Molecular Immunology 110 (2019) 40–47

Contents lists available at ScienceDirect

Molecular Immunology

journal homepage: www.elsevier.com/locate/molimm

Natural killer cells and anti-tumor immunity

Sandra E. Nicholson*, Narelle Keating, Gabrielle T. Belz

Walter and Eliza Hall Institute of Medical Research, Melbourne, 3052, Australia; and Department of Medical Biology, University of Melbourne, Melbourne, 3010, Australia

ARTICLE INFO

Keywords:
Cancer
ILCs
NK cells
IL-15
SOCS proteins
CIS
SOCS2

ABSTRACT

Immune checkpoint inhibitors harness the power of the immune system to fight cancer. The clinical success achieved with antibodies against the inhibitory T cell receptors PD-1 and CTLA4 has focused attention on the possibility of manipulating other immune cells, in particular those involved in innate immunity. Here we review the role of innate lymphoid cells (ILCs) and their contribution to tumor immunity. As the prototypical ILC, the natural killer (NK) cell has an intrinsic ability to detect and kill cancer cells. NK cells are dependent on the cytokine interleukin (IL)-15 for their development and effector activity. We discuss the role of the Suppressor of cytokine (SOCS) proteins in negatively regulating IL-15 and NK cell responses and the potential for targeting these small intracellular regulators as new immune checkpoints.

1. Introduction

Immunotherapies that target inhibitory receptors on immune cells, such as programmed cell-death protein 1 (PD1) and cytotoxic T-lymphocyte protein 4 (CTLA-4), have dramatically impacted patient outcomes and the lives of many patients with otherwise untreatable disease have been prolonged. However, as this new and exciting frontier advances it is apparent that some patients are refractory to treatment and that not all cancers can be treated with the current immunotherapies (reviewed in (Emens et al., 2017)). Further, after an initial response to anti-PD1 therapy, tumors can acquire resistance associated with loss of major histocompatibility complex (MHC)I expression, and inactivation of antigen presentation or interferon (IFN) signaling (JAK1 and JAK2 inactivating mutations) (Zaretsky et al., 2016). The success and limitations of the current immunotherapies have spurred the search for alternative approaches and in particular, attention has turned to innate lymphoid cells such as the natural killer (NK) cells, which as their name suggests, possess an intrinsic ability to detect and kill transformed cells.

2. Innate lymphoid cell subsets

Innate lymphoid cells (ILCs) are a group of immune cells derived from a common lymphoid progenitor (CLP) and are distinguished from adaptive immune cells by the lack of classical features including various lineage markers and recombined antigen-specific receptors.

Although the first members of the ILC family, the lymphoid tissue-inducer (LTi) cells and NK cells were discovered more than 22 years ago (Kiessling et al., 1975a; Herberman et al., 1975; Kiessling et al., 1975b; Mebius et al., 1997), ILCs have only recently been recognized as a highly diverse group of cells, essentially akin to a new population of effector lymphocytes. They include NK cells and at least three different ILC subsets (ILC1, ILC2, ILC3) that are distinguished by lineage-defining transcription factors, their capacity to rapidly produce 'signature' cytokines, along with other functional attributes (Fig. 1**A**). Many of these subsets are highly enriched at mucosal sites (e.g. ILC3 and ILC2), whilst other subsets are found predominantly in the blood or largely confined to tissues (ILC1s and some NK cells). Similar to adaptive immune cells, ILCs are involved in host resistance to pathogens and tumor 'immunosurveillance', but perhaps one of the most important and unexpected roles of ILCs is in the maintenance of mucosal and tissue homeostasis and in tissue repair (Monticelli et al., 2011).

Group 1 ILCs are generally characterized by their ability to secrete IFN-γ and reliance on the transcription factor T-bet (encoded by Tbx21). This group includes both NK cells ($CD49b^+CD49a^-$) that express eomesodermin (Eomes) and tissue-resident ILC1s ($CD49b^-CD49a^+$) that generally lack Eomes expression. This distinction however, is not as clearly demarcated as initially thought. Group 2 ILCs depend on the transcription factors GATA-binding protein 3 (GATA3) for their development and maintenance and Rorα during activation and expansion (Hoyler et al., 2013; Hoyler et al., 2012;

Abbreviations: CAR, chimeric antigen receptor; CLP, Common lymphoid progenitor; IL, interleukin; ILCs, Innate lymphoid cells; JAK, Janus Kinase; KIR, kinase inhibitory region; LTi, lymphoid tissue-inducer; MHC, major histocompatibility complex; NCR (NKp46), Natural Cytotoxicity triggering Receptor 1; NK, natural killer; PEST, sequence rich in proline (P) glutamic acid (G) serine (S) and threonine (T); Rbx2, RING (Really Interesting New Gene) box protein 2; SOCS, Suppressor of Cytokine Signaling; STAT, Signal Transducer and Activator of Transcription

* Corresponding author.
E-mail addresses: snicholson@wehi.edu.au (S.E. Nicholson), belz@wehi.edu.au (G.T. Belz).

https://doi.org/10.1016/j.molimm.2017.12.002
Received 29 July 2017; Received in revised form 20 November 2017; Accepted 1 December 2017
Available online 09 December 2017
0161-5890/

