

# Natural killer cell deficiency

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**List of Design Committee Members:** Jordan S. Orange, MD, PhD  
**Activity Objectives**

1. To report the classification system of natural killer (NK) cell disorders.
2. To discuss the roles of the NK cell in innate immunity.
3. To summarize the clinical characteristics of NK cell deficiency.
4. To describe newly discovered genetic mutations associated with NK cell disorders.

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Natural killer (NK) cells are part of the innate immune defense against infection and cancer and are especially useful in combating certain viral pathogens. The utility of NK cells in human health has been underscored by a growing number of persons who are deficient in NK cells and/or their functions. This can be in the context of a broader genetically defined congenital immunodeficiency, of which there are more than 40 presently known to impair NK cells. However, the abnormality of NK cells in certain cases represents the majority immunologic defect. In aggregate, these conditions are termed NK cell deficiency. Recent advances have added clarity to this diagnosis and identified defects in 3 genes that can cause NK cell deficiency, as well as some of the underlying biology. Appropriate consideration of these diagnoses and patients raises

the potential for rational therapeutic options and further innovation. (*J Allergy Clin Immunol* 2013;132:515-25.)

**Key words:** *Natural killer cells, innate immunity, natural killer cell deficiency, primary immunodeficiency, cytotoxicity*

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Natural killer (NK) cells are lymphocytes of the innate immune system that are best known for their ability to mediate cytotoxicity and produce cytokines after the ligation of germline-encoded activation receptors.<sup>1</sup> As a result, they have long been considered part of the innate immune system but do have some newly appreciated adaptive roles as well.<sup>2</sup> NK cells are best known for innate defense against viral infections and in tumor cell surveillance but are also increasingly recognized for participating in immunoregulation, coordination of immunity, and modulation of autoreactivity. NK cells are lymphocytes and major members of the innate lymphoid cell family,<sup>3</sup> which develop from CD34<sup>+</sup> hematopoietic cells in the bone marrow and undergo terminal maturation in secondary lymphoid tissues.<sup>4</sup> In humans NK cells are classically identified by the absence of the T-cell receptor complex and the presence of neural cell adhesion molecule (denoted CD56 according to the cluster designation system). The majority of peripheral blood NK cells express low levels of CD56 as well as an IgG Fc receptor FcγRIIIA (CD16).<sup>4</sup> These are considered

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*Abbreviations used*

ADCC:	Antibody-dependent cell-mediated cytotoxicity
CNKD:	Classical natural killer cell deficiency
CTL:	Cytotoxic T lymphocyte
CMV:	Cytomegalovirus
DOCK8:	Dedicator of cytokinesis 8
FNKD:	Functional natural killer cell deficiency
HPV:	Human papillomavirus
HSV:	Herpes simplex virus
MCM:	Minichromosome maintenance
NK:	Natural killer
NKD:	Natural killer cell deficiency
PID:	Primary immunodeficiency
VZV:	Varicella zoster virus

mature and are referred to as CD56<sup>dim</sup> NK cells (which means that the fluorescent intensity for CD56 is slightly increased compared with that seen in cells negative for CD56, Fig 1). A minority of peripheral blood NK cells express high levels of CD56 without expressing CD16 and are considered to comprise a developmentally immature but functionally enabled NK cell subset (otherwise known as CD56<sup>bright</sup>, Fig 1).<sup>5,6</sup>

NK cells characteristically express a wide range of receptors, some of which are rather specific in their expression. These include receptors capable of inducing either activation or inhibitory signals. NK cell activities are accessed after a favorable balance of activation over inhibitory signals is achieved in their recognition of a target.<sup>7</sup>

Regarding their role in infectious diseases, NK cells specialize in defense against certain T-cell elusive pathogens, most notably viruses.<sup>8</sup> Many viruses have evolved strategies to evade the cytotoxic T lymphocyte (CTL) response by specifically downregulating class I MHC in the infected cell.<sup>9</sup> Although this allows the virus to prevent its host cell from presenting viral protein-derived peptides to virus-specific CTLs, it also makes the infected cell more susceptible to NK cell defenses. Although NK cells are triggered by a large number of activation receptors, they are restrained by an extensive family of inhibitory receptors that can be ligated by class I MHC.<sup>10</sup> The most well known in humans is that of the killer cell immunoglobulin-like receptor family. As an example of the effect of NK cells, some viruses have further evolved specific NK cell evasion mechanisms to interfere with the killer cell immunoglobulin-like receptor system, including virus-encoded class I decoy molecules.<sup>11</sup> The viruses that seem to be best targeted by NK cell-mediated defenses are those of the herpesvirus family, which are notorious for downmodulating class I MHC.

