CIS and TGF-β regulatory pathways influence immunity to bacterial infection

Timothy R. McCulloch | Gustavo R. Rossi | Jaring Schreuder | Gabrielle T. Belz | Timothy J. Wells | Fernando Souza-Fonseca-Guimaraes

1University of Queensland Diamantina Institute, Faculty of Medicine, The University of Queensland, Woolloongabba, QLD, Australia
2Australian Infectious Diseases Research Centre, University of Queensland, Brisbane, QLD, Australia

Abstract

Immunotherapy has revolutionized cancer therapy by reactivating tumour-resident cytotoxic lymphocytes. More recently, immunotherapy has emerged to restore immunity against infectious agents, including bacterial infections. Immunotherapy primarily targets inhibitory pathways in T cells, however interest in other effector populations, such as natural killer (NK) cells, is growing. We have previously discovered that NK cell metabolism, proliferation and activation can be neutralized through the immunosuppressive transforming growth factor (TGF)-β pathway by inducing plasticity of NK cells and differentiation into innate lymphoid cell (ILC1)-like subsets. NK cells are also regulated through cytokine-inducible SH2-containing protein (CIS), which is induced by interleukin (IL)-15 and is a potent intracellular checkpoint suppressing NK cell survival and function. Targeting these two distinct pathways to restore NK cell function has shown promise in cancer models, but their application in bacterial infection remains unknown. Here, we investigate whether enhancement of NK cell function can improve anti-bacterial immunity, using Salmonella Typhimurium as a model. We identified conversion of NK cells to ILC1-like for the first time in the context of bacterial infection, where TGF-β signalling contributed to this plasticity. Future study should focus on identifying further drivers of ILC1 plasticity and its functional implication in bacterial infection model. We further describe that CIS-deficient mice displayed enhanced pro-inflammatory function and dramatically enhanced anti-bacterial immunity. Inhibition of CIS may present as a viable therapeutic option to enhance immunity towards bacterial infection.

Keywords

cellular plasticity, CIS, innate lymphoid cell 1, natural killer cells, TGF-βR signalling

Abbreviations: CFU, Colony forming units; CIS, Cytokine-inducible SH2-containing protein (CIS); CTLA-4, Cytotoxic T-lymphocyte-associated protein-4; IFN, Interferon; IL, Interleukin; ILC, Innate lymphoid cells; JAK, Janus kinase; mTOR, Mammalian target of rapamycin; NK, Natural killer; PD-1, Programmed cell death protein-1; STAT5, Signal transducer and activator of transcription 5; TGF, Transforming growth factor; TGF-βRII, Transforming growth factor-beta receptor II.
INTRODUCTION

Natural killer (NK) cells are cytotoxic innate lymphocytes which have well described roles in the host defence against viral pathogens and cancer [1]. Yet their ability to contribute to immunity in many bacterial infections, including Salmonella enterica serovar Typhimurium (S. Typhimurium), remains unclear. NK cells have the potential to promote anti-Salmonella immunity through direct killing of infected cells and activation of infected cells by proinflammatory cytokine production, namely interferon (IFN)-γ. Previous studies in a murine model of S. Typhimurium infection have indicated that NK cells can contribute to protective IFN-γ and disease clearance in immunocompromised mice, but are otherwise dispensable when CD4+ T cells are present [2]. The idea of NK cell redundancy when adaptive lymphocytes are present has also been suggested in human disease [3]. This suggests that the standard NK cell depletion model using anti-NK1.1 or anti-asialo-GM1 antibodies is likely insufficient to gauge the full potential of NK cells to promote antibacterial immunity in otherwise immunocompetent hosts. Depletion studies do not account for the impact of NK cell regulation during infection through the action of immunoregulatory cytokines such as interleukin (IL)-10 and transforming growth factor (TGF)-β, NK cell lymphopenia and inhibitory immune checkpoints. Targeting these mechanisms of regulation by immunotherapy may allow NK cells to participate in immunity where they may otherwise have been incapable.