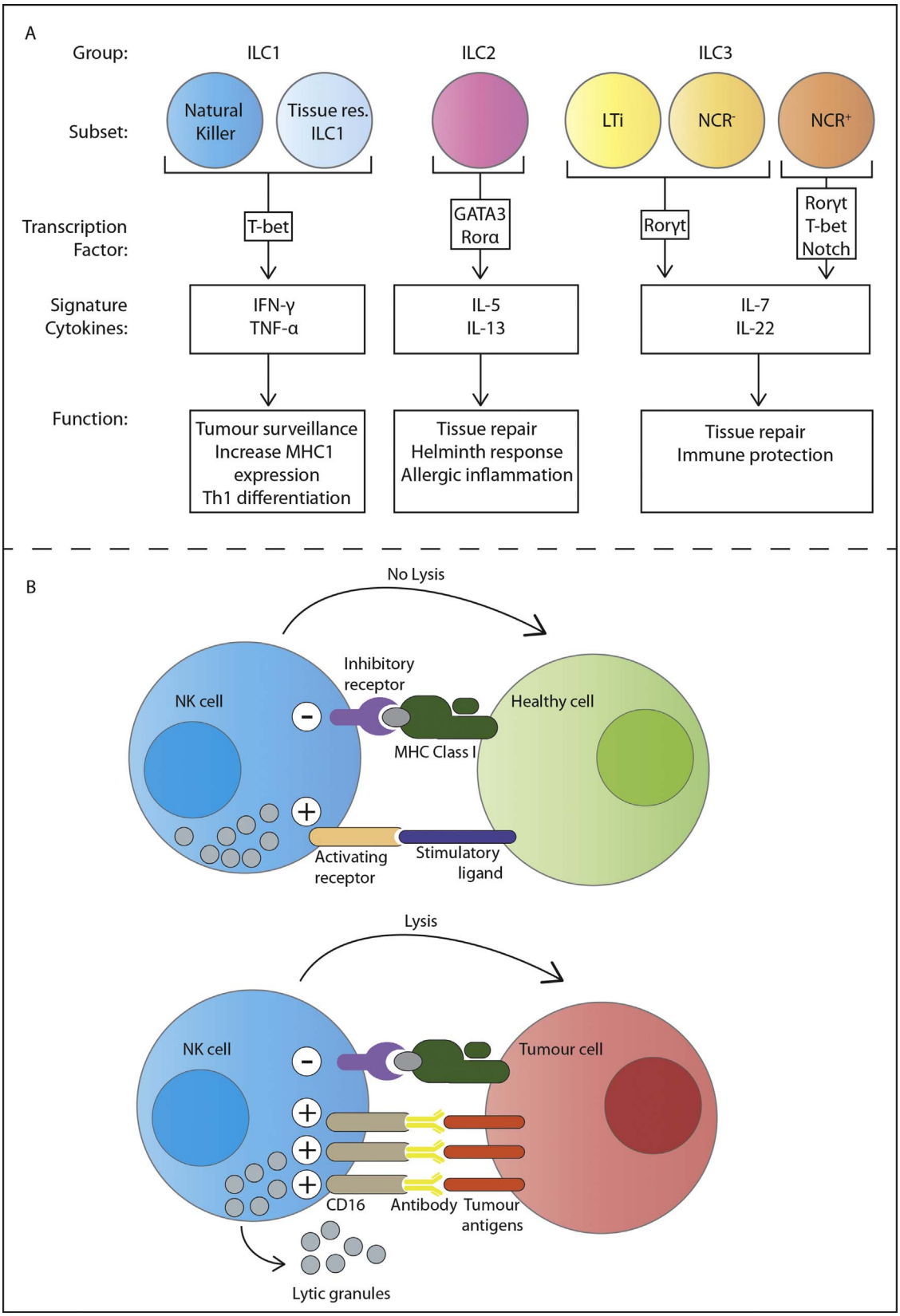

Fig. 1. Potential pro- and anti-tumorigenic functions of ILC subsets in cancer.
(**A**) Schematic showing the different innate lymphoid subsets and their functional roles. (**B**) NK cells effect tumor removal through a number of mechanisms including immunosurveillance *via* registration of altered MHC class I ligand expression and lytic mechanisms to kill transformed cells. Simultaneously, cellular stress results in the upregulation of ligands for activating NK cell receptors on the tumor cell. Both these events contribute to NK cell activation and the cytolytic effector functions that kill the target tumor cell. However, as the tumor progresses, mutations may arise which upregulate ligands for inhibitory receptors and/or result in loss of ligands for activating receptors. In this way, the tumor evades NK cell immunosurveillance. A major challenge is to identify the means to 'activate' NK cells. The role and impact of other ILC subsets is far less clear, with each subset potentially able to both promote the development or escape of tumor cells and restrain tumor growth. Due to the continually changing nature of the tumor microenvironment, these roles are likely to evolve over time.

Mjosberg et al., 2012; Wong et al., 2012). They mainly secrete the cytokines IL-5 and IL-13, but also produce IL-4, IL-9 and amphiregulin and have been implicated in tissue repair, helminth responses and allergic inflammation (Fallon et al., 2006; Moro et al., 2010; Wilhelm et al., 2011).

Group 3 ILCs are made up of multiple subsets, all of which depend on the transcription factor Rorγt, even at steady-state. These include LTi cells, and two populations of ILC3s which differ in their expression of the natural cytotoxicity triggering receptor 1, NCR (or NKp46, encoded by *Ncf1*). NCR^+ ILC3s depend on T-bet and Notch signaling for their development, whilst NCR^- ILC3s lack this requirement (Rankin et al., 2013; Klose et al., 2013). At steady-state in man, NCR^+ cells comprise ~5% of lymphocytes but are fewer in mice. Nevertheless, NCR^+ ILC3s found in mucosal tissues significantly outnumber classical NK cells (Klose et al., 2014; Satoh-Takayama et al., 2008; Vonarbourg et al., 2010; Sanos et al., 2009). ILC3s lack cytotoxic machinery such as perforin, granzyme B and molecules involved in triggering target killing and instead secrete IL-17 and IL-22 to initiate pathways of immune protection and tissue repair.

2.1. Involvement of ILCs in tumor defense

Immunosurveillance has long been thought to be the fundamental role of NK cells, which can spontaneously kill cells that pose a danger to the host (Guillerey et al., 2016; Morvan and Lanier, 2016). The development of a malignancy, however, is associated with a diverse and continuously changing spectrum of infiltrating cells, including different types of ILCs which may play an ambiguous role in tissue repair *vs.* driving tumor development. Furthermore, our interpretation of studies establishing the dominant role of NK cells may be confounded by the failure to recognize that early analyses didn't account for NK cell diversity, nor did they distinguish other ILC1 subsets; necessitating a renewed investigation of the ILC contributions to tumor immunity.

2.2. Potential tumorigenic and protective roles of other ILC subsets

Our current understanding of the contributions of non-NK cell ILCs to tumor development is quite limited, particularly as we don't fully understand the temporal changes in the ILC types and phenotypes within the tumor microenvironment that might be conducive to tumor eradication. The cytokine profiles expressed by the different ILCs, however, suggest that they may well drive particular stages of tumor development, maintenance or elimination.

ILC1s, like NK cells, produce both IFN-γ and TNF-α (Bernink et al., 2013; Fuchs et al., 2013). IFN-γ can significantly improve the anti-tumor responses of macrophages, NK cells and T cells by augmenting the expression of MHCI, driving the differentiation of Th1 cells and impairing tumor cell proliferation and angiogenesis. For example, it was recently shown that $NK1.1^+CD49a^+CD103^+$ ILC1-like cells expanded in response to cellular transformation and could lyse tumor cells in a granzyme B/TRAIL-dependent manner (Dadi et al., 2016). This study, however, didn't distinguish between the actions of ILC1s and cytotoxic T cells, thus the main effector population remains unclear. In a separate study, a similar ILC1 population which was regulated independently from NK cells was implicated in tumor immunosurveillance (Dadi et al., 2016). T cells responding in this same microenvironment acquired many similar features to ILC1 cells, including the expression of NK1.1, CD49a and CD103. The two populations were thus designated as 'unconventional type-1-like innate lymphoid cells' and 'type 1 innate-like T cells'. Both populations were dependent on IL-15 and exhibited cytotoxicity *in vitro* and although ILC1s accumulated during tumor formation, the contribution of each cell type *in vivo* remains uncertain (Dadi et al., 2016).

The apparent plasticity between ILC1 and ILC3 phenotypes adds an unanticipated complication to unravelling the role of different ILCs in tumorigenesis. IFNγ-producing ILC1-like cells that have previously exhibited an ILC3 signature may fulfil a cytotoxic role in response to IL-12 and thus form a tumor-repressive subset (Eisenring et al., 2010). $NKp46^+$ $CD56^+CD3^-$ ILCs have been described in man that exhibit low cytotoxicity but produce IL-22 and show an expression profile that overlaps with NK cells (Crome et al., 2017). These ILCs are purported to suppress T cell expansion as the addition of activating NKp46 antibodies to *in vitro* cultured tumor infiltrating lymphocytes abrogated the ILC suppression. Whether this reveals the essential function of the regulatory ILCs or addresses the role of NKp46 in cell-cell interactions in these cultures is not clear. Indeed, the function of these ILCs will need to be further examined *in vivo* as current assertions are largely extrapolated from *in vitro* analyses.

The inherent plasticity of ILC populations is further illustrated by a recent study showing NK cells can be converted into $CD49a^+$ $CD49b^+$ ILC1 intermediate and $CD49a^+$ $CD49b^-$ ILC1 populations, in response to TGFβ in the tumor microenvironment (Gao et al., 2017). The inability of these ILC1 populations to control tumor cell growth highlights the evolution of tumors to evade the immune response (Gao et al., 2017).

3. Natural killer cells

NK cells can spontaneously kill cells that are infected with a pathogen or exhibit markers of 'foreignness' such as tumor cells. Indeed, the lytic functions of NK cells mediated *via* granzyme B and perforin have dominated investigations of their role in anti-cancer defense (Voskoboinik et al., 2006). However, multiple other mechanisms that induce target cell apoptosis exist, including Fas/FasL and TRAIL/TRAIL receptor triggering. In addition, NK cells can regulate multiple cell types, including dendritic cells, through the secretion of cytokines (e.g. IFN-γ and TNF-α) and other growth factors. Although NK cells are armed to rapidly kill cells without the requirement for prior sensitization or antigen, regulation of this capacity is effected by the activatory (e.g. NKG2D, NKp30, NKp44, 2B4, DNAM-1 and CD16) (Vivier et al., 2008; Martinet et al., 2015; Martinet and Smyth, 2015; Yokoyama and Plougastel, 2003; Huntington et al., 2013) and MHC class I-mediated inhibitory receptors (e.g. various Ly49 receptors, leukocyte immunoglobulin-like receptors, KLRG1) (Robbins et al., 2002; Colonna et al., 1997; Cosman et al., 1997) (Fig. 1**B**).

As an innate immune cell armed with intrinsic cell killing ability, NK cells have been the subject of intense interest as an immunotherapeutic target. Tumor infiltrating NK cells are associated with improved patient prognosis and survival for a variety of different cancers (Coca et al., 1997; Ishigami et al., 2000; Takanami et al., 2001; Villegas et al., 2002). These data are supported by a seminal 11-year prospective cohort study conducted in Japan which found an inverse correlation between increased cancer incidence and existing NK cell cytotoxicity (Imai et al., 2000).

NK cells possess a number of characteristics that make them particularly attractive. Unlike T cells, NK cells don't require an adaptive immune response to kill and the absence of the TCR means there is no associated risk of graft-versus-host disease (GVHD). Their lack of clonal expansion may also limit toxicity due to excessive cytokine production. However, NK cells within the tumor microenvironment are subject to the same suppressive factors that dampen down receptors on T cells, often resulting in a quenching of their capacity to eliminate tumor cells. Approaches that augment the anti-cancer effector properties by bypassing receptor downregulation are a major focus for potential immunotherapies.