Viruses can make infected host cells more susceptible to NK cell activities in ways other than simply decreasing NK cell inhibition. NK cells have activation receptors that can directly recognize particular viral antigens, such as certain natural cytotoxicity receptors, which can bind viral hemagglutinin.<sup>12</sup> Some viruses are capable of inducing the expression of specific host cell stress molecules that can serve as ligands for NK cell activation receptors, thus representing an important paradigm by which NK cells combat disease. In this light malignant cell transformation can also induce these cell stress-associated ligands, which, when compounded by the fact that many cancer cells lose class I MHC expression in evading tumor-specific CTL responses, emphasizes the role of NK cells in tumor surveillance.<sup>13</sup>

After activation, NK cells are capable of 3 main functions to participate in immune defense. The first and best characterized is the ability to mediate contact-dependent killing of target cells. This involves the mobilization of highly specialized organelles in NK cells known as lytic granules that contain the pore-forming molecule perforin and death-inducing enzymes, such as granzymes.<sup>14</sup> Once a killing program is triggered in an NK cell, the lytic granules are transported to the interface formed with the targeted cell, and their contents are secreted onto it. This function of cytotoxicity can be accessed by NK cell activation receptors as an innate immune defense or by recognition of IgG-opsonized cells through CD16 to enable antibody-dependent cell-mediated cytotoxicity (ADCC). Through ADCC, NK cells have an intimate interface with adaptive immunity and also enable the functions of certain therapeutic mAbs.<sup>15</sup>

The second function of NK cells is the production of soluble factors to promote direct antitumor effects, as well as to further induce or regulate immunity. These include a wide variety of cytokines, chemokines, and other regulators. NK cells are probably best appreciated within this category for their ability to produce IFN- $\gamma$  that has both antiviral and immune-enhancing capabilities.

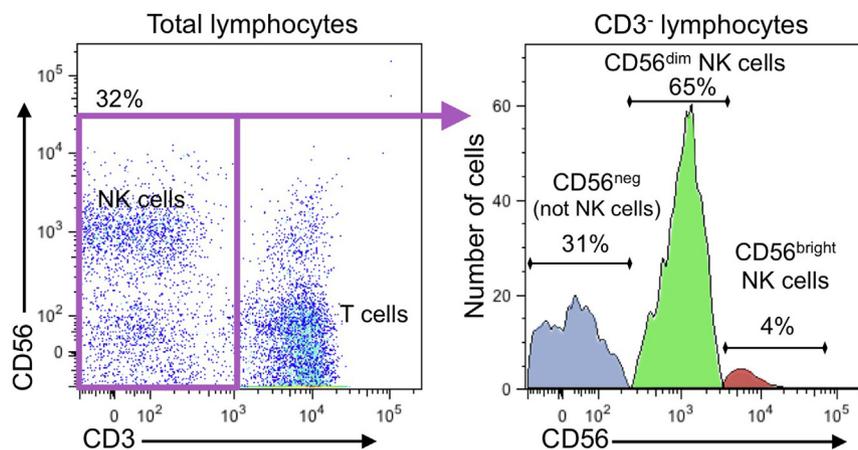
The third but less appreciated function of NK cells is that of promoting and regulating immunity through contact-dependent costimulatory and regulatory mechanisms. NK cells express or can be induced to express a large number of relevant costimulatory and regulatory ligands and can localize to key immunoregulatory sites, including secondary lymphoid tissues in which these contact-dependent contributions to immune responses can be affected.<sup>1</sup>

Although NK cells and their diverse functions serve important roles in numerous animal models of disease, they are also associated with human clinical conditions. However, perhaps the clearest demonstration of the value of NK cells to humans derives from their deficiency. Natural killer cell deficiency (NKD) represents a small but increasingly appreciated subset of primary immunodeficiencies (PIDs) that present challenges both in diagnosis and clinical management.<sup>16</sup> NKD also provides great insight into the value of and mechanisms underlying human NK cell functions.<sup>17-19</sup> As an overarching theme, patients with NKD have susceptibility to herpesviruses, as well as select other viral pathogens. There are also distinct genetically defined PIDs that include an effect on NK cells and their functions.<sup>17-20</sup> Many of these diseases also include susceptibility to viral infection and are informative from a mechanistic standpoint because they delineate specific molecular requirements of human NK cells.

This review will provide an overview of the substantive advances made in the understanding of NKD. It will also recap PIDs affecting NK cells to build on previous reviews of this topic.

## NKD DEFINITION

To be considered an NKD, the impact upon NK cells need represent the major immunologic abnormality in the patient. Although many diseases, drugs, infections, and physiologic states can affect NK cell numbers, function, or both, the NKD diagnosis is reserved for abnormalities that are fixed over time and not secondary in nature. Specifically, NKD should be inherent and hardwired. In several cases a genetic mechanism responsible for NKD has been identified. It is also anticipated that the majority of true NKD will be monogenic given the overall rarity and effect of lacking NK cells, and/or their functions.



