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IMMUNE DEFICIENCY AND DYSREGULATION (CAROLINE KUO, SECTION EDITOR)

The Impact of Immunodeficiency on NK Cell Maturation and Function

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Abstract

Purpose of Review Natural killer cells are innate lymphoid cells (ILCs) that play critical roles in human host defense and are especially useful in combating viral pathogens and malignancy.

Recent Findings The NK cell deficiency (NKD) is particularly underscored in patients with a congenital immunodeficiency in which NK cell development or function is affected. The classical NK cell deficiency (cNKD) is a result of absent or a profound decrease in the number of circulating NK cells. In contrast, functional NKD (fNKD) is characterized by abnormal NK cell function but with normal number of NK cells. The combined immune deficiencies with significant impact on NK cells are not considered classical or functional NK cell deficiencies. In these disorders, the impairment of NK cells represents an important aspect of the overall immunodeficiency. In turn, this leads to improved insights on the NK cell development and function.

Summary Here, we detail the NK cell biology based upon recent natural killer cell defects described in combined immune deficiencies.

Keywords Natural killer cells · NK cell deficiency

Introduction

In humans, the importance of NK cell function and homeostasis is illuminated by the primary immunodeficiency diseases in which the NK cell is an affected population [1, 2••, 3]. This is distinct from the classification of pure natural killer cell deficiency (NKD), classical or functional [3, 4]. For the diagnosis of a primary NKD, there is an absence of secondary causes (medication use, malignancy, or infection) and other PID that can affect the NK cell have been excluded [5]. A classical NK deficiency presents with a clinical history of recurrent and severe viral infections. It is a deficiency in NK cell number, less than 1% of circulating lymphocytes, accompanied by decreased or impaired NK cell function validated at different time points [1, 5–7]. On the other hand, NK cell numbers are normal in patients with suspected functional NK cell deficiency; these patients have abnormal NK cell function [5, 8–10]. True NK cell deficiency affects NK cell development, function, or both in isolation of other major immunologic effects [5, 6].

By definition, the combined immune deficiencies with significant NK cell impact are not classical or functional NK cell deficiencies as the NK cell component represents a portion of the overall immunodeficiency [3, 5, 6]. Patients with these PIDs have susceptibility to viral infections and represent a mechanistic contribution to the field of NK cell biology. Many monogenic causes of NKD have been defined previously unknown requirements for human NK cell differentiation, maturation, and function [3, 4]. These have been well reviewed elsewhere; here, we will focus on an overview on primary natural killer cell deficiency and emerging concepts in NK cell biology based upon recent NK cell studies in patients with combined immune deficiencies.

This article is part of the Topical Collection on *Immune Deficiency and Dysregulation*

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Table 1 NK cell deficiency classification

Gene (protein)	Inheritance (mutation)	NK cell (lytic function)	NK cell (number)	CD56^{bright} (number)	CD56^{dim} (number)	Associated immune phenotype	References
Classical NK cell deficiency							
GATA2 (GATA2)	AD	↓	Normal or ↓	Absent/severe ↓	Normal or ↓	Monocytopenia, B cell and CD4 lymphopenia, neutropenia	[7, 11–14]
IRF8 (IRF8)	AR	↓	↓	Normal or ↑	↓	Abnormal DC phenotype	[1]
Functional NK cell deficiency							
FCGR3A (CD16)	AR	↓	Normal	Normal	Normal	None	[8–10]
DNA helicase complex deficiencies							
MCM4 (MCM4)	AR	↓	↓	Normal or ↑	↓	None	[15–17]
GINS1 (GINS1)	AR	↓	Absent/severe ↓	Absent/severe ↓	Absent/severe ↓	Chronic neutropenia	[18, 19]
RTEL1 (RTEL1)	AR	↓	↓	ND	ND	None	[20]
Combine immune deficiency with significant NK cell impact							
STAT1 (STAT1)	AD (GOF)	↓	↓	Normal or ↓	↓	Low T_H1 and T_H17 immunity	[2••, 4–33]
STAT5b (STAT5b)	AR	↓	Severe ↓	Normal or ↓	Severe ↓	Decreased Treg immunity	[34, unpublished]
PIK3D (p110δ)	AD (GOF)	↓	↓	Normal	↓	B cell lymphopenia (transitional B cells ↑), T cell lymphopenia	[35••]
RASGRP1 (RASGRP1)	AD	↓	↓	Normal or ↓	↓	B cell lymphopenia (transitional B cells ↑), CD4 lymphopenia	[36••]
RAG (RAG1–2)	AR	Normal	Normal or ↑	↑	↓	T and B cell lymphopenia	[37••]