The adoptive transfer of both autologous and allogeneic NK cells has been tested in clinical trials and shown to be safe (Locatelli et al., 2014; Rezvani and Rouce, 2015). However, efficacy in this setting appears to be limited, possibly due to the short lifespan of NK cells *in vivo* and because they are still subject to inhibition by suppressive factors in the tumor environment. Leukemic cell lines such as the NK92 line which was derived from a malignant non-Hodgkin's lymphoma, have been

widely used as an allogeneic therapy. Use of these transformed cell lines necessitates irradiation of cells prior to use, which in turn, contributes to the limited persistence *in vivo*. Efforts to counteract this and boost the activity of NK cells include engineering them to express activating receptors and chimeric antigen receptors (CARs) which either express tumor antigens or activating ligands (reviewed by Rezvani and colleagues (Rezvani et al., 2017)). Recent strategies include the generation of cord blood-derived NK cells which express CAR-CD19 (for B cell lymphoma), IL-15, CD16 and an inducible caspase 9; the latter so that cells can be pharmacologically killed post-transfusion (Liu et al., 2017). The co-administration of NK enhancing cytokines such as IL-15 has also been trialed with mixed results and this approach is discussed in more detail below.

3.1. IL-15 regulation of NK cell development and activity

NK cell homeostasis and function requires exposure to cytokines such as IL-2, IL-12 and IL-15. IL-15 is critical not only for NK cell development, but also for NK cell proliferation, survival and effector function. It is primarily produced by dendritic cells and monocytes in response to IFN in the tumor microenvironment and high intratumoral IL-15 levels are associated with increased disease-free survival in colorectal cancer (Mlecnik et al., 2014).

IL-15 is a four-α-helical bundle cytokine belonging to the IL-2 family (IL-2, IL-4, IL-7, IL-9, IL-15, IL-21). It is uniquely pre-assembled together with its cognate receptor (IL-15Rα) in the golgi and expressed on the surface of cells such as monocytes and dendritic cells for *trans*-presentation to adjacent NK cells and CD8 memory T cells; signaling is initiated on IL-15 binding to the IL-2 receptor complex, which is comprised of β and γ subunits (Mortier et al., 2008). IL-2 also signals through this complex, however it binds first to a specific IL-2R subunit (IL-2Rα) on the surface of the target cell (Fig. 2). IL-2 and IL-15 have both overlapping and unique activities; for instance, IL-2 is required for maintaining Tregs in the periphery and IL-15 contributes to the maintenance of memory T cells. These cytokine-specific actions can partly be explained by differential expression of the respective α-chains, whilst the relatively high affinity of IL-15 for IL-15Rα and its persistence in the local cellular environment may alter the kinetics or strength of signal generated (Waldmann, 2015). All other family members signal through a heterodimeric complex consisting of a ligand-specific alpha-chain and the common gamma chain (γ_c) (Fig. 2**)** (Liao et al., 2013).

The importance of IL-15 for NK cell development and function is underscored by the severely reduced NK cell numbers in *Il-2rβ*$^{-/-}$ mice (Suzuki et al., 1997) and the absence of NK cells in *Il-2rγ*$^{-/-}$ mice (DiSanto et al., 1995). The severity of these phenotypes is greater than the reduced NK cell function found in *Il-2*$^{-/-}$ mice (Kundig et al., 1993), and no doubt stems from effective loss of both IL-2 and IL-15 signaling. Administration of neutralizing IL-15 antibodies to rhesus macaques resulted in loss of peripheral NK cells, evidence that the role of IL-15 in maintaining NK cell population is conserved in primates (DeGottardi et al., 2016).

IL-15 signaling is initiated by activation of the JAK1 and JAK3 tyrosine kinases that are constitutively associated with the IL-2Rβ and IL-2Rγ subunits, respectively. The activated JAKs phosphorylate tyrosine residues within the receptor subunits which then act as docking sites for secondary signaling molecules such as STAT3 and STAT5 and other SH2-containing proteins such as GRB2 and Shc. STAT5 is the predominant STAT activated by IL-15 and is critical for NK cell development and survival; NK cell-specific deletion of the *Stat5* gene in mice results in an almost complete loss of NK cells (Eckelhart et al., 2011). The STAT5 transcriptional program induces expression of the SOCS family proteins, the critical negative regulators of JAK-STAT signaling.

Patients with mutations in *Il-2rγ* and *Jak3* suffer from severe combined immune deficiencies (SCID) and lack T lymphocytes and NK cells as a result of loss of signaling by the IL-2 family cytokines (Macchi et al., 1995; Noguchi et al., 1993; Russell et al., 1995). The lack of circulating NK cells in these patients can be primarily attributed to loss of IL-15 signaling, further evidence that the importance of IL-15 for NK cell development translates to humans (Jouanguy et al., 2013). Similarly, there are rare reports of patients with *Stat5B* mutations who, in addition to growth hormone-related defects, have immunodeficiencies associated with reduced NK cell numbers (Bernasconi et al., 2006; Pugliese-Pires et al., 2010).

4. The suppressor of cytokine signaling (SOCS) proteins

There are eight canonical SOCS proteins characterized by a central SH2 domain and a C-terminal SOCS box motif (Hilton et al., 1998). They can be differentiated by the presence of a short (CIS, SOCS1, 2, 3) or long, N-terminal region (SOCS4, 5, 6, 7; 270–385 residues). The SOCS box motif recruits an E3 ubiquitin ligase complex consisting of Elongins B and C, RING box 2 (Rbx2) and the scaffold Cullin-5 (Zhang et al., 1999). Broadly speaking the SOCS proteins can be thought of as adaptors that target an SH2-bound protein for ubiquitination and consequently, proteasomal degradation (Linossi and Nicholson, 2012).

SOCS1 and SOCS3 contain a unique kinase-inhibitory region (KIR)

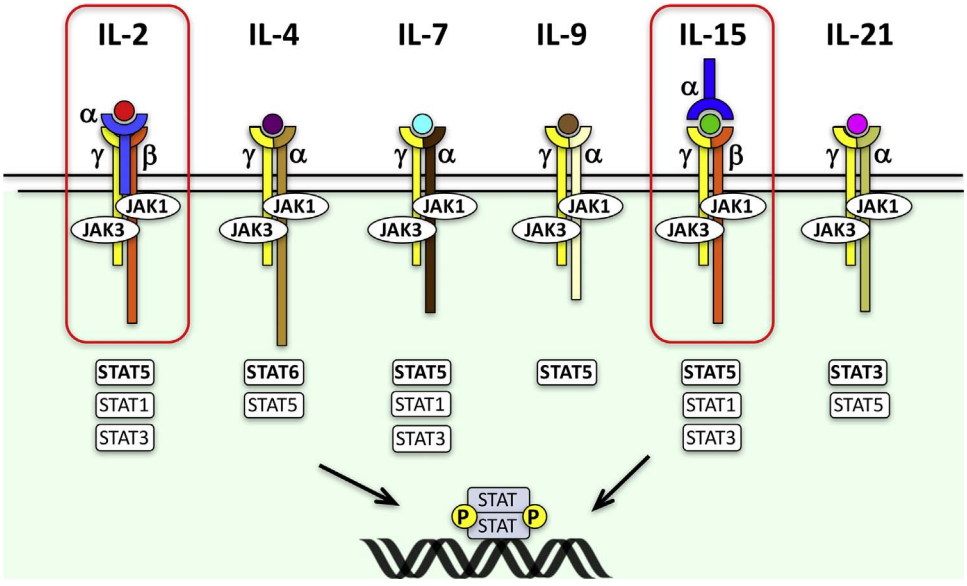

Fig. 2. Schematic showing receptor complexes activated by IL-2 family cytokines.
All IL-2 family cytokines bind to a specific alpha chain (α) and then signal through interaction with the common gamma (γ) chain. In addition, IL-2 and IL-15 share the IL-2Rβ subunit, with IL-15 uniquely *trans*-presented together with its alpha subunit on the surface of adjacent cells. The associated JAK1 and JAK3 protein kinases are activated and phosphorylate the receptor cytoplasmic domains, which recruits the STAT transcriptional activators. The predominant STAT protein activated by each cytokine is highlighted in bold, but this will vary from cell type to cell type.

which interacts with a GQM amino acid motif on JAK1, JAK2 and JAK3, partially blocking the substrate binding site, resulting in the inhibition of JAK catalytic activity (Babon et al., 2012; Sasaki et al., 1999). SOCS3 and CIS also contain a unique unstructured insertion within the SH2 domain, which does not disrupt the SH2 fold. In SOCS3 this has been shown to be a true PEST motif (rich in proline, glutamic acid, serine and threonine) and regulates SOCS3 stability (Babon et al., 2006). The function of the insertion in CIS is unknown.