**FIG 1.** Flow cytometry depicting peripheral blood NK cell subsets according to CD56. PBMCs from a healthy donor were evaluated by using fluorescence-activated cell sorting, and gated lymphocytes were analyzed for expression of CD3 and CD56 (left). NK cells are CD56<sup>+</sup>/CD3<sup>-</sup>. To illustrate the range of CD56 expression, all CD3<sup>-</sup> cells (non-T cells) were gated (purple box, left) and displayed as a histogram (right). Here there are 3 clear populations: CD56<sup>neg</sup> (blue peak), which are largely not NK cells; CD56<sup>dim</sup> NK cells (green peak); and CD56<sup>bright</sup> NK cells (red peak). In this example the CD56<sup>bright</sup> NK cells represent 4% of the CD3<sup>-</sup> cells or 5.8% of the total NK cells (CD56<sup>dim</sup> plus CD56<sup>bright</sup> cells). Experimental credit is given to Dr Emily Mace, Baylor College of Medicine.

NKDs can be divided into 2 major types depending on whether NK cells are present in the peripheral blood.<sup>19</sup> Classical natural killer cell deficiency (CNKD) is defined as an absence of NK cells and their function among peripheral blood lymphocytes. Functional natural killer cell deficiency (FNKD) is defined as the presence of NK cells within peripheral blood lymphocytes having defective NK cell activity. In other words, in patients with CNKD, NK cells are absent, and in patients with FNKD, NK cells are present but do not work. It should be re-emphasized that in both patients with CNKD and those with FNKD, the NK cell abnormality is the major immunologic deficit resulting in inadequate host defense. CNKD and FNKD are further subdivided based on the particular gene that is responsible for the phenotype, if identified (Table I). Although both diagnoses are presently considered quite rare, a definitive estimate of prevalence is currently unavailable.

To be more specific, there are several important features in considering the diagnosis of CNKD or FNKD (Table II): (1) that the defect is stable over time; (2) that secondary causes of NK cell abnormalities are excluded as a cause (eg, related to medication use, malignancy, or infection); (3) that other known PID syndromes that can affect NK cell numbers and function are considered and at least rationally excluded; (4) that, for the purposes of an NKD diagnosis, NK cells should be considered as those lymphocytes that are CD3<sup>-</sup>CD56<sup>+</sup>; (5) that to be considered CNKD, NK cells must be present at 1% or less of peripheral blood lymphocytes; and (6) that functional evaluation of NK cells is considered by using reliable and validated assays on at least 3 occasions separated by 1 month each (the <sup>51</sup>Cr release cytotoxicity assay with K562 target cells is recommended, although normative ranges differ among clinical laboratories). An algorithm outlining an approach to a patient with suspected NKD is presented in Fig 2.

NKD should be clearly distinguished from any human abnormalities of NKT cells. NKT cells are a subset of T cells that express certain NK cell-surface determinants.<sup>21</sup> They are not NK cells and are thus not part of a consideration of NKD. The presence or absence of NKT cells has been part of prior classifications of NKD (in what was previously referred to as absolute

NKD),<sup>17,18</sup> but enough progress has been made regarding the biological and developmental understanding of NKT cells that they can be uncoupled from any present consideration of NKD. Thus the consideration of NKD should be specifically to NK cells and according to either the CNKD or FNKD subtype.<sup>19</sup> In this light, as further understanding of historical published patients and cohorts improves, the classification of certain cases is also subject to change.

### CNKD

CNKD is characterized by the absence of both NK cells and their functions among peripheral blood lymphocytes. The single most well known case is that published in 1989 in the *New England Journal of Medicine* of an adolescent girl with multiple severe or disseminated herpesvirus infections, including varicella pneumonia, disseminated cytomegalovirus (CMV), and herpes simplex virus (HSV).<sup>22</sup> She was stably deficient in NK cell cytotoxic activity, as measured by using K562 killing assays, and lacked “classical” CD56<sup>+</sup>/CD3<sup>-</sup> NK cells among PBMCs, as determined by means of flow cytometry. This original case has served as the “typical” example of an NKD and led to continued interest in pursuit of additional patients and answers.

Since this initial clear description of CNKD, there have been at least 18 additional patients described phenotypically, representing a total of 12 unrelated families.<sup>22-31</sup> Of this group, 42% (8/19) died prematurely. Fifty-three percent (10/19) have been described as experiencing severe consequences of herpesvirus infections, with cases present in 67% of the families represented. Of these, severe varicella zoster virus (VZV) was most common, occurring in 27% of patients, but CMV, EBV, and HSV were all represented. Unusual consequences of human papillomavirus (HPV) infection was identified in 16%, and fungal infections were identified in 10%. A number of patients (21%) experienced malignancies, including an EBV-driven smooth muscle tumor, HPV-related cancers, and leukemia. Two patients have been successfully treated with hematopoietic stem cell transplantation,<sup>29,32</sup> whereas

TABLE I. NKD classification

NKD type	Peripheral blood NK cells (CD3 <sup>-</sup> CD56 <sup>+</sup> )	CD56 <sup>dim</sup> NK cell subset	CD56 <sup>bright</sup> NK cell subset	NK cell function	Infectious susceptibility	Gene defect	Inheritance
CNKD	Absent or very low	?	?	Absent	Herpesviruses	?	?
Subtype 1 (CNKD1)	Absent or very low	Absent or very low	Absent	Absent	VZV, HSV, CMV, HPV, mycobacteria	<i>GATA2</i>	AD
Subtype 2 (CNKD2)	Absent or very low	Absent or very low	Preserved	Absent		<i>MCM4</i>	AR
FNKD	Present	Should be present	Should be present	Absent or severely decreased	Herpesviruses, HPV	?	?
Subtype 1 (FNKD1)	Present	Present	Present	Decreased	HSV, EBV, HPV	<i>FCGR3A</i>	AR

AD, Autosomal dominant; AR, autosomal recessive.