Blockade of immunoregulatory molecules though immunotherapy has revolutionized cancer therapy. The classic targets are surface immune checkpoint molecules, such as programmed cell death protein-1 (PD-1), and cytotoxic T-lymphocyte-associated protein-4 (CTLA-4), which bind to their ligand on infected or malignant cells to ablate lymphocyte function. Blockade of these receptors can reverse this immune suppression and restore lymphocyte function [4]. However, interest is rising in targeting other types of molecules, including regulatory cytokines and intracellular immune checkpoints. TGF-β is a pleiotropic cytokine of the TGF superfamily which has potent regulatory effects on NK cells by repressing mammalian target of rapamycin (mTOR) [5] and converting them to an innate lymphoid cell (ILC1)-like phenotype [6]. Also of particular importance in NK cells is cytokine-inducible SH2-containing protein (CIS), which acts as a negative regulator of IL-15 signalling to limit NK cell proliferation and pro-inflammatory function [7]. IL-15 signalling leads to phosphorylation of Janus kinase (JAK)1 and 3, and subsequent recruitment and phosphorylation of signal transducer and activator of transcription (STAT)5. This allows STAT5 to then translocate to the nucleus to transcribe variety of genes associated with NK cell function and survival [8, 9]. Also transcribed is Cish, encoding the protein CIS, which ubiquitates JAK1 and JAK3, targeting them for proteasomal degradation. In this way, CIS acts as a negative feedback loop to limit IL-15 signalling and NK cell activation [7, 10]. Inhibition of the CISH and TGF-β regulatory pathways in bacterial infection may restore the function of NK cells, enhancing their ability to contribute towards anti-bacterial immunity.

In the face of antimicrobial resistance, which is leading to infections that are increasingly difficult to treat, immunotherapy is arising as a potential alternative or conjunctural to traditional antimicrobial therapy [11]. In this study, we use a ‘gain of function’ model to investigate whether NK cell function could be enhanced or restored during infection to boost anti-bacterial immunity. Our group and others have focused on the improvement of NK cell function through the deletion of the receptor for immunoregulatory molecule TGF-β [5, 6, 12], or CIS [7]. Here, we investigate whether suppressing signalling of immune checkpoints TGF-β and CIS in a new transgenic mouse model could act independently or synergistically to increase the magnitude of NK anti-bacterial function against Salmonella infection.

RESULTS

NK cells are depleted and converted to ILC1-like cells during S. Typhimurium infection

To investigate NK cells during S. Typhimurium infection, mice were infected with the attenuated mutant strain, SL3261, to cause a chronic, invasive infection. At day 10 postinfection, spleens and livers were taken to observe phenotypic changes in immune cells. Of note, we observed upregulation of the collagen binding protein CD49a in NK cells in both the spleen (Figure 1a,b) and liver (Figure 1c,d) of infected mice. Upregulation of CD49a on NK cells has previously been linked to a phenotypic switch from conventional NK (cNK) to tissue resident ILC1-like cells [6, 13]. Indeed, further phenotyping of these cells from infected liver showed a decrease in classical NK markers Eomes and CD62L, and an increase of ILC1 markers CD226 and CD200R in CD49a+ NK cells compared with CD49a− (Figure S1a–f). We also observed significant NK cell lymphopenia in the blood (Figure 1e) and spleen (Figure 1f) of infected mice compared with uninfectecl. NK cell depletion was not seen in the liver of infected mice, where normal NK numbers were persevered (Figure 1g). Collectively, this data indicates that NK cells are considerably affected during S. Typhimurium
infection, namely by conversion to ILC1-like cells and organ specific depletion.

ILC1-like show phenotypic properties of ILC1 but derive from cNK

We hypothesized that the CD49a⁺ CD49b⁺ ILC1-like cells derived from cNK cells, as has been observed in tumours [6] and Toxoplasma infection [14]. To address whether this was the case, immunodeficient mice (Rag2⁻/⁻ × Il2rg⁻/⁻, lacking T, B and NK cells) were reconstituted with cNK cells isolated from the spleens of Eomes-mCherry reporter mice (Figure 2a). Seven days posttransfer, mice were either infected with S. Typhimurium or left as uninfected controls. After a further 7 days postadoptive cell transfer, CD49a⁺ CD49b⁺ ILC1-like cells were observed in the livers of NK reconstituted mice (Figure 2b), indicating that cNK cells indeed give rise to ILC1-like cells. Consistent with previous study by our group [6], ILC1-like cells were found even in uninfected mice, suggesting the naïve liver has an environment conducive to ILC plasticity. However, infected livers had significantly less cNK and more ILC1-like cells compared with naïve livers (Figure 2c), likely due to upregulation of external conversion factors such as TGF-β during infection. Similar to our previous
observations in the tumour microenvironment [6], the transferred NK cells also showed further signs of a switch to ILC1-like cells, including downregulation of Eomes and CD62L, and upregulation of CD226 and CD200R (Figure 2d). Thus, this confirmed our hypothesis that ILC1-like cells derived from cNK cells during S. Typhimurium infection.