4.1. SOCS proteins act as immune checkpoints in tumor immunity

CIS was first discovered by Yoshimura and colleagues as a cytokine-inducible SH2-containing protein (Yoshimura et al., 1995). While CIS was induced by a number of cytokines and was able to inhibit signaling from the same cytokines when overexpressed (Yoshimura et al., 1995; Aman et al., 1999; Matsumoto et al., 1997; Dif et al., 2001), deletion of the gene encoding CIS (*Cish*) appeared to have very little impact *in vivo* (Marine et al., 1999). A second *Cish*-deficient mouse strain was predisposed to pulmonary inflammation and showed increased susceptibility to experimental allergic asthma, as a consequence of enhanced IL-2/STAT5 and IL-4/STAT6 signaling (Yang et al., 2013). Although regulation of the IL-2Rβ/γ_c complex was implied, the protein/s targeted by CIS were not identified in this study. Recently, several laboratories (including ours) have since highlighted a role for CIS as an intracellular regulator in CD8 T cells and NK cells which restrains anti-tumor immunity.

Cish is a STAT5 response gene and is transcriptionally upregulated by IL-2 and IL-15 in both T cells and NK cells (Delconte et al., 2016). Expression is also rapidly induced in response to TCR signaling (Li et al., 2000; Palmer et al., 2015). *Cish*-deficient CD8 T cells show enhanced expansion on TCR engagement; this IL-2-independent response was suggested to occur via CIS targeting PLCγ-1 for proteasomal degradation. Further, adoptive transfer of *Cish*-deficient CD8 cells was protective in a subcutaneous B16 melanoma model (Palmer et al., 2015). Paradoxically, this is in stark contrast to the enhanced TCR signaling and responses observed in *Cish*-transgenic CD4 cells (Li et al., 2000). The discrepancy between these studies remains unresolved, but might indicate differential expression of CIS target proteins. Alternatively, high CIS levels might “mop up” the E3 components, limiting the activity of other SOCS box proteins.

Genetic deletion of *Cish* didn’t impact on NK cell homeostasis however, it did enhance IL-15 signaling responses in cultured NK cells, leading to increased proliferation, production of IFNγ and effector proteins and killing of target cells (Delconte et al., 2016). This correlated with increases in IL-2Rβ levels, phosphorylated and total JAK1 and downstream signaling. The CIS-SH2 domain bound with high affinity to a phosphopeptide corresponding to the JAK activation loop and *in vitro,* inhibited JAK1 kinase activity. Consistent with previous studies, CIS also interacted with peptides derived from the IL-2Rβ, supporting the hypothesis that CIS interacts at multiple points within the IL-15 receptor complex, efficiently blocking further kinase activity and targeting the receptor complex, including the associated JAK kinases, for proteasomal degradation (Delconte et al., 2016) (Fig. 3). Importantly, *Cish*$^{-/-}$ mice were dramatically protected in various experimental and spontaneous metastasis models and the adoptive transfer of *Cish*$^{-/-}$ NK cells was sufficient to confer protection (Delconte et al., 2016).

A number of questions remain unresolved. For instance, why would CIS target different proteins in NK and CD8 T cells? And why would CIS, rather than SOCS1 (which is also induced by IL-15 and interacts with JAK1 and the IL-2Rβ (Delconte et al., 2016; Sporri et al., 2001; Cornish et al., 2003)) be the critical regulator of IL-15 signaling in NK cells, particularly when SOCS1 regulates IL-15 in CD8 T cells (Ilangumaran et al., 2003)? The converse also holds true − why is it that SOCS1 (Cornish et al., 2003), but not CIS, regulates IL-2 signaling in CD8 T cells? Much of this could be explained by differential expression of the two proteins; currently we are hampered by the lack of tools to quantitatively compare absolute levels. Alternatively, there may be additional activation steps, such as post-translational modification, that are required to fully active CIS and/or SOCS1 or which regulate their turnover. Finally, given that CIS lacks the KIR region found in SOCS1 and SOCS3, exactly how CIS inhibits JAK1 activity is also unclear. This will no doubt be better understood as we gain a structural understanding of the binding interfaces between SOCS and their target proteins.

These studies reveal an unequivocal role for CIS as an immune checkpoint in mice. Critically, we now need to confirm that the role of CIS is functionally conserved in human immune cells. If it is, CIS inhibitors could be developed as a novel immunotherapy with the potential to synergistically enhance the effector activity of both CD8 T cells and NK cells.

SOCS2 also appears to have a role in various cancers (Letellier and Haan, 2016). Although it was initially discovered as a key regulator of growth hormone, prolactin and IGF signaling (Metcalf et al., 2000), further investigation suggests that its targets and functions are more diverse, with roles in neurodevelopment, Th1/Th2 immunity, sepsis and diabetes. Although reports are often conflicting, SOCS2 may limit tumor growth, with low *Socs2* expression correlating with advanced disease in a number of cancers (Letellier and Haan, 2016).

SOCS2 has also been implicated in regulating NK cell-mediated tumor immunity and appears to limit NK cell differentiation independently of effector function, with *Socs2*-deficient (*Socs2*$^{-/-}$) mice exhibiting increased numbers of NK cells in the bone marrow and spleen. Consistent with this, *Socs2*$^{-/-}$ mice were protected in the experimental B16 melanoma lung metastasis model (Kim et al., 2017). Although the authors demonstrated an increased NK cell presence in *Socs2*$^{-/-}$ lungs, they did not definitively demonstrate that enhanced *in vivo* anti-tumor immunity was due to the increased NK cell number (Kim et al., 2017). Indeed, a recent study by Nirschl et al. (Nirschl et al., 2017), suggests that IFNγ-induction of SOCS2 in migratory dendritic cell (DC)s dampens the adaptive immune response, limiting T cell immunity and raising the possibility that enhanced antigen presentation by DCs could contribute to the *in vivo* response attributed to *Socs2*$^{-/-}$ NK cells (Kim et al., 2017). The relative contribution of these findings to various tumor models needs to be clarified and further work is required to understand the proteins targeted by SOCS2. The protein target/s in DCs were not identified, whilst Kim et al., suggested that SOCS2 could directly regulate JAK2 (Kim et al., 2017). Interestingly, within the canonical SOCS family, CIS and SOCS2 share the highest amino acid homology across their SH2 domains, yet appear to exert quite specific cellular effects, despite being induced by an overlapping set of cytokines. This points to an underlying level of specificity which as yet, we don’t understand.

We again need to consider the relevance of these findings to human tumor immunity. The study by Nirschl et al. (Nirschl et al., 2017), elegantly demonstrated conservation of the *Socs2* signature in melanoma infiltrating human CD11c+ mononuclear phagocytes. However, the impact of deleting *Socs2* in human DCs still needs to be investigated. Other studies have suggested that loss of SOCS2 expression correlates with disease progression, although in many instances it may be a prognostic marker, rather than contributing to the etiology of disease and the specific levels of SOCS2 in tumor versus immune cells requires further investigation.