AD autosomal dominant, *AR* autosomal recessive, *GOF* gain of function, *ND* not determinate

Primary NK Cell Deficiency: Classical (CNKD) and Functional (FNKD) NK Cell Deficiency

A classical NK deficiency is a deficiency in cell number, less than 1% of circulating lymphocytes, accompanied by decreased or impaired NK cell function validated at different time points [5, 6]. In these deficiencies, there is an NK cell intrinsic impairment. For example, patients with GATA2 deficiency are characterized with monocytopenia and B cell lymphopenia, and some patients have a phenotype that is restricted to the NK cell compartment with accompanying clinical hallmarks of NK cell deficiency, including severe and recurrent herpesviral infections [7, 11–14] (Table 1). Herpesviral susceptibility is the hallmark of biallelic *IRF8* deficiency which is characterized by unusual life-threatening susceptibility to EBV infection. Studies revealed lower frequency of NK cells and impaired terminal maturation of NK cells, with a stable over-representation of the $CD56^{bright}$ subset [1].

CD16 deficiency is a typical functional NK cell deficiency, characterized by a susceptibility to viral infections with normal NK cell numbers but abnormal NK cell function [8–10]. The low-affinity IgG receptor (CD16) is encoding by *FCGR3A* gene and the c.230T>A transversion described in these patients leads to L66H substitution, located in the first extracellular Ig domain of CD16. This alteration does not affect the expression of CD16 nor does it affect the binding of the FC receptor. CD16-L66H also retains the capacity for recognition of the first Ig domain by the more commonly used antibody anti-CD16 3G8 mAb. The mutation affects an epitope of the second Ig domain that is recognized by the anti-CD16 mAb B73.1 that had a previously unknown function [8–10]. Importantly, the absence of $CD16^{+}$ NK cells using the mAb B73.1 does not affect NK cell development [9]. The *FCGR3A* gene sequencing must be applied to confirm the L66H mutation, because patients without this mutation but with decreased B73.1 epitope expression have been reported [21].

An interesting new paradigm in human NK cell development has recently arisen due to a highly conserved NKD that arises from mutations in the eukaryotic DNA helicase complex. In eukaryotic, the replicative helicase complex is fully ensembled in S phase and is composed of the heterohexameric ring-shaped MCM 2–7 complex, the Cdc45 protein, and the tetrameric GINS complex [22]. MCM4 (MCM complex number 4) deficiency was described in patients from consanguineous nomadic Irish families with a history of recurrent HSV and VZV infections and a lower count of NK cells, and is associated with growth retardation and adrenal insufficiency [15–17]. Further NK cell phenotype studies demonstrated that the absence of MCM4 affects the generation of mature NK cells with a selective loss of $CD56^{dim}$ NK subset resulting in an elevated $CD56^{bright}/CD56^{dim}$ cell ratio [15]. In addition, the counts of $perforin^{+}$, $CD57^{+}$, and $CD94^{med/lo/neg}$ NK cells were decreased, demonstrating that MCM4 protein is essential for the terminal NK cell maturation. The compound heterozygous mutations in GINS1 are associated with growth retardation (intra- and extrauterine), severe viral infections, and complete loss of circulating NK cells [18]. The clinical manifestations observed in these patients include bacterial infections, severe viral infections (as CMV, VZV, HSV, rotavirus, adenovirus, and RSV), hypothyroidism, osteosarcoma, and eczema [18]. The absence of GINS1 affects both $CD56^{bright}$ and $CD56^{dim}$NK cell subsets with a loss of circulating NK cells and low proliferation and survival in response to IL-2 and IL-15 [19].