TGF-β contributes to NK to ILC1-like conversion in S. Typhimurium infection

NK conversion to ILC1-like cells has been previously shown to be dependent primarily on TGF-β [6]. To investigate if this was the case in our model, the NK/ILC1-like status was investigated by flow cytometry in mice with a conditional deletion of the TGF-β receptor II (TGF-βRII) specifically within NK cells (Ncr1cre/C2 TgfbRIIfl/fl) infected with S. Typhimurium. As expected, conditional deletion of TGF-β signalling within NK cells resulted in significantly reduced percentages of ILC1-like cells in both the spleen (Figure 3a,b) and liver (Figure 3c,d) of infected mice. ILC1-like cells were not completely ablated, indicating that TGF-β drives this conversion in concert with other signals in our infection model. TGF-β signalling was also targeted therapeutically using the TGF-β receptor 1 kinase inhibitor galunisertib in infected mice, however we observed no changes to bacterial burdens or NK cell to ILC1-like conversion in galunisertib treated mice compared with untreated controls (Figure S2).

Deletion of CISH, but not conditional deletion of TgfbRII, results in enhanced anti-bacterial immunity

We predicted that both depletion of NK cells and their conversion into ILC1-like could potentially act to dampen the ability of NK cells to contribute to bacterial clearance. Further, we hypothesized that reversing these two phenotypes could enhance anti-bacterial immunity. To address the ILC1-like conversion, we infected mice with conditional deletion of the TGF-βRII specifically within NK cells (Ncr1cre x TgfbRIIfl/fl) infected with S. Typhimurium. To address the NK cell depletion, we infected Cish knockout mice (Cish−/−, encoding the intracellular checkpoint molecule CIS). As CIS has been reported to limit NK cell survival and proliferation [7, 10], we proposed that its deletion could enhance proliferation to help maintain NK cell numbers during infection. We also infected mice with both CIS deficiency and conditional deletion of TGF-β signalling within NK cells (Ncr1cre x TgfbRIIfl/fl x Cish−/−) to
determine if deletion of both pathways simultaneously could have a synergistic effect. Surprisingly, \textit{Ncr1}^{cre} \times \textit{TgfbR}^{II}^{fl/fl} mice did not show a reduced bacterial load in either the spleen (Figure 4a) or the liver (Figure 4b) compared with wild-type controls. Conversely, \textit{Cish}^{-/-} mice exhibited a significant reduction in bacterial burden in both organs (Figure 4a,b). The combination of both NK cell gain-of-function deletions did not synergize to further reduce bacterial burden (Figure 4a,b). Thus CIS, but not TGF-β signalling in NK cells, acts as an immune checkpoint to restrict anti-bacterial immunity.

The rationale behind including \textit{Cish}^{-/-} mice was that we predicted deletion of CIS would prevent NK cell lymphopenia, therefore NK cell numbers were analysed in the spleens and livers of infected mice. However, deletion of CIS was not able to enhance NK cell numbers in either the spleen (Figure 4c) or liver (Figure 4d) of infected mice compared with \textit{Ncr1}^{cre} controls. Surprisingly, deletion of the TGF-β receptor II from NK cells resulted in significantly reduced numbers in both organs (Figure 4c,d), suggesting TGF-β actually plays a protective role on NK cell survival during bacterial infection.