5. Concluding remarks

Historically, SOCS proteins have not been attractive therapeutic targets. SOCS1 and SOCS3 are important negative regulators of inflammatory signaling and to be beneficial, agents would need to enhance (rather than inhibit) SOCS activity – a hard task for an intracellular protein. In addition, the SH2 domains and SOCS box-associated E3 ligases are functionally conserved across the family. The

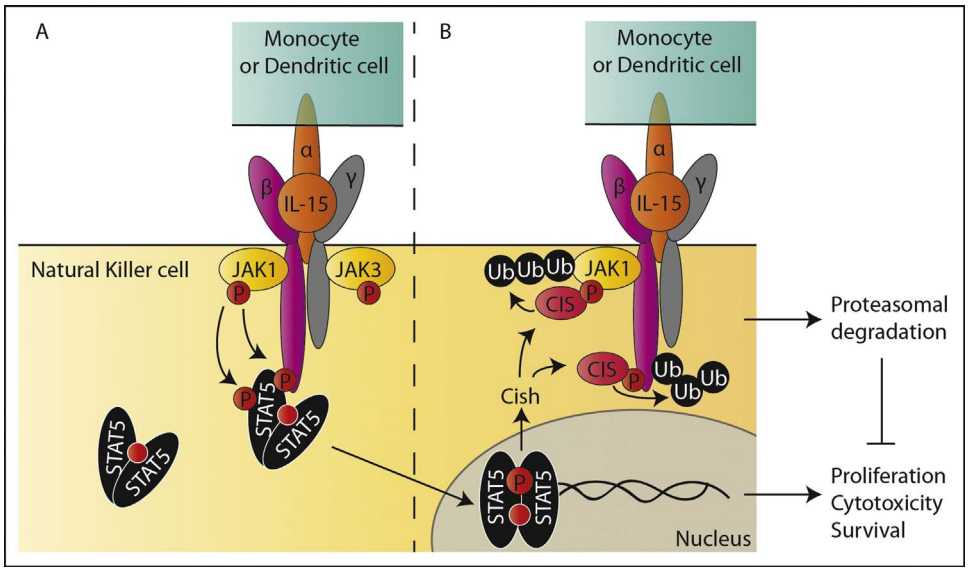

Fig. 3. CIS is an immune checkpoint that regulates IL-15 signaling.
A) In response to IL-15 stimulation, receptor-associated JAK 1 and JAK3 undergo auto- and/or *trans*-phosphorylation and phosphorylate tyrosine residues within the receptor subunits. These create docking sites for the STAT-SH2 domains. Tyrosine phosphorylation of STAT dimers enables their translocation to the nucleus, promoting transcription of target genes that drive NK cell survival, proliferation and cytotoxicity.
B) *Cish* is also a transcriptional target of STAT5. Induced expression of CIS inhibits JAK-STAT5 signaling by binding to the IL-2Rβ and JAKs, targeting them for proteasomal degradation in a classic negative feedback loop to limit NK cell proliferation, cytotoxicity and survival.

studies highlighted here suggest that inhibition of CIS and SOCS2 could be a useful treatment modality in cancer, either in hematological malignancies or in the context of enhancing immune surveillance post-surgery. Used in combination with current immunotherapies, SOCS inhibitors may help combat the development of resistance and tumor escape. Alternatively, SOCS inhibitors could be used to expand and activate allogeneic NK cells prior to adoptive transfer.

The real challenge lies with the inherent difficulty in targeting SH2 domains. SH2 domains not only share a common mode of binding to phosphotyrosine-containing sequences, which makes achieving specificity difficult, but this is compounded by the challenge of delivering compounds which mimic the negative charge equivalent to a phosphate, across the cell membrane. To date, no SH2 inhibitor has progressed to the clinic, however the development of specific inhibitors of the STAT3 and STAT5-SH2 domains which display *in vivo* activity (Cumaraswamy et al., 2014; Zhang et al., 2012; Auzenne et al., 2012), suggest that this is an achievable aim.

The context and specificity of JAK inhibitors also needs to be considered and their use approached with caution. In various breast cancer models, JAK1/2 inhibition was shown to have the dual effect of limiting STAT signaling in both the tumor cells and infiltrating immune cells, with the net result an increase in metastatic disease that correlated with reduced NK cell maturation and functionality (Bottos et al., 2016). Further, STAT inhibition may also impact on tumor vascularization, by relieving STAT repression of VEGFA production in NK cells (Gotthardt et al., 2016). These bystander effects on infiltrating immune cells could potentially be countered by administration of either IL-15 (Bottos et al., 2016) or agents (such as SOCS inhibitors) that enhance the response to IL-15.

Acknowledgments

GTB is supported by an Elizabeth Blackburn NHMRC Fellowship (#516711). The authors were supported in part by an NHMRC IRIISS grant 361646 and a Victorian State Government Operational Infrastructure Scheme grant.

References

Aman, M.J., Migone, T.S., Sasaki, A., Ascherman, D.P., Zhu, M., Soldaini, E., Imada, K., Miyajima, A., Yoshimura, A., Leonard, W.J., 1999. CIS associates with the interleukin-2 receptor beta chain and inhibits interleukin-2-dependent signaling. J. Biol. Chem. 274, 30266–30272.

Auzenne, E.J., Klostergaard, J., Mandal, P.K., Liao, W.S., Lu, Z., Gao, F., Bast Jr., R.C., Robertson, F.M., McMurray, J.S., 2012. A phosphopeptide mimetic prodrug targeting the SH2 domain of Stat3 inhibits tumor growth and angiogenesis. J. Exp. Ther. Oncol. 10, 155–162.

Babon, J.J., McManus, E.J., Yao, S., DeSouza, D.P., Mielke, L.A., Sprigg, N.S., Willson, T.A., Hilton, D.J., Nicola, N.A., Baca, M., Nicholson, S.E., Norton, R.S., 2006. The structure of SOCS3 reveals the basis of the extended SH2 domain function and identifies an unstructured insertion that regulates stability. Mol. Cell 22, 205–216.

Babon, J.J., Kershaw, N.J., Murphy, J.M., Varghese, L.N., Laktyushin, A., Young, S.N., Lucet, I.S., Norton, R.S., Nicola, N.A., 2012. Suppression of cytokine signaling by SOCS3: characterization of the mode of inhibition and the basis of its specificity. Immunity 36, 239–250.

Bernasconi, A., Marino, R., Ribas, A., Rossi, J., Ciaccio, M., Oleastro, M., Ornani, A., Paz, R., Rivarola, M.A., Zelazko, M., Belgorosky, A., 2006. Characterization of immunodeficiency in a patient with growth hormone insensitivity secondary to a novel STAT5b gene mutation. Pediatrics 118, e1584–e1592.

Bernink, J.H., Peters, C.P., Munneke, M., te Velde, A.A., Meijer, S.L., Weijer, K., Hreggvidsdottir, H.S., Heinsbroek, S.E., Legrand, N., Buskens, C.J., Bemelman, W.A., Mjosberg, J.M., Spits, H., 2013. Human type 1 innate lymphoid cells accumulate in inflamed mucosal tissues. Nat. Immunol. 14, 221–229.

Bottos, A., Gotthardt, D., Gill, J.W., Gattelli, A., Frei, A., Tzankov, A., Sexl, V., Wodnar-Filipowicz, A., Hynes, N.E., 2016. Decreased NK-cell tumour immunosurveillance consequent to JAK inhibition enhances metastasis in breast cancer models. Nat. Commun. 7, 12258.

Coca, S., Perez-Piqueras, J., Martinez, D., Colmenarejo, A., Saez, M.A., Vallejo, C., Martos, J.A., Moreno, M., 1997. The prognostic significance of intratumoral natural killer cells in patients with colorectal carcinoma. Cancer 79, 2320–2328.

Colonna, M., Navarro, F., Bellon, T., Llano, M., Garcia, P., Samaridis, J., Angman, L., Cella, M., Lopez-Botet, M., 1997. A common inhibitory receptor for major histocompatibility complex class I molecules on human lymphoid and myelomonocytic cells. J. Exp. Med. 186, 1809–1818.

Cornish, A.L., Chong, M.M., Davey, G.M., Darwiche, R., Nicola, N.A., Hilton, D.J., Kay, T.W., Starr, R., Alexander, W.S., 2003. Suppressor of cytokine signaling-1 regulates signaling in response to interleukin-2 and other gamma c-dependent cytokines in peripheral T cells. J. Biol. Chem. 278, 22755–22761.

Cosman, D., Fanger, N., Borges, L., Kubin, M., Chin, W., Peterson, L., Hsu, M.L., 1997. A novel immunoglobulin superfamily receptor for cellular and viral MHC class I molecules. Immunity 7, 273–282.

Crome, S.Q., Nguyen, L.T., Lopez-Verges, S., Yang, S.Y., Martin, B., Yam, J.Y., Johnson, D.J., Nie, J., Pniak, M., Yen, P.H., Milea, A., Sowamber, R., Katz, S.R., Bernardini, M.Q., Clarke, B.A., Shaw, P.A., Lang, P.A., Berman, H.K., Pugh, T.J., Lanier, L.L., Ohashi, P.S., 2017. A distinct innate lymphoid cell population regulates tumor-associated T cells. Nat. Med. 23 (3), 368–375. http://dx.doi.org/10.1038/nm.4278.

Cumaraswamy, A.A., Lewis, A.M., Geletu, M., Todic, A., Diaz, D.B., Cheng, X.R., Brown, C.E., Laister, R.C., Muench, D., Kerman, K., Grimes, H.L., Minden, M.D., Gunning, P.T., 2014. Nanomolar-potency small molecule inhibitor of STAT5 protein. ACS Med. Chem. Lett. 5, 1202–1206.