In humans, the regulator of telomere length 1 (RTEL1) deficiency is associated with Hoyeraal-Hreidarsson syndrome, a severe form of the telomerapathy dyskeratosis congenita that is characterized by bone marrow failure, intrauterine growth retardation, and developmental delay [23–25]. Immunologic analysis showed normal T and B cell counts and function with decreased NK cell counts and impaired NK cell cytotoxicity [20]. Mutations in the DNA helicase complex proteins underscore the sensitivity of human NK cells to DNA damage and their pivotal role for normal NK cell development and function.

Combined Immune Deficiency with Significant NK Cell Impact

The combined immune deficiencies are not considered CNKD or FNKD because the NK cell component represents a minor portion of the overall immunodeficiency [5]. These patients have impaired NK cell development, lower NK cell cytotoxicity, or both [5]. Although the NK cell defects in these disorders contribute substantially to the disease burden, the defect does not solely impair NK cells. For example, leukocyte adhesion disorder type 1 (LAD-1) is not commonly thought to affect NK cells as it is classically considered a neutrophil trafficking defect. However, NK cell cytotoxicity is impaired due to reduced convergence of lytic granules to the microtubular organizing center (MTOC) because CD18 is a critical component of LFA-1 needed for IS activation [26, 27].

STAT1 GOF Deficiency

In humans, signal transducer and activator of transcription 1 (STAT1) gain-of-function (GOF) disease was first described as a genetic cause for the chronic mucocutaneous candidiasis (CMC) [28]. Using whole-exome sequencing strategies, many other groups have identified heterozygous gain-of-function mutations in *STAT1* [28, 29]. These mutations are mostly located in the coiled-coil (CCD) or DNA-binding (DBD)

domains and lead to an excess of pSTAT1-driven target gene transcription and significantly higher levels of phosphorylated STAT1 (pSTAT1), resulting in an increased STAT1 response to type I and II interferons [29–31]. Typically, patients develop a broader range of infections such as staphylococcal, mycobacterial, and streptococcal infections; disseminated dimorphic fungal infections (*Coccidioides inmitis* and *Histoplasma capsulatum*); and viral infections [28, 30, 32]. Recently, an international cohort study that included 274 STAT1-GOF patients showed that 38% of these patients developed at least one viral infection by varicella zoster virus (VZV), cytomegalovirus (CMV), or Epstein-Barr virus (EBV) [28, 32]. The significant viral susceptibility observed in patients with *STAT1 GOF* mutations led us to investigate the phenotype and function of NK cells from these patients. There is a distinct NK cell phenotype owing to an impairment of NK cell terminal maturation and function [2••, 33] (Table 1). In that line, these patients had a decreased frequency of NK cells in peripheral blood. In addition, an extended analysis of NK cell phenotype showed an immature poorly functional $CD56^{dim}$ NK cell subset, which retained expression of CD94, CD117, and NKG2A molecules associated with the more immature $CD56^{bright}$ subset. Furthermore, the $CD56^{dim}$ NK cell subset from these patients had impaired expression of the NK cell components needed for the elimination of infected cells including CD16, perforin, and CD57 [2••]. The impaired expression of CD16 and perforin correlated with a decreased or absent NK cell cytolytic capacity [2••].

STAT1 GOF patients who have been treated with the Jak inhibitor, ruxolitinib, showed a reduction in their burden of CMC and hyper-responsiveness to type I and II interferons, normalization of their T_H1 and follicular T helper responses, and remission of their autoimmune-mediated cytopenias [38, 39]. In NK cells, ruxolitinib restored perforin expression in $CD56^{dim}$ NK cells and partially rescued NK cell cytotoxic function in patients with STAT1 GOF [2••] indicating that restoring STAT1 homeostasis may have a critical role in NK cell biology.

STAT5b Deficiency

Autosomal recessive STAT5b deficiency is associated with severe growth hormone (GH)-resistant growth failure and combined primary immunodeficiency [40–42]. These patients develop recurrent bacterial and viral infections, and their immunophenotype reveals lymphopenia, decreased regulatory T cells, and decreased NK cells [41, 43, 44]. The low numbers of NK cells leave these patients susceptible to recurrent viral infections. In addition, the impaired homeostasis of T regulatory cells results in autoimmunity and inflammatory disease. The germline loss of STAT5b should be distinguished from the somatic mutations in *STAT5b* associated with lymphocytic leukemia as well as the recently characterized novel variant N642H gain-of-function mutation shown to be associated with eosinophilia, urticaria, dermatitis, and diarrhea [45].