TABLE II. Features of NKD\*

NK cell abnormalities represent the major immunologic abnormality.†  
Defect is stable over time.‡  
Secondary causes of NK cell abnormalities are excluded.§  
Exclude broader PIDs known to include an NK cell defect.||  
NK cells are evaluated as CD3<sup>-</sup>/CD56<sup>+</sup> cells.¶  
In patients with CNKD, NK cells are ≤1% of peripheral blood lymphocytes.#  
Abnormal functional evaluations are repeatable.\*\*

\*NKD overall characteristics include both CNKD and FNKD, except where specified.

†Some gene mutations will affect other immune cells, but to be considered an NKD, the major effect on the patient should be derivative from the NK cell deficit.

‡It is of the essence that the NK cell defect be consistent in the absence of a genetic abnormality known to cause NKD.

§Considering medications, malignancy, HIV infection, and severe physiologic or emotional stress.

||See Table III.

¶Most clinical laboratories use a reagent that identifies NK cells as CD56-PE<sup>+</sup> or CD16-PE<sup>+</sup>. This is adequate for initial assessments.

#The decreased population of NK cells should be stably decreased and not simply represent a single low value.

\*\*Repetition of assays should be performed by using a reliable validated assay (<sup>51</sup>Cr-release assay against K562 target cells is recommended for screening) on 3 occasions separated by a minimum of 1 month each applying laboratory-specific normative ranges.

I died during the process.<sup>17,22</sup> Other causes of death included EBV (n = 2), CMV (n = 1), VZV (n = 1), cancer (n = 2), and mycobacterial infection (n = 1).

Further scientific advances have enabled the identification of 2 genetic mechanisms underlying CNKD. Thus it is appropriate to refer to the CNKD subtypes according to genetic mechanisms. The 2 presently identified genetic causes of CNKD can be labeled CNKD1 and CNKD2. Additional numeric designations (eg, CNKD3 and CNKD4) should be reserved for subsequent independent genetic mechanisms. CNKD without an identified genetic mechanism should be referred to as CNKD (Table I). Each of the 2 known genetic causes of CNKD is considered more specifically below.

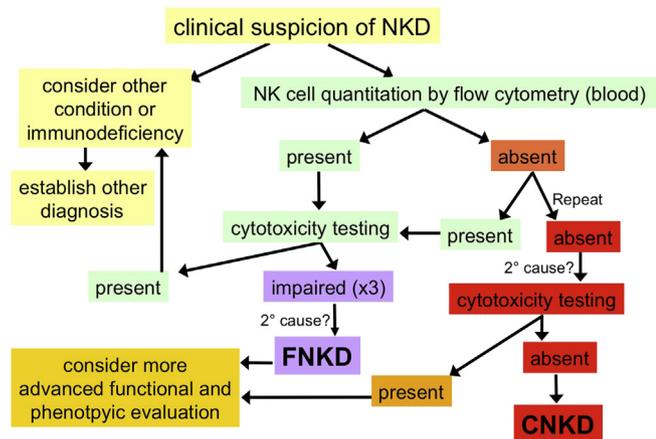
### CNKD1

Because the molecular mechanism of the 1989 CNKD case has been identified, arguably representing the original description of a CNKD,<sup>22</sup> it is given the CNKD1 designation. CNKD1 is caused by *GATA2* haploinsufficiency.<sup>33</sup> Although *GATA2* mutations can lead to a wide variety of clinical and immunologic phenotypes, there is a subset of patients who present with hallmarks of NKD, including the patient reported in 1989.<sup>33</sup> *GATA2* is a ubiquitously expressed hematopoietic transcription factor that

promotes numerous genes of relevance and allows for survival and maintenance of hematopoietic cell subsets. A substantial number of *GATA2*-deficient patients present with infectious phenotypes characteristic of NKD, including 78% with HPV and 33% with severe or atypical manifestations of herpesviruses.<sup>34</sup> The latter includes disseminated VZV, CMV, and HSV. In several cases these infections have been ascribed as a cause of death, most notably HPV-derived anogenital cancers.