Dadi, S., Chhangawala, S., Whitlock, B.M., Franklin, R.A., Luo, C.T., Oh, S.A., Toure, A., Pritykin, Y., Huse, M., Leslie, C.S., Li, M.O., 2016. Cancer immunosurveillance by tissue-resident innate lymphoid cells and innate-like t cells. Cell 164, 365–377.

DeGottardi, M.Q., Okoye, A.A., Vaidya, M., Talla, A., Konfe, A.L., Reyes, M.D., Clock, J.A., Duell, D.M., Legasse, A.W., Sabnis, A., Park, B.S., Axthelm, M.K., Estes, J.D., Reiman, K.A., Sekaly, R.P., Picker, L.J., 2016. Effect of anti-IL-15 administration on t cell and NK cell homeostasis in rhesus macaques. J. Immunol. 197, 1183–1198.

Delconte, R.B., Kolesnik, T.B., Dagley, L.F., Rautela, J., Shi, W., Putz, E.M., Stannard, K., Zhang, J.G., Teh, C., Firth, M., Ushiki, T., Andoniou, C.E., Degli-Esposti, M.A., Sharp, P.P., Sanvitale, C.E., Infusini, G., Liau, N.P., Linossi, E.M., Burns, C.J., Carotta, S., Gray, D.H., Seillet, C., Hutchinson, D.S., Belz, G.T., Webb, A.I., Alexander, W.S., Li, S.S., Bullock, A.N., Babon, J.J., Smyth, M.J., Nicholson, S.E., Huntington, N.D., 2016.