That we found deletion of \textit{Cish} did not prevent bacterial driven-NK cell depletion and considering the \textit{Cish}^{-/-} mouse was a whole mouse knock out as opposed to a conditional deletion, this suggested that the enhanced anti-bacterial immunity in these mice may have been independent of NK cells. To investigate whether the enhanced immunity in \textit{Cish}^{-/-} mice was NK cell dependent, wild-type or \textit{Cish}^{-/-} mice were treated with anti-NK1.1 to deplete NK cells. No differences were observed between any groups in the spleen (Figure 4e). In the liver, \textit{Cish} deletion was able to significantly reduce bacterial burdens as shown previously. Bacterial burdens in \textit{Cish}^{-/-} mice with NK cell depletion were significantly reduced compared with wild-type mice with NK depletion, and showed no difference compared with \textit{Cish}^{-/-} treated with isotype control (Figure 4f). Taken together, this suggests that the phenotype observed in \textit{Cish}^{-/-} mice was independent of NK cell function. In cancer models, \textit{Cish}^{-/-} NK cells exhibit enhanced maturation, however this was not evident in our infection model, further suggesting the phenotype was not dependent on NK cells (Figure S3). In addition, IL-6 was the only cytokine significantly increased
in the plasma of Cish−/− mice at day two compared with wild-type controls (Figure S4), suggesting that the phenotype observed following Cish deletion may primarily act through myeloid cells in this infection. Furthermore, no significant increase in cytokine levels were observed at day nine postinfection (Figure S4). Thus, while our results show that CIS is a potent checkpoint in anti-bacterial immunity, we found no evidence that the reduction in bacterial burden observed in Cish−/− mice were due to enhancement of NK cell function.

**DISCUSSION**

The purpose of this study was to gain a better understanding of how NK cells are regulated during bacterial infection. NK cells are increasingly being recognized as a promising immunotherapy target in settings of cancer [15], however their potential as a target during acute or chronic bacterial infection is unknown. Immunotherapy for treating bacterial infections, particularly in the case of antibiotic resistance, is an emerging field [11], for which NK-mediated immunotherapy may also show viability. Identification of specific regulatory molecules and checkpoints acting on NK cells during bacterial infection could uncover novel immunotherapy targets to enhance NK-mediated bacterial immunity.

Our finding that NK cells were converted to ILC1-like cells during S. Typhimurium was not unexpected. This conversion has previously been observed in other disease settings including cancer [5, 6, 16] and parasitic infection [14]. Further, *Salmonella* subspecies are known to actively drive macrophage polarization towards an anti-inflammatory (or M2-like) phenotype [17], promoting the production of regulatory cytokines such as IL-10 and TGF-β [18] which may be driving NK cells conversion. By using transgenic mouse models, we found that TGF-β contributed to conversion of NK to ILC1-like cells, however, was not the only driver in our model. The TGF-β superfamily is a large group of over 33 regulatory proteins which have both distinct and overlapping functions [19]. Therefore, it is reasonable to assume that other members of the TGF-β superfamily could also drive this conversion, which has already been observed by our group in the case of Activin A [12]. Our lack of findings using the therapeutic inhibitor galunisertib, which targets the TGF-β receptor 1 kinase, may also be hindered by inconsistencies in the literature on the appropriate dosing of this drug in mouse models. Dosing regimens published in murine studies range from 10 mg/kg every second day by i.p. injection [12] to 150 mg/kg twice daily by oral gavage [20]. Whether underdosing was a defining factor in our results is unknown. Regardless, the remaining factor, or factors, driving NK to ILC1-like conversion would need to be identified before definitively determining whether this conversion limits anti-bacterial immunity. Conversely, this plasticity may even play a protective role by preventing NK cell depletion. Upregulation of CD49a in tissue resident memory T cells is critical for their survival by allowing binding to collagen and preventing apoptosis [21, 22]. This could also be the case for CD49a+ NK cells, and would be consistent with our data showing enhanced depletion of TGF-βRII deficient NK cells.

This study also found that deletion of CIS improved control of S. Typhimurium infection. CIS is a SOCS protein which functions as a negative regulator of cytokine signalling. In the case of NK cells, activation of STAT5 by IL-15 signalling induces expression of CIS, which acts via...
a classic negative feedback loop to limit IL-15 signalling and NK cell overstimulation [7]. However, STAT5 signalling is also active in other cell types in response to signalling by cytokines other than IL-15, including IL-2 and GM-CSF. CIS also inhibits T cell receptor signalling [23]. Thus, CIS has also been shown to regulate additional immune cells in addition to NK cells including ILC2s [24], CD4+ and CD8+ T cells [23, 25], neutrophils [26] and macrophages [27, 28]. Considering this was a whole mouse knockout of CIS, we cannot be sure of which cell type, or combinations of cell types, benefited from CIS deletion to promote anti-bacterial immunity. We found no evidence to confirm our initial hypothesis that CIS deletion acted through NK cell enhancement. This does, however, open an exciting new area of research where further investigation of the CIS pathway may uncover specific cell types in which CIS acts as an immune check-point during bacterial infection.

