a tetramer, regulating expression of specific genes. Moreover, STATs can regulate gene expression by recruiting chromatin modifiers and/or inducing tri-dimensional reorganization of the DNA, promoting the juxtaposition of the proper elements necessary for the downstream gene transcription. Finally, JAK inhibitors and their specificity for JAKs are shown.