CIS is a potent checkpoint in NK cell-mediated tumor immunity. Nat. Immunol. 17, 816–824.
DiSanto, J.P., Muller, W., Guy-Grand, D., Fischer, A., Rajewsky, K., 1995. Lymphoid development in mice with a targeted deletion of the interleukin 2 receptor gamma chain. Proc. Natl. Acad. Sci. U. S. A. 92, 377–381.
Dif, F., Saunier, E., Demeneix, B., Kelly, P.A., Edery, M., 2001. Cytokine-inducible SH2-containing protein suppresses PRL signaling by binding the PRL receptor. Endocrinology 142, 5286–5293.
Eckelhart, E., Warsch, W., Zebedin, E., Simma, O., Stoiber, D., Kolbe, T., Rulicke, T., Mueller, M., Casanova, E., Sexl, V., 2011. A novel Ncr1-Cre mouse reveals the essential role of STAT5 for NK-cell survival and development. Blood 117, 1565–1573.
Eisenring, M., vom Berg, J., Kristiansen, G., Saller, E., Becher, B., 2010. IL-12 initiates tumor rejection via lymphoid tissue-inducer cells bearing the natural cytotoxicity receptor NKp46. Nat. Immunol. 11, 1030–1038.
Emens, L.A., Ascierto, P.A., Darcy, P.K., Demaria, S., Eggermont, A.M.M., Redmond, W.L., Seliger, B., Marincola, F.M., 2017. Cancer immunotherapy: opportunities and challenges in the rapidly evolving clinical landscape. Eur. J. Cancer 81, 116–129.
Fallon, P.G., Ballantyne, S.J., Mangan, N.E., Barlow, J.L., Dasvarma, A., Hewett, D.R., McIlgorm, A., Jolin, H.E., McKenzie, A.N., 2006. Identification of an interleukin (IL)-25-dependent cell population that provides IL-4, IL-5, and IL-13 at the onset of helminth expulsion. J. Exp. Med. 203, 1105–1116.
Fuchs, A., Vermi, W., Lee, J.S., Lonardi, S., Gilfillan, S., Newberry, R.D., Cella, M., Colonna, M., 2013. Intraepithelial type 1 innate lymphoid cells are a unique subset of IL-12- and IL-15-responsive IFN-gamma-producing cells. Immunity 38, 769–781.
Gao, Y., Souza-Fonseca-Guimaraes, F., Bald, T., Ng, S.S., Young, A., Ngiow, S.F., Rautela, J., Straube, J., Waddell, N., Blake, S.J., Yan, J., Bartholin, L., Lee, J.S., Vivier, E., Takeda, K., Messaoudene, M., Zitvogel, L., Teng, M.W.L., Belz, G.T., Engwerda, C.R., Huntington, N.D., Nakamura, K., Holzel, M., Smyth, M.J., 2017. Tumor immunoevasion by the conversion of effector NK cells into type 1 innate lymphoid cells. Nat. Immunol. 18, 1004–1015.
Gotthardt, D., Putz, E.M., Grundschober, E., Prchal-Murphy, M., Straka, E., Kudweis, P., Heller, G., Bago-Horvath, Z., Witalisz-Siepracka, A., Cumaraswamy, A.A., Gunning, P.T., Strobl, B., Muller, M., Moriggl, R., Stockmann, C., Sexl, V., 2016. STAT5 is a key regulator in NK cells and acts as a molecular switch from tumor surveillance to tumor promotion. Cancer Discov. 6, 414–429.
Guillerey, C., Huntington, N.D., Smyth, M.J., 2016. Targeting natural killer cells in cancer immunotherapy. Nat. Immunol. 17, 1025–1036.
Herberman, R.B., Nunn, M.E., Lavrin, D.H., 1975. Natural cytotoxic reactivity of mouse lymphoid cells against syngeneic acid allogeneic tumors. I. Distribution of reactivity and specificity. Int. J. Cancer 16, 216–229.
Hilton, D.J., Richardson, R.T., Alexander, W.S., Viney, E.M., Willson, T.A., Sprigg, N.S., Starr, R., Nicholson, S.E., Metcalf, D., Nicola, N.A., 1998. Twenty proteins containing a C-terminal SOCS box form five structural classes. Proc. Natl. Acad. Sci. U. S. A. 95, 114–119.
Hoyler, T., Klose, C.S., Souabni, A., Turqueti-Neves, A., Pfeifer, D., Rawlins, E.L., Voehringer, D., Busslinger, M., Diefenbach, A., 2012. The transcription factor GATA-3 controls cell fate and maintenance of type 2 innate lymphoid cells. Immunity 37, 634–648.
Hoyler, T., Connor, C.A., Kiss, E.A., Diefenbach, A., 2013. T-bet and Gata3 in controlling type 1 and type 2 immunity mediated by innate lymphoid cells. Curr. Opin. Immunol. 25, 139–147.
Huntington, N.D., Nutt, S.L., Carotta, S., 2013. Regulation of murine natural killer cell commitment. Front. Immunol. 4, 14.
Ilangumaran, S., Ramanathan, S., Ning, T., La Rose, J., Reinhart, B., Poussier, P., Rottapel, R., 2003. Suppressor of cytokine signaling 1 attenuates IL-15 receptor signaling in CD8+ thymocytes. Blood 102, 4115–4122.
Imai, K., Matsuyama, S., Miyake, S., Suga, K., Nakachi, K., 2000. Natural cytotoxic activity of peripheral-blood lymphocytes and cancer incidence: an 11-year follow-up study of a general population. Lancet 356, 1795–1799.
Ishigami, S., Natsugoe, S., Tokuda, K., Nakajo, A., Che, X., Iwashige, H., Aridome, K., Hokita, S., Aikou, T., 2000. Prognostic value of intratumoral natural killer cells in gastric carcinoma. Cancer 88, 577–583.
Jouanguy, E., Gineau, L., Cottineau, J., Beziat, V., Vivier, E., Casanova, J.L., 2013. Inborn errors of the development of human natural killer cells. Curr. Opin. Allergy Clin. Immunol. 13, 589–595.
Kiessling, R., Klein, E., Wigzell, H., 1975a. Natural killer cells in the mouse. I. Cytotoxic cells with specificity for mouse Moloney leukemia cells. Specificity and distribution according to genotype. Eur. J. Immunol. 5, 112–117.
Kiessling, R., Klein, E., Pross, H., Wigzell, H., 1975b. Natural killer cells in the mouse. II. Cytotoxic cells with specificity for mouse Moloney leukemia cells. Characteristics of the killer cell. Eur J Immunol 5, 117–121.
Kim, W.S., Kim, M.J., Kim, D.O., Byun, J.E., Huy, H., Song, H.Y., Park, Y.J., Kim, T.D., Yoon, S.R., Choi, E.J., Jung, H., Choi, I., 2017. Suppressor of cytokine signaling 2 negatively regulates NK cell differentiation by inhibiting JAK2 activity. Sci. Rep. 7, 46153.
Klose, C.S., Kiss, E.A., Schwierzeck, V., Ebert, K., Hoyler, T., d'Hargues, Y., Goppert, N., Croxford, A.L., Waisman, A., Tanriver, Y., Diefenbach, A., 2013. A T-bet gradient controls the fate and function of CCR6-RORgammat+ innate lymphoid cells. Nature 494, 261–265.
Klose, C.S., Flach, M., Mohle, L., Rogell, L., Hoyler, T., Ebert, K., Fabiunke, C., Pfeifer, D., Sexl, V., Fonseca-Pereira, D., Domingues, R.G., Veiga-Fernandes, H., Arnold, S.J., Busslinger, M., Dunay, I.R., Tanriver, Y., Diefenbach, A., 2014. Differentiation of type 1 ILCs from a common progenitor to all helper-like innate lymphoid cell lineages. Cell 157, 340–356.
Kundig, T.M., Schorle, H., Bachmann, M.F., Hengartner, H., Zinkernagel, R.M., Horak, I., 1993. Immune responses in interleukin-2-deficient mice. Science 262, 1059–1061.
Letellier, E., Haan, S., 2016. SOCS2: physiological and pathological functions. Front. Biosci. (Elite Ed.) 8, 189–204.
Li, S., Chen, S., Xu, X., Sundstedt, A., Paulsson, K.M., Anderson, P., Karlsson, S., Sjogren, H.O., Wang, P., 2000. Cytokine-induced Src homology 2 protein (CIS) promotes T cell receptor-mediated proliferation and prolongs survival of activated T cells. J. Exp. Med. 191, 985–994.
Liao, W., Lin, J.X., Leonard, W.J., 2013. Interleukin-2 at the crossroads of effector responses, tolerance, and immunotherapy. Immunity 38, 13–25.
Linossi, E.M., Nicholson, S.E., 2012. The SOCS box-adapting proteins for ubiquitination and proteasomal degradation. IUBMB Life 64, 316–323.
Liu, E., Tong, Y., Dotti, G., Shaim, H., Savoldo, B., Mukherjee, M., Orange, J., Wan, X., Lu, X., Reynolds, A., Gagea, M., Banerjee, P., Cai, R., Bdaiwi, M.H., Basar, R., Muftuoglu, M., Li, L., Marin, D., Wierda, W., Keating, M., Champlin, R., Shpall, E., Rezvani, K., 2017. Cord blood NK cells engineered to express IL-15 and a CD19-targeted CAR show long-term persistence and potent anti-tumor activity. Leukemia. http://dx.doi.org/10.1038/leu.2017.226.
Locatelli, F., Moretta, F., Brescia, L., Merli, P., 2014. Natural killer cells in the treatment of high-risk acute leukaemia. Semin. Immunol. 26, 173–179.
Macchi, P., Villa, A., Giliani, S., Sacco, M.G., Frattini, A., Porta, F., Ugazio, A.G., Johnston, J.A., Candotti, F., O'Shea, J.J., et al., 1995. Mutations of Jak-3 gene in patients with autosomal severe combined immune deficiency (SCID). Nature 377, 65–68.
Marine, J.C., McKay, C., Wang, D., Topham, D.J., Parganas, E., Nakajima, H., Pendeville, H., Yasukawa, H., Sasaki, A., Yoshimura, A., Ihle, J.N., 1999. SOCS3 is essential in the regulation of fetal liver erythropoiesis. Cell 98, 617–627.
Martinet, L., Smyth, M.J., 2015. Balancing natural killer cell activation through paired receptors. Nat. Rev. Immunol. 15, 243–254.
Martinet, L., Ferrari De Andrade, L., Guillerey, C., Lee, J.S., Liu, J., Souza-Fonseca-Guimaraes, F., Hutchinson, D.S., Kolesnik, T.B., Nicholson, S.E., Huntington, N.D., Smyth, M.J., 2015. DNAM-1 expression marks an alternative program of NK cell maturation. Cell Rep. 11, 85–97.
Matsumoto, A., Masuhara, M., Mitsui, K., Yokouchi, M., Ohtsubo, M., Misawa, H., Miyajima, A., Yoshimura, A., 1997. CIS, a cytokine inducible SH2 protein, is a target of the JAK-STAT5 pathway and modulates STAT5 activation. Blood 89, 3148–3154.
Mebius, R.E., Rennert, R., Weissman, I.L., 1997. Developing lymph nodes collect CD4+ CD3- LTb+ cells the can differentiate to APC, NK cells, and follicular cells but not t or B cells. Immunity 7, 493–504.
Metcalf, D., Greenhalgh, C.J., Viney, E., Willson, T.A., Starr, R., Nicola, N.A., Hilton, D.J., Alexander, W.S., 2000. Gigantism in mice lacking suppressor of cytokine signalling-2. Nature 405, 1069–1073.
Mjosberg, J., Bernink, J., Peters, C., Spits, H., 2012. Transcriptional control of innate lymphoid cells. Eur. J. Immunol. 42, 1916–1923.
Mlecnik, B., Bindea, G., Angell, H.K., Sasso, M.S., Obenauf, A.C., Fredriksen, T., Lafontaine, L., Bilocq, A.M., Kirilovsky, A., Tosolini, M., Berger, A., Fridman, W.H., Rafii, A., Valge-Archer, V., Pages, F., Speicher, M.R., Galon, J., 2014. Functional network pipeline reveals genetic determinants associated with in situ lymphocyte proliferation and survival of cancer patients. Sci. Transl. Med. 6, a237.
Monticelli, L.A., Sonnenberg, G.F., Abt, M.C., Alenghat, T., Ziegler, C.G., Doering, T.A., Angelosanto, J.M., Laidlaw, B.J., Yang, C.Y., Sathaliyawala, T., Kubota, M., Turner, D., Diamond, J.M., Goldrath, A.W., Farber, D.L., Collman, R.G., Wherry, E.J., Artis, D., 2011. Innate lymphoid cells promote lung-tissue homeostasis after infection with influenza virus. Nat. Immunol. 12, 1045–1054.
Moro, K., Yamada, T., Tanabe, M., Takeuchi, T., Ikawa, T., Kawamoto, H., Furusawa, J., Ohtani, M., Fujii, H., Koyasu, S., 2010. Innate production of T(H)2 cytokines by adipose tissue-associated c-Kit(+)Sca-1(+) lymphoid cells. Nature 463, 540–544.
Mortier, E., Woo, T., Advincula, R., Gozalo, S., Ma, A., 2008. IL-15Ralpha chaperones IL-15 to stable dendritic cell membrane complexes that activate NK cells via trans presentation. J. Exp. Med. 205, 1213–1225.
Morvan, M.G., Lanier, L.L., 2016. NK cells and cancer: you can teach innate cells new tricks. Nat. Rev. Cancer 16, 7–19.
Nirschl, C.J., Suarez-Farinas, M., Izar, B., Prakadan, S., Dannenfelser, R., Tirosh, I., Liu, Y., Zhu, Q., Devi, K.S.P., Carroll, S.L., Chau, D., Rezaee, M., Kim, T.G., Huang, R., Fuentes-Duculan, J., Song-Zhao, G.X., Gulati, N., Lowes, M.A., King, S.L., Quintana, F.J., Lee, Y.S., Krueger, J.G., Sarin, K.Y., Yoon, C.H., Garraway, L., Regev, A., Shalek, A.K., Troyanskaya, O., Anandasabapathy, N., 2017. IFNgamma-dependent tissue-immune homeostasis is co-opted in the tumor microenvironment. Cell 170, 127–141 (e115).
Noguchi, M., Yi, H., Rosenblatt, H.M., Filipovich, A.H., Adelstein, S., Modi, W.S., McBride, O.W., Leonard, W.J., 1993. Interleukin-2 receptor gamma chain mutation results in X-linked severe combined immunodeficiency in humans. Cell 73, 147–157.
Palmer, D.C., Guittard, G.C., Franco, Z., Crompton, J.G., Eil, R.L., Patel, S.J., Ji, Y., Van Panhuys, N., Klebanoff, C.A., Sukumar, M., Clever, D., Chichura, A., Roychoudhuri, R., Varma, R., Wang, E., Gattinoni, L., Marincola, F.M., Balagopalan, L., Samelson, L.E., Restifo, N.P., 2015. Cish actively silences TCR signaling in CD8+ T cells to maintain tumor tolerance. J. Exp. Med. 212, 2095–2113.
Pugliese-Pires, P.N., Tonelli, C.A., Dora, J.M., Silva, P.C., Czepielewski, M., Simoni, G., Arnhold, I.J., Jorge, A.A., 2010. A novel STAT5 B mutation causing GH insensitivity syndrome associated with hyperprolactinemia and immune dysfunction in two male siblings. Eur. J. Endocrinol. 163, 349–355.
Rankin, L.C., Groom, J.R., Chopin, M., Herold, M.J., Walker, J.A., Mielke, L.A., McKenzie, A.N., Carotta, S., Nutt, S.L., Belz, G.T., 2013. The transcription factor T-bet is essential for the development of NKp46(+) innate lymphocytes via the Notch pathway. Nat. Immunol. 14, 389–395.
Rezvani, K., Rouce, R.H., 2015. The application of natural killer cell immunotherapy for the treatment of cancer. Front. Immunol. 6, 578.
Rezvani, K., Rouce, R., Liu, E., Shpall, E., 2017. Engineering natural killer cells for cancer