As mentioned above, the original patient with CNKD1 died from complications of a hematopoietic stem cell transplantation that was performed to treat aplastic anemia. As is now appreciated, aplastic anemia can be a late complication of having a *GATA2* mutation. In this light, a *GATA2* mutation causes a variable clinical syndrome that is viewed by some as a progressive immunologic exacerbation that evolves over decades and can include deficiency of monocytes and dendritic cells.<sup>35,36</sup> Six patients with *GATA2* mutations have received hematopoietic stem cell transplantation, with 5 successes, but it is unclear whether these were NK cell–predominant cases.<sup>37</sup> What is also presently unclear in patients with *GATA2* mutations is whether the NKD occurs first, is a hardwired component of the mutation, or is just more pronounced in some patients. In this light, it is interesting that in a more comprehensive recent survey of human *GATA2* mutation, HPV infection was the main infectious phenotype in the first decade of life.<sup>38</sup> This suggests that the abnormal NK cell defenses might represent an early and even inherent aspect of this genetic disease.

Recently, a specific analysis of NK cells in patients with *GATA2* mutations presenting with phenotypes suggestive of NKD has been performed.<sup>33</sup> Half of these had immunologic phenotypes consistent with CNKD, with 1% or less NK cells among peripheral blood lymphocytes. Although some of these patients had NK cells in their peripheral blood, in all cases NK cell cytotoxicity was defective, even when abundant NK cells were present. Thus some patients with *GATA2* mutations appear to be more characteristic of an FNKD. That said, it is presently unclear whether these patients might eventually progress toward a total loss of NK cells. The experimental NK cell studies performed in CNKD1 have provided some additional insight and guidance. Even when NK cells were present, the developmentally immature minority CD56<sup>bright</sup> NK cell subset was uniformly absent. This could be recapitulated experimentally when differentiating NK cells *in vitro* from patients' CD34<sup>+</sup> hematopoietic stem cells.<sup>33</sup> In healthy donor NK cells, the highest expression of *GATA2* is found in the CD56<sup>bright</sup> subset,<sup>33</sup> suggesting that the absence of this important intermediate in patients represents an inherent abnormality. In aggregate, these findings suggest a specific and important role for *GATA2* in either a key phase of NK cell



**FIG 2.** Diagnostic algorithm for NKD. An algorithmic approach to a patient suspected of having NKD is presented. Initial steps include considering alternative diagnoses because they are statistically more likely, as well as quantifying NK cells and their function in peripheral blood. Abnormal results should be repeated with a time interval of approximately 1 month. Absent NK cells are defined as 1% or less of peripheral blood lymphocytes. Cytotoxicity testing for screening is recommended by using the <sup>51</sup>Cr-release assay with K562 target cells; normative ranges differ between laboratories, and laboratory-specific ranges should be considered. Secondary (2°) causes should be considered as explanations for abnormalities and thus excluding an inherent NKD. More advanced functional and phenotypic testing is presently in the domain of research-level interventions.

development or in NK cell survival. Further experimental work will hopefully gain insight from these patients and their particular mutations to better understand the role of GATA2 in NK cell biology.

Until further clarity can be gained surrounding the natural history and biology of GATA2 patients with reference to NK cells, the subset with an NK cell-specific presentation should be considered to have CNKD1, given the original patient described. However, clinicians should be aware of the potential for the with NK cell-specific GATA2 patients to present as having FNKD.

### CNKD2

A familial form of CNKD was defined in 2006 and has been listed as “natural killer cell deficiency, familial isolated” in the Online Mendelian Inheritance in Man database as entry number 609981.<sup>26</sup> The original report described a large consanguineous Irish cohort, one of whom had recurrent EBV-driven lymphoproliferative disease despite having evidence of intact adaptive immunity against EBV. Two other family members had recurrent viral infections. All 3 had 1% or fewer NK cells in peripheral blood. All also had intrauterine growth retardation, and some had adrenal insufficiency. The family was evaluated genetically by using microsatellite homozygosity mapping, and the locus was linked to chromosome 8 (8p11.23-q11.21). Each of the genes in this region was sequenced, and the minichromosome maintenance 4 (*MCM4*) gene was identified as appropriately segregating with the clinical phenotype in an autosomal recessive pattern.<sup>39</sup> Two additional Irish families with similar phenotypes were also identified as having the same mutations in *MCM4* and thus presumably deriving from a common founder effect.<sup>39,40</sup> One of the scientific groups sharing in this discovery approached the topic

because of the endocrinologic manifestations but arrived at the same molecular, immunologic, and mechanistic conclusion.<sup>40</sup>

The *MCM4* gene encodes MCM complex member 4. It is a member of the MCM2 to MCM7 protein complex that enables helicase function during DNA replication. The MCM complex is recruited to DNA origin of recognition sites to direct DNA unwinding and ultimately polymerization.<sup>41</sup> *MCM4* is widely expressed, and its function is considered essential for most cells. Complete *MCM4* deficiency is embryonically lethal in mice.<sup>42</sup>

Thus an obvious question is why specific mutations in *MCM4* result in CNKD2. The answer is not presently clear, but some evidence has been experimentally defined in patients’ cells and other *in vitro* systems. First, fibroblasts and lymphocytes from patients with *MCM4* mutations appropriately assemble an MCM complex but demonstrate excessive DNA breaks after aphidicolin<sup>39</sup> or diepoxybutane<sup>40</sup> stress, respectively. The fibroblast abnormality was shown to be complemented by reintroducing wild-type *MCM4* expression *in vitro*.<sup>39</sup> Thus it must follow that certain human cell types rely more intensely on MCM function or some particular aspect of the mutated region of MCM4. Presumably these cells would include either NK cells or cells that support NK cell development, as well as certain key cells of the endocrine system.