The NK cell activation through of IL-15 leads to the recruitment and phosphorylation of STAT5 proteins, as a principal downstream effector of this signaling pathway. This activation induces NK cell development and is required for the terminal maturation of fully functional NK cells [46, 47]. We studied the NK cells of patients previously reported with STAT5b homozygous deletion of a single G at the junction of exon13–intron13 [40]. There is a dramatic decrease in total NK cell numbers in peripheral blood with decreased natural and ADCC NK cell cytotoxicity (unpublished). Further phenotypic assay of NK cells from these patients identified a poorly functional $CD56^{dim}$ NK cell subset with impaired terminal maturation; these Stat5b-deficient $CD56^{dim}$ NK cells were characterized with abnormal expression of developmental markers as CD16, CD57, CD94, CD117, CD215 (IL-15Rα), and perforin which leads to the decrease in NK cell cytolytic activity. In addition, the absence of STAT5b affects the cytokine regulation in the $CD56^{bright}$ NK cell subset ([34] and unpublished). Since IL-15 signaling is critical to NK cell development and maturation, impairment of STAT5b has detrimental effects on NK cell development, maturation, and activation.

PI3K100δ Deficiency

Activated phosphoinositide 3-kinase δ syndrome (APDS) is caused by gain-of-function (GOF) mutations in the *PIK3CD* gene, which encodes the p110δ catalytic subunit of phosphoinositide 3-kinase δ (PI3Kδ) [48, 49]. PI3Kδ is expressed by leukocytes and plays a critical role in their proliferation, survival, and activation [50–52]. Mutations in PI3K110δ affect the interaction with the p85α-regulatory subunit and induce a constitutive membrane association that leads to hyperactivation of PI3K110δ signaling [48, 49, 53].

APDS is an autosomal dominant combined immunodeficiency characterized by multiple clinical manifestations which include a higher susceptibility to sinopulmonary infections, lymphadenopathy, hepatosplenomegaly, and approximately 50% of these patients developed persistent, severe, or recurrent viral infections as EBV, CMV, HSV, and VZV infections [54]. There is impairment of the lymphocyte compartment, including reduced CD4 T cell numbers with increased CD8 counts with poor function, and expansion of transitional B cells [48, 49]. PI3K110δ GOF patients also have decreased NK cell frequency with an abnormal NK cell phenotype and poor function [35••]. In NK cells, PI3K110δ plays a critical role in signaling downstream of activation and KIR receptors [55, 56]; moreover, PI3K signaling induces antibody-

dependent cellular cytotoxicity (ADCC) mediated by CD16 [57].

Recently, our group showed there is abnormal terminal NK cell maturation in the NK cells of PI3K110δ GOF patients. The $CD56^{bright}$ NK cell subset had decreased expression of CD62L and CD127, and in turn, an immature $CD56^{dim}$ NK cell subset arises with low expression of CD16 and higher expression of NKG2A (usually expressed on $CD56^{bright}$ NK cells). These poorly differentiated NK cells lead to impaired functional capacity as a result of a decrease in conjugation with target cells and impaired MTOC polarization and actin accumulation at the immunological synapse (IS) [35••]. This causes a reduction in the downstream activation of ERK proteins in the effector stage of cytotoxicity after IS engagement. Collectively, these effects lead to impaired NK cell lytic function.

RASGRP1 Deficiency

In 2016, Salzer and colleagues reported a patient with a homozygous nonsense variant in *RASGRP1* [36••]. Clinically, this patient had recurrent episodes of pneumonia that resulted in bronchiectasis as well as herpetic lesions, and severe growth failure. In addition, immunological studies revealed a progressive decrease of mature B cells and an increase in transitional B cells. There is an inverted ratio of $CD4^+$ to $CD8^+$ T cells and a persistent $CD4^+$ T lymphopenia [36••]. Ras guanyl nucleotide-releasing protein 1 (RASGRP1) is a member of the RasGRP family [58]. This guanine-nucleotide exchange factor is involved in lymphocyte development and function [59].