Curiously, our results from the CIS knockout mouse differ from other studies. Despite being a negative regulator of immune function, CIS has previously been shown to mediate early control of tuberculosis infection in a mouse model [28]. In humans, single nucleotide polymorphisms (SNPs) in CISH have been associated with increased susceptibility to tuberculosis, malaria and bacteremia [29]. However, the functional implications of these SNPs on immune response were not established in the study. These previous results may be explained by observations in which excessive inflammation worsens infectious disease. This can be epitomized by the curious case of anti-PD1 therapy in tuberculosis, which exacerbates disease severity and reactivates latent infection [30, 31]. Thus, in the case of tuberculosis, immune enhancement does not always lead to greater immunity. That we have found CIS deletion enhances anti-Salmonella immunity, where others have found it impedes anti-tuberculosis immunity, may be explained by underlying differences in the physiology and pathology of these two pathogens. These differences must be rigorously examined if any pharmacological targeting of CIS signalling is to be trialled to treat bacterial infections.

In summary, we have been able to expand the current knowledge of ILC plasticity to show for the first-time evidence of conversion of NK cells to ILC1-like cells during bacterial infection. However, the precise drivers of conversion as well as the functional relevance this plasticity has on infection outcome remains elusive. We have also shown that CIS is a potent immune checkpoint in anti-Salmonella immunity. However, expression of CIS is also conserved across immune cell types including myeloid cells, and thus the exact cell type or mechanism where CIS acts on to restrict bacterial clearance could not be addressed in this current study. Future study is warranted to elucidate how CIS inhibition enacts its effects, and whether pharmacological inhibition of this molecule could enhance anti-bacterial immunity, which may be particularly relevant in the face of rising antimicrobial resistance.

METHODS

Mouse models

C57BL/6 mice were purchased from Animal Resources Centre (ARC). Ncr1cre × TgfbRIIfl/fl mice were used as conditional TgfbRII-deficiency specific to NK cells, obtained by crossing as previous described by our group [6]. CISH−/− were maintained on a C57BL/6 background [7]. To obtain a double deficient mouse model, Ncr1cre × TgfbRIIfl/fl were back crossed with CISH−/− to obtain a Ncr1cre × TgfbRIIfl/fl × CISH−/− mice strain. Ncr1cre mice were considered wild-type controls for some experiments. Ncr1crecre × McI1fl/fl and Rag2−/− × Il2rg−/− mice were used as NK cell deficient controls [32]. Eomes-mCherry reporter mice on a C57BL/6 background were used for NK cell adoptive cell transfer experiments [33]. All experiments were performed using cells from age/sex matched cohort of mice (age range 8–12 weeks). Cohort sizes are described in each figure legends, where power calculations were used to estimated sample size needed to achieve statistical significance at a 50% change in bacterial burden or immune parameters with a power of 0.80 and Type I error (alpha) of 0.05. For infection studies, mice with no detectable bacterial load at the end of the experiment were considered to have not taken up infection and were excluded from further analysis. All experiments were approved by the University of Queensland’s Animal Ethics Committees.

Bacterial strains and in vivo infections

Mice were infected with an attenuated aroA mutant strain of Salmonella enterica serovar Typhimurium, SL3261 [34]. For in vivo infection, bacteria were grown at 37°C with shaking in Lysogeny broth (LB) for 16 to 18 h. OD600 was used to enumerate bacteria, before being diluted to the appropriate concentration in PBS. Mice were infected by intraperitoneal (i.p) injection with 5 × 10⁶ colony forming units (CFU) of SL3261 in 200 μl and sacrificed at the described times post-infection. The TGF-β receptor I inhibitor galunisertib (LY2157299, SelleckChem, Houston, TX) was given at a dose of 10 mg/kg by i.p infection, as described
previously [12]. To deplete NK cells, appropriate mice were treated with 100 μg of anti-NK1.1 antibody (PK136, BioXCell, Lebanon NH) or isotype control (2AE, BioXCell) on days −3, 0, 3 and 8 relative to infection.