As a second experimental clue, although patients were found to have substantively reduced numbers of NK cells, the depletion was reflected entirely within the CD56<sup>dim</sup> NK cell subset. This population contains the mature perforin-containing NK cells, and interestingly, the entirety of the CD56<sup>bright</sup> NK cell subset was preserved and potentially even increased.<sup>39</sup> This finding generates 2 hypotheses to explain the effect of *MCM4* mutation on NK cells in patients with CNKD2. The first is derivative from the fact that the CD56<sup>bright</sup> NK cell subset contains the immature population that can serve as a developmental intermediate to CD56<sup>dim</sup> NK cells.<sup>5</sup> Thus *MCM4* mutation might be interrupting NK cell development at the CD56<sup>bright</sup> stage. The second hypothesis is that patients’ CD56<sup>dim</sup> NK cells are generated but have decreased survival in the face of the *MCM4* mutation and thus are short-lived in patients with CNKD2. In this light, decreased NK cell survival was documented in both NK cell subsets.<sup>39</sup> At present, either hypothesis is viable. Further work will likely determine which is correct and discern the surprising role of either the MCM complex, or *MCM4* itself, in NK cells.

Diagnostically, CNKD2 can be suspected in the context of increased percentages of CD56<sup>bright</sup> NK cells (of total NK cells) in patients with endocrine abnormalities, growth abnormalities, or both. These patients also have decreased NK cell cytotoxic function,<sup>26,39</sup> but it is presently unclear whether this is a feature of (1) extremely low total NK cell numbers, (2) decreased presence of mature CD56<sup>dim</sup> perforin-containing NK cells, or (3) an inherent inability of *MCM4*-mutated NK cells to kill.

### Future of CNKD

It is expected that other genetic mechanisms underlying CNKD will be discovered in the near future. A CNKD2 “phenocopy” was described in a nonconsanguineous French cohort, and these patients had growth retardation and facial abnormalities.<sup>25</sup> One died of disseminated CMV infection. The patient studied had a very small peripheral blood NK cell population that contained a preponderance of immature NK cells (defined in this report as CD56<sup>+</sup>/CD16<sup>-</sup> cells). Interestingly, separate studies

of cultured patients' T cells showed impaired IL-2- and IL-15-dependent survival.<sup>43</sup> This is relevant because IL-15 in particular is a clear requirement for NK cell development and homeostasis.<sup>4</sup> A separate family described more than 30 years ago also has a similar NK cell phenotype. These patients did not have growth retardation or endocrinologic abnormalities. There were 3 affected family members, all of whom experienced severe EBV infection.<sup>44</sup> One case was immediately fatal, whereas a second (female) patient died later of progressive pulmonary decline. A third family member survived and has persistent NK cell abnormalities with near-absent cytotoxicity and a preponderance of immature CD56<sup>bright</sup> NK cells (J. S. Orange, unpublished data).<sup>44</sup> A separate, recently reported spontaneous patient with pediatric melanoma and opportunistic fungal infection was also found to have an abnormal transition from CD56<sup>bright</sup> to CD56<sup>dim</sup> NK cells.<sup>45</sup> It is presently unclear as to whether the molecular pathways affected in these cohorts will functionally overlap with that of CNKD2. That said, neither of the 2 family cohorts have been defined to have *MCM4* mutations (despite this having been evaluated in one of the cases, unpublished results), and thus it is likely that the CNKD category will encompass additional genetic mechanisms that affect the CD56<sup>bright</sup> to CD56<sup>dim</sup> NK cell transition.

Additional detailed study of other patients suggests that, as in patients with CNKD1, the CD56<sup>bright</sup> to CD56<sup>dim</sup> transition will not be the only step in NK cell differentiation or homeostasis that is targeted by disease-causing mutations. An example is a recently described girl with an EBV-driven smooth muscle tumor. This patient had absent NK cell cytotoxic activity and less than 1% of NK cells in peripheral blood but a normal ratio of CD56<sup>bright</sup> and CD56<sup>dim</sup> NK cells within the few that were identified.<sup>31</sup> However, her NK cells had some abnormal developmental signatures because there were no CD57-expressing NK cells (a marker of terminal NK cell differentiation) and an increase in CD117<sup>+</sup> NK cell counts (a hallmark of immaturity). This patient did not have *MCM4* or *GATA2* mutations (J. S. Orange, unpublished data). Thus it is probable that CNKD will comprise an array of genetic abnormalities that have the potential to selectively affect distinct steps in NK cell differentiation or NK cell subset survival. Further delineation of these will likely affect our understanding of not only this growing group of patients but also NK cell biology overall.