RASGRP1 is required for mitogen-activated protein kinase (MAPK) activation in the signaling cascade important to NK cell cytotoxicity [60]. The NK cell defect associated to *RASGRP1* mutations does not affect the frequency of NK cells or the ratio $CD56^{bright}/CD56^{dim}$ NK cells. Nor, does it impair the normal expression of activation receptors, granzyme B and perforin in RASGRP1-deficient NK cells. The defect occurs during lytic granule convergence to the MTOC and actin polymerization [36••]. Thus, the impaired NK cell cytotoxicity occurs due to defective formation of a mature NK cell immunological synapse.

RAG-Leaky SCID

The recombinase activating genes (RAG) proteins play a critical role in the V(D) J recombination process leading to the assembly of immunoglobulin, and T cell receptor (TCR) chains [61]. However, *RAG* genes are not required for NK cell development [62]. In humans, mutations in RAG proteins are associated with distinct clinical and immunological phenotypes [63, 64], including severe combined immunodeficiency characterized by loss of T and B cells but with normal numbers of NK cells ($T^-B^-NK^+$ SCID) [65], Omenn syndrome ($T^+B^-NK^+$ OS) [66], atypical SCID (AS) [67–69], and combined immunodeficiency with granuloma and/or autoimmunity (CID-G/A) [70–73]. However, the severity correlates with the residual recombination activity of the mutant-RAG proteins [74], affecting the T and B repertoires [62], whereas the NK cell development is unaffected.

A recent study with a large cohort of patients with $T^-B^-NK^+$ SCID, OS, AS, and CID, showed that mutations in RAG affect the terminal NK cell maturation [37••]. The immune phenotype classically showed T and B lymphopenia, and the patients presented with severe viral infections such as HPV, VZV, CMV, EBV, adenovirus, and rhinovirus. It is important to note that the circulating NK cell counts were normal or higher in these patients compared to healthy donors [37••]. However, NK cell phenotype analysis showed that patients with SCID due to RAG or NHEJ defects had an abnormal distribution of NK cell subsets. There was an accumulation of the $CD56^{bright}CD16^{neg}$ subset and an intermediate bright/dim stage, $CD56^{bright}CD16^{int}$, both of which affected the mature transition to $CD56^{dim}CD16^+$ NK cells [37••]. In addition, the $CD56^{bright}$ NK cell subset displayed an abnormal expression of developmental markers and NK inhibitory receptors [37••]. Interestingly, the more severe *RAG* mutations correlated with a more profound NK cell defect.

Treatment for NK Cell Deficiencies

For patients with NKD, the therapeutic focus has been on the susceptibility to herpes viral infections, so prophylactic antiviral medication is the most commonly used intervention. With the rapidly growing use of new-targeted biologic therapies, advances in treating NK cell deficiencies will emerge. In the case of GATA2 deficiency, cytokine treatment with IFN-α showed a partial induction of NK cell cytotoxic capacity and resolution of some clinical wart burden to enhance activation of antiviral defense [7]. Hematopoietic stem cell transplantation (HSCT) has been employed to treat patients with GATA2 deficiency. [75, 76] For combined immunodeficiencies, understanding the underlying mechanism resulting in impaired NK cells will drive the decisions for therapeutic intervention and possible reversal of disease manifestations. Patients with APDS have improvement in NK cell function with mTOR inhibition using rapamycin [35••]. Our recent study demonstrated that patients with *STAT1 GOF* mutations have restored perforin expression in $CD56^{dim}$ NK cells and partial rescue of NK cell cytotoxic function with a Jak inhibitor [2••]. With the exception of children with severe combined immunodeficiency, HSCT is reserved for patients who have severe disease progression such that they fail immunomodulation or do not

have precise targeted therapies to treat the underlying mechanism. New gene therapy and gene editing technology will give patients without an option for HSCT opportunity for a cure. Great strides are now being made in pre-clinical studies that have promise to evolve into clinical trials [77, 78].

Conclusion

A large number of primary immunodeficiency and primary immune regulation diseases include a NK cell defect. As we continue the rapid pace of novel gene discovery and uncouple the mechanism of known diseases, we are at an era where we can make great strides in the mechanistic contribution to the field of NK cell biology.

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Compliance with Ethical Standards

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