Murine tissue collection

Blood samples were taken from mice by retro-orbital bleeds into EDTA-coated tubes. Tubes were centrifuged at 1500 g for 15 min, and plasma removed from cell pellet. Plasma samples were stored at −20°C until analysis. At the experimental endpoint, mice were euthanized by CO2 asphyxiation. Spleens and livers were dissected and held in PBS until processing. Bacterial counts were enumerated from organs by homogenizing samples in 0.1% Triton-X (Sigma-Aldrich, Burlington, MA) in PBS before serially diluting in PBS and plating on LB agar plates.

Flow cytometry

Spleens and livers were passed through a 70 or 100 μm cell strainer, respectively, in cold fluorescence-activated cell sorting (FACS) buffer (PBS containing 2% fetal bovine serum and 2 mM EDTA). Leukocytes were enriched using 37.5% Percoll solution (GE Healthcare, Uppsala, Sweden) and red blood cells lysed with Ammonium-Chloride-Potassium (ACK) lysis buffer (Biolgend, San Diego CA). Dead cells were stained with Fixable Viability Stain 440UV (1:1000 in PBS, Becton Dickinson, Franklin Lakes, NJ) for 15 min at room temperature. Fc receptors were blocked by incubation for 15 min in Fc Blocking Reagent (1:100 in FACS buffer, Miltenyi Biotec, Bergisch Gladbach, Germany). Single-cell suspensions were stained with the indicated fluorescent antibodies on ice for 45 min. For intracellular cytokine staining, cells were fixed and permeabilized using the FoxP3/Transcription Factor Staining Buffer Set (eBioscience, San Diego CA), then stained for 60 min with the indicated fluorescent antibodies on ice for 45 min. For intracellular cytokine staining, cells were fixed and permeabilized using the FoxP3/Transcription Factor Staining Buffer Set (eBioscience, San Diego CA), then stained for 60 min with the indicated fluorescent antibodies on ice for 45 min.

Measurement of cytokines

IFN-γ titers were determined from murine serum samples using a Mouse IFN-γ ELISA set (Becton Dickinson) as per the manufacturer’s instructions. Other cytokines were determined using Cytometric Bead Array (Becton Dickinson) as per the manufacturer’s instructions.

Statistics

Statistical analysis was performed using GraphPad Prism Software v9 (GraphPad Software, San Diego, CA). Statistical tests were performed for experiments as indicated in figure legends, and error bars represent SEM. Levels of statistical significance are expressed as p values.

ORCID

Timothy R. McCulloch https://orcid.org/0000-0003-0247-7374
Fernando Souza-Fonseca-Guimaraes https://orcid.org/0000-0002-2037-8946

AUTHOR CONTRIBUTIONS

Timothy R. McCulloch and Fernando Souza-Fonseca-Guimaraes designed research and wrote the paper. Timothy R. McCulloch, Fernando Souza-Fonseca-Guimaraes and Gustavo R. Rossi, performed research and Timothy R. McCulloch analysed data. Jaring Schreuder and Gabrielle T. Belz provided critical mouse models and advice in assays. Timothy J. Wells and Fernando Souza-Fonseca-Guimaraes supervised work.

ACKNOWLEDGEMENTS

We thank all the members of the Guimaraes and Wells laboratories; Prof. N. D. Huntington, Prof. S. Bell, Prof. M. Sweet and Prof. A. Yoshimura for discussion, comments, and advice in this project; Prof. E. Vivier for providing the Nkp46Cre mice; and Profs. J. Ihle and E. Parganas for providing the CIS knockout mice. This research was carried out at the Translational Research Institute, Woolloongabba, QLD 4102, Australia. The Translational Research Institute is supported by grants from the Australian and Queensland Governments. Open access publishing facilitated by The University of Queensland, as part of the Wiley - The University of Queensland agreement via the Council of Australian University Librarians. [Correction added on 05 July 2022, after first online publication: CAUL funding statement has been added.]

FUNDING INFORMATION

This study is supported by project grants from the National Health and Medical Research Council (NHMRC) of
Australia (#1140406 to Fernando Souza-Fonseca-Guimaraes). Fernando Souza-Fonseca-Guimaraes is funded by a UQ Diamantina Institute Laboratory Start-Up Package, a US Department of Defense—Breast Cancer Research Program—Breakthrough Award Level 1 (#BC200025), a grant (1158085) awarded through the Priority-driven Collaborative Cancer Research Scheme and co-funded by Cancer Australia and Cure Cancer and a ANZSA Sarcoma Research Grant (supported by Kicking Goals for Xav, Stoney's Steps and Stop Sarcoma).