## FNKD

FNKD describes patients with normal numbers of NK cells present in peripheral blood but ones that are functionally disabled in the face of otherwise effective immunity.<sup>46-51</sup> An example of a well-known PID that results in absent NK cell function (cytotoxicity) would be perforin deficiency.<sup>52</sup> However, perforin deficiency is not considered an FNKD because it also abrogates the lytic function of CTLs. Thus the FNKD label is reserved for an effect on NK cells in relative isolation. It is anticipated that the FNKD category will be extensive, but this has been more difficult to ascertain because the screening assay is one of cellular function. A recent study evaluating patients with severe and recurrent herpesvirus infections identified 5 such patients with functional abnormalities,<sup>47</sup> which was reflective of an historical study of similarly affected patients.<sup>48</sup> The modern immunologic resolution applied in the current study suggested that specific phenotypic and functional aberrations might be present in each of the patients with functional deficiency, but further analysis is needed.

Although some of the patients with CNKD can present with NK cells in the peripheral blood, this might be akin to the "leaky" severe combined immunodeficiency phenomenon and requires further study of the natural history of those particular patients/mutations. It is also possible that some patients having CNKD that interferes with the CD56<sup>bright</sup> to CD56<sup>dim</sup> transition will present with peripheral blood total NK cell numbers within the normal range. Because that would be a feature of increased immature NK cell counts with a paucity of mature cells, it is recommended that those patients (having abnormalities of NK cell development) be considered in the CNKD category.

However, the overarching theme in patients with FNKD is one of herpesvirus susceptibility, with the most common being HSV1.<sup>47,48,50</sup> However, abnormal susceptibility to or consequences of EBV, VZV, HPV, and respiratory viruses have all been described in patients with FNKD.<sup>46,49,51,53</sup> That said, there is likely some degree of selection bias in that in the majority only patients with abnormal susceptibility to herpesviruses have been studied. Because there is presently 1 known genetic defect underlying FNKD, the same nomenclature as for CNKD should be used (Table I), with the first being designated FNKD1 and subsequent numbers reserved for additional genetic discoveries (eg, FNKD2 and FNKD3).

## FNKD1

The single known gene defect that causes FNKD is that of a particular mutation of the *FCGR3A* gene encoding CD16.<sup>49-51</sup> As introduced above, CD16 is the NK cell IgG Fc receptor and is best known for enabling ADCC. Thus far, FNKD1 has been described in 3 unrelated families, the first 2 almost 20 years ago. One had severe recurrent HSV stomatitis and recurrent herpetic whitlow.<sup>50</sup> A second had progressive EBV infection and severe VZV infection requiring systemic therapy.<sup>49</sup> Both had recurrent viral respiratory tract infections. A third patient was recently described and had EBV-driven monocentric Castleman disease and recalcitrant cutaneous warts.<sup>51</sup> All patients had decreased spontaneous NK cell cytotoxicity against K562 target cells, but surprisingly, none had abnormal ADCC.

The mutation underlying FNKD1 is recessive, rare, and predicts homozygous substitution of leucine with a histidine at the 66th amino acid of CD16 (L66H).<sup>49-51</sup> This alteration is in the distal immunoglobulin domain in the extracellular portion of CD16, which is not required for IgG binding (a function of the proximal immunoglobulin domain).<sup>54</sup> The distal immunoglobulin domain has been recently shown to function in linking CD16 to the NK cell costimulatory receptor CD2.<sup>51</sup> Thus the patient mutation does not affect ADCC but does impair CD16 from being used as a costimulatory receptor when CD2 is ligated in the context of spontaneous NK cell cytotoxicity. The L66H mutation does not abrogate surface expression of CD16 but destroys an epitope present in the distal immunoglobulin domain recognized by the anti-CD16 mAb B73.1.<sup>49-51</sup> However, the mutant CD16 is still recognized by the more commonly used anti-CD16 3G8 mAb. Thus these 2 mAbs can be used in screening for patients with this mutation by using flow cytometry because those affected will have NK cells that are recognized by 3G8 but not B73.1. However, *FCGR3A* gene sequencing must be applied to confirm this because patients with decreased NK cell expression of the B73.1 epitope without *FCGR3A* mutations have been identified.<sup>55</sup>