immunotherapy. Mol. Ther. 25 (8), 1769–1781. http://dx.doi.org/10.1016/j.ymthe.2017.06.012.
Robbins, S.H., Nguyen, K.B., Takahashi, N., Mikayama, T., Biron, C.A., Brossay, L., 2002. Cutting edge: inhibitory functions of the killer cell lectin-like receptor G1 molecule during the activation of mouse NK cells. J. Immunol. 168, 2585–2589.
Russell, S.M., Tayebi, N., Nakajima, H., Riedy, M.C., Roberts, M.J., Migone, T.S., Noguchi, M., Markert, M.L., Buckley, R.H., O'Shea, J.J., Leonard, W.J., 1995. Mutation of Jak3 in a patient with SCID: essential role of Jak3 in lymphoid development. Science 270, 797–800.
Sanos, S.L., Bui, V.L., Mortha, A., Oberle, K., Heners, C., Johner, C., Diefenbach, A., 2009. ROR(t and commensal microflora are required for the differentiation of mucosal interleukin IL22-producion NKp46+ cells. Nat. Immunol. 10, 83–91.
Sasaki, A., Yasukawa, H., Suzuki, A., Kamizono, S., Syoda, T., Kinjyo, I., Sasaki, M., Johnston, J.A., Yoshimura, A., 1999. Cytokine-inducible SH2 protein-3 (CIS3/SOCS3) inhibits Janus tyrosine kinase by binding through the N-terminal kinase inhibitory region as well as SH2 domain. Genes Cells 4, 339–351.
Satoh-Takayama, N., Vosshenrich, C.A., Lesjean-Pottier, S., Sawa, S., Lochner, M., Rattis, F., Mention, J.J., Thiam, K., Cerf-Bensussan, N., Mandelboim, O., Eberl, G., Di Santo, J.P., 2008. Microbial flora drives interleukin 22 production in intestinal NKp46+ cells that provide innate mucosal immune defense. Immunity 29, 958–970.
Sporri, B., Kovanen, P.E., Sasaki, A., Yoshimura, A., Leonard, W.J., 2001. JAB/SOCS1/SSI-1 is an interleukin-2-induced inhibitor of IL-2 signaling. Blood 97, 221–226.
Suzuki, H., Duncan, G.S., Takimoto, H., Mak, T.W., 1997. Abnormal development of intestinal intraepithelial lymphocytes and peripheral natural killer cells in mice lacking the IL-2 receptor beta chain. J. Exp. Med. 185, 499–505.
Takanami, I., Takeuchi, K., Giga, M., 2001. The prognostic value of natural killer cell infiltration in resected pulmonary adenocarcinoma. J. Thorac. Cardiovasc. Surg. 121, 1058–1063.
Villegas, F.R., Coca, S., Villarrubia, V.G., Jimenez, R., Chillon, M.J., Jareno, J., Zuil, M., Callol, L., 2002. Prognostic significance of tumor infiltrating natural killer cells subset CD57 in patients with squamous cell lung cancer. Lung Cancer 35, 23–28.
Vivier, E., Tomasello, E., Baratin, M., Walzer, T., Ugolini, S., 2008. Functions of natural killer cells. Nat. Immunol. 9, 503–510.
Vonarbourg, C., Mortha, A., Bui, V.L., Hernandez, P.P., Kiss, E.A., Hoyler, T., Flach, M., Bengsch, B., Thimme, R., Holscher, C., Honig, M., Pannicke, U., Schwarz, K., Ware, C.F., Finke, D., Diefenbach, A., 2010. Regulated expression of nuclear receptor RORgammat confers distinct functional fates to NK cell receptor-expressing RORgammat(+) innate lymphocytes. Immunity 33, 736–751.
Voskoboinik, I., Smyth, M.J., Trapani, J.A., 2006. Perforin-mediated target-cell death and immune homeostasis. Nat. Rev. Immunol. 6, 940–952.
Waldmann, T.A., 2015. The shared and contrasting roles of IL2 and IL15 in the life and death of normal and neoplastic lymphocytes: implications for cancer therapy. Cancer Immunol. Res. 3, 219–227.
Wilhelm, C., Hirota, K., Stieglitz, B., Van Snick, J., Tolaini, M., Lahl, K., Sparwasser, T., Helmby, H., Stockinger, B., 2011. An IL-9 fate reporter demonstrates the induction of an innate IL-9 response in lung inflammation. Nat. Immunol. 12, 1071–1077.
Wong, S.H., Walker, J.A., Jolin, H.E., Drynan, L.F., Hams, E., Camelo, A., Barlow, J.L., Neill, D.R., Panova, V., Koch, U., Radtke, F., Hardman, C.S., Hwang, Y.Y., Fallon, P.G., McKenzie, A.N., 2012. Transcription factor RORalpha is critical for nuocyte development. Nat. Immunol. 13, 229–236.
Yang, X.O., Zhang, H., Kim, B.S., Niu, X., Peng, J., Chen, Y., Kerketta, R., Lee, Y.H., Chang, S.H., Corry, D.B., Wang, D., Watowich, S.S., Dong, C., 2013. The signaling suppressor CIS controls proallergic T cell development and allergic airway inflammation. Nat. Immunol. 14, 732–740.
Yokoyama, W.M., Plougastel, B.F., 2003. Immune functions encoded by the natural killer gene complex. Nat. Rev. Immunol. 3, 304–316.
Yoshimura, A., Ohkubo, T., Kiguchi, T., Jenkins, N.A., Gilbert, D.J., Copeland, N.G., Hara, T., Miyajima, A., 1995. A novel cytokine-inducible gene CIS encodes an SH2-containing protein that binds to tyrosine-phosphorylated interleukin 3 and erythropoietin receptors. EMBO J. 14, 2816–2826.
Zaretsky, J.M., Garcia-Diaz, A., Shin, D.S., Escuin-Ordinas, H., Hugo, W., Hu-Lieskovan, S., Torrejon, D.Y., Abril-Rodriguez, G., Sandoval, S., Barthly, L., Saco, J., Homet Moreno, B., Mezzadra, R., Chmielowski, B., Ruchalski, K., Shintaku, I.P., Sanchez, P.J., Puig-Saus, C., Cherry, G., Seja, E., Kong, X., Pang, J., Berent-Maoz, B., Comin-Anduix, B., Graeber, T.G., Tumeh, P.C., Schumacher, T.N., Lo, R.S., Ribas, A., 2016. Mutations associated with acquired resistance to PD-1 blockade in melanoma. N. Engl. J. Med. 375, 819–829.
Zhang, J.G., Farley, A., Nicholson, S.E., Willson, T.A., Zugaro, L.M., Simpson, R.J., Moritz, R.L., Cary, D., Richardson, R., Hausmann, G., Kile, B.T., Kent, S.B., Alexander, W.S., Metcalf, D., Hilton, D.J., Nicola, N.A., Baca, M., 1999. The conserved SOCS box motif in suppressors of cytokine signaling binds to elongins B and C and may couple bound proteins to proteasomal degradation. Proc. Natl. Acad. Sci. U. S. A. 96, 2071–2076.
Zhang, X., Yue, P., Page, B.D., Li, T., Zhao, W., Namanja, A.T., Paladino, D., Zhao, J., Chen, Y., Gunning, P.T., Turkson, J., 2012. Orally bioavailable small-molecule inhibitor of transcription factor Stat3 regresses human breast and lung cancer xenografts. Proc. Natl. Acad. Sci. U. S. A. 109, 9623–9628.