**CONFLICT OF INTEREST**
The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

**DATA AVAILABILITY STATEMENT**
All data is public available in the Dryad Data Repository and datasets has been assigned a unique digital object identifier (DOI): 10.5061/dryad.zs7h44jb7. Data can be also found in this link: https://datadryad.org/stash/share/B9rMzd25Bx6vGT2aZiQZ_i6kIE3bHNt1Y1z-_CBUFES.

**REFERENCES**

1. Huntington ND, Cursons J, Rautela J. The cancer–natural killer cell immunity cycle. Nat Rev Cancer. 2020;20(8):437–54.

2. Kupz A, Scott TA, Belz GT, Andrews DM, Greyer M, Lew AM, et al. Contribution of Thy1+ NK cells to protective IFN-γ production during Salmonella Typhimurium infections. Proc Natl Acad Sci U S A. 2013;110(6):2252–7.

3. Vély F, Barlogis V, Vallentin B, Neven B, Piperoglou C, Ebbo M, et al. Evidence of innate lymphoid cell redundancy in humans. Nat Immunol. 2016;17(11):1291–9.

4. Robert C. A decade of immune-checkpoint inhibitors in cancer therapy. Nat Commun. 2020;11(1):3801.

5. Viel S, Marcais A, Guimaraes FS-F, Loftus R, Rabilloud J, Grau M, et al. TGF-beta inhibits the activation and functions of NK cells by repressing the mTOR pathway. Sci Signal. 2016;9(415):ra19.

6. Gao Y, Souza-Fonseca-Guimaraes F, Bald T, Ng SS, Young A, Ngiov SF, et al. Tumor immunoevasion by the conversion of effector NK cells into type 1 innate lymphoid cells. Nat Immunol. 2017;18(9):1004–10.

7. Delconte RB, Kolesnik TB, Dagley LF, Rautela J, Shi W, Putz EM, et al. CIS is a potent checkpoint in NK cell-mediated tumor immunity. Nat Immunol. 2016;17(7):816–24.

8. Ma A, Koka R, Burkett P. Diverse functions of IL-2, IL-15, and IL-7 in lymphoid homeostasis. Annu Rev Immunol. 2006;24:657–79.

9. Rautela J, Souza-Fonseca-Guimaraes F, Hediyeh-Zadeh S, Delconte RB, Davis MJ, Huntingdon ND. Molecular insight into targeting the NK cell immune response to cancer. Immunol Cell Biol. 2018;96(5):477–84.

10. Delconte RB, Guitard G, Goh W, Hediyeh-Zadeh S, Hennessy RJ, Rautela J, et al. NK cell priming from endogenous homeostatic signals is modulated by CIS. Front Immunol. 2020;11:75.

11. McCulloch TR, Wells T, Souza-Fonseca-Guimaraes F. Towards efficient immunotherapy for bacterial infection. Trends Microbiol. 2022;30(2):158–69.

12. Rautela J, Dagley LF, De Oliveira CC, Schuster IS, Hediyeh-Zadeh S, Delconte RB, et al. Therapeutic blockade of activin-A improves NK cell function and antitumor immunity. Sci Signal. 2019;12(596).

13. Marquardt N, Kekäläinen E, Chen P, Lourda M, Wilson JN, Scharenberg M, et al. Unique transcriptional and protein-expression signature in human lung tissue-resident NK cells. Nat Commun. 2019;10(1):1–12.

14. Park E, Patel S, Wang Q, Andhey P, Zaitsev K, Porter S, et al. Toxoplasma gondii infection drives conversion of NK cells into ILC1-like cells. Elife. 2019;8:e47605.

15. Souza-Fonseca-Guimaraes F, Cursons J, Huntington ND. The emergence of natural killer cells as a major target in cancer immunotherapy. Trends Immunol. 2019;40:142–58.

16. Hawke LG, Mitchell BZ, Ormiston ML. TGF-β and IL-15 synergize through MAPK pathways to drive the conversion of human NK cells to an innate lymphoid cell 1-like phenotype. J Immunol. 2020;204(12):3171–81.

17. Stapels DAC, Hill PWS, Westermann AJ, Fisher RA, Thurston TL, Saliba AE, et al. Salmonella persisters underpin host immune defenses during antibiotic treatment. Science (80–). 2018;362(6419):1156–60.

18. Jaslow SL, Gibbs KD, Fricke WF, Wang L, Pittman KJ, Mammel MK, et al. Salmonella activation of STAT3 signaling by SarA effector promotes intracellular replication and production of IL-10. Cell Rep. 2018;23(12):3525–36.

19. Morikawa M, Derynck R, Miyazono K. TGF-β and the TGF-β family: context-dependent roles in cell and tissue physiology. Cold Spring Harb Perspect Biol. 2016;8(5):a021873.

20. Gunderson AJ, Yamazaki T, McCarty K, Fox N, Phillips M, Alice A, et al. TGFβ suppresses CD8+ T cell expression of CXCRI3 and tumor trafficking. Nat Commun. 2020;11(1):1749.

21. Richter MV, Topham DJ. The α1β1 integrin and TNF receptor II protect airway CD8+ effector T cells from apoptosis during influenza infection. J Immunol. 2007;179(8):5054–63.

22. Ray SJ, Franki SN, Pierce RH, Dimitrova S, Koteliansky V, Sprague AG, et al. The collagen binding α1β1 integrin VLA-1 regulates CD8 T cell-mediated immune protection against heterologous influenza infection. Immunity. 2004;20(2):167–79.

23. Palmer DC, Guittard GC, Franco Z, Crompton JG, Eil RL, Patel SJ, et al. CISH actively silences TCR signaling in CD8α+ T cells to maintain tumor tolerance. J Exp Med. 2015;212(12):2971–81.

24. Mcculloch ET AL.
inflammatory arthritis and is negatively regulated by CIS.

J Exp Med. 2020;217(5):e20191421.

27. Shoger KE, Cheemalavagu N, Cao YM, Michalides BA, Chaudhri VK, Cohen JA, et al. CISH attenuates homeostatic cytokine signaling to promote lung-specific macrophage programming and function. Sci Signal. 2021;14(698):eabe5137.

28. Carow B, Gao Y, Terán G, Yang XO, Dong C, Yoshimura A, et al. CISH controls bacterial burden early after infection with Mycobacterium tuberculosis in mice. Tuberculosis. 2017;107:175–80.

29. Khor CC, Vannberg FO, Chapman SJ, Guo H, Wong SH, Walley AJ, et al. CISH and susceptibility to infectious diseases. N Engl J Med. 2010;362(22):2092–101.

30. Barber DL, Sakai S, Kudchadkar RR, Fling SP, Day TA, Vergara JA, et al. Tuberculosis following PD-1 blockade for cancer immunotherapy. Sci Transl Med. 2019;11(475):1–8.

31. Kauffman KD, Sakai S, Lora NE, Namasivayam S, Baker PJ, Kamenyeva O, et al. PD-1 blockade exacerbates Mycobacterium tuberculosis infection in rhesus macaques. Sci Immunol. 2021;6(55):eabf3861.

32. Sathe P, Delconte RB, Souza-Fonseca-Guimaraes F, Seillet C, Chopin M, Vandenberg CJ, et al. Innate immunodeficiency following genetic ablation of Mcl1 in natural killer cells. Nat Commun. 2014;5:4539.

33. Zhang P, Lee JS, Gartlan KH, Schuster IS, Comerford I, Varelias A, et al. Eomesodermin promotes the development of type 1 regulatory T (T[RI]) cells. Sci Immunol. 2017;2(10).

34. Hoiseth SK, Stocker BAD. Aromatic-dependent Salmonella typhimurium are non-virulent and effective as live vaccines. Nature. 1981;291(5812):238–9.

**SUPPORTING INFORMATION**

Additional supporting information may be found in the online version of the article at the publisher’s website.

---

**How to cite this article:** McCulloch TR, Rossi GR, Schreuder J, Belz GT, Wells TJ, Souza-Fonseca-Guimaraes F. CIS and TGF-β regulatory pathways influence immunity to bacterial infection. Immunology. 2022;167(1):54–63. [https://doi.org/10.1111/imm.13516](https://doi.org/10.1111/imm.13516)