**TABLE III.** PID diseases with an NK cell abnormality

Disease	Gene(s)*	NK cell defect	Mechanism†	Infectious susceptibility	Reference‡
Diseases impairing NK cell development or survival					
X-linked SCID	<i>IL2RG</i>	Absent NK cells	IL-15 receptor signaling	Multiple infections	61
Autosomal recessive SCID	<i>JAK3</i>	Absent or low NK cell numbers	IL-15 receptor signaling	Multiple infections	62
	<i>IKZF1</i>		Developmental transcriptional programs		63
	<i>ADA</i>	Metabolic requirements	64		
	<i>MTHFD1</i>	Metabolic requirements	64		
IPEX-like syndrome with growth hormone deficiency	<i>STAT5B</i>	Absent or low NK cells	IL-15 receptor signaling	Multiple infections	65
Bloom syndrome	<i>BLM</i>	Cytotoxicity	Unclear	Fungi, bacteria	66
Fanconi anemia	<i>FANCA-G</i>	Low NK cells	Bone marrow impairment	Multiple infections	67
Dyskeratosis congenita	<i>DKC1</i>	Low NK cells	Bone marrow impairment	Multiple infections	68
Diseases impairing the mechanics of cytotoxicity					
FHL2	<i>PRF1</i>	Cytotoxicity	Absent perforin	Herpesviruses	52
FHL3	<i>UNC13D</i>	Cytotoxicity	Lytic granules cannot dock at synaptic membrane	Herpesviruses	69
FHL4	<i>STX11</i>	Cytotoxicity	Lytic granules cannot fuse with synaptic membrane	Herpesviruses	70
FHL5	<i>STXBP2</i>	Cytotoxicity	Lytic granules cannot fuse with synaptic membrane	Herpesviruses	71
Chediak-Higashi syndrome	<i>LYST</i>	Cytotoxicity	Abnormal lytic granule biogenesis	EBV, fungi	72
Griscelli syndrome type 2	<i>RAB27A</i>	Cytotoxicity	Lytic granules cannot detach from microtubules	Herpesviruses, bacteria	73
Hermansky-Pudlik syndrome	<i>AP3B1</i>	Cytotoxicity	Abnormal lytic granule biogenesis	Herpesviruses, bacteria	74
	<i>BLOC1S6</i>		Abnormal lytic granule biogenesis		75
Papillon-Lefevre syndrome	<i>CTSC</i>	Cytotoxicity	Ineffective maturation of lytic machinery	Herpesviruses, bacteria	76
Wiskott-Aldrich syndrome	<i>WASP</i>	Cytotoxicity	Defective actin organization at immunological synapse	Herpesviruses, multiple infections	77
	<i>WIPF1</i>		Defective actin organization at immunological synapse		78
Autosomal recessive hyper-IgE syndrome	<i>DOCK8</i>	Cytotoxicity	Defective actin accumulation at immunological synapse	HPV, multiple infections	59
May-Hegglin anomaly	<i>MYH9</i>	Cytotoxicity	Defective lytic granule positioning	Intracellular bacteria	79
Leukocyte adhesion deficiency type I	<i>ITGB2</i>	Cytotoxicity	Defective target cell binding and lytic granule organization	Multiple infections	58
Leukocyte adhesion deficiency type III	<i>FERMT3</i>	Cytotoxicity	Defective target cell binding and NK cell activation	Multiple infections	80
Diseases inherently impairing signaling for cytotoxicity					
XLP type 1	<i>SH2D1A</i>	Cytotoxicity	Receptor-induced NK cell activation (CD244)	EBV	81
XLP type 2	<i>XIAP</i>	Low numbers ± cytotoxicity	Unclear	EBV	82
Non-X-linked lymphoproliferative syndrome	<i>ITK</i>	Low numbers ± cytotoxicity	Unclear	EBV	83
PLC-γ-associated immunodeficiency	<i>PLCG2</i>	Degranulation	Reduced activation-induced calcium flux	Respiratory infections	60
PKC-δ deficiency	<i>PRKCD</i>	Cytotoxicity	Unclear	EBV	84
CRAC channel deficiency	<i>ORAI1</i>	Degranulation	Activation-induced calcium flux for degranulation	Multiple infections	85
	<i>STIM1</i>		Activation-induced calcium flux for degranulation		86
NEMO deficiency	<i>IKBKG</i>	Cytotoxicity	Activation signaling and NF-κB activation	Mycobacteria, bacteria, CMV	87
ALPS (caspase 8 deficiency)	<i>CASP8</i>	Cytotoxicity	Activation signaling and NF-κB activation	Herpesviruses, bacteria	88
STAT1 deficiency	<i>STAT1</i>	Cytotoxicity and cytokine production	Activation-induced transcription	HSV, CMV, fungi, mycobacteria	89
Diseases impairing other functions					
Bare lymphocyte syndrome	<i>TAP1</i>	Cytotoxicity	Aberrant NK cell licensing	Multiple infections	90
	<i>TAP2</i>				91

(Continued)

TABLE III. (Continued)

Disease	Gene(s)*	NK cell defect	Mechanism†	Infectious susceptibility	Reference‡
Severe congenital neutropenia	<i>ELANE</i>	Cytotoxicity	Homeostasis and terminal differentiation via neutrophils	Bacteria	92
X-linked hyper-IgM-I	<i>CD40LG</i>	Cytotoxicity	Unclear	Enteroviruses, bacteria, pneumocystis	93
Netherton syndrome	<i>SPINK5</i>	Cytotoxicity	Unclear	Cutaneous infections	94
IL-12/IL-12 receptor deficiency	<i>IL12B</i> <i>IL12RB1</i>	Cytokine production, cytotoxicity	Deficient IL-12 signaling	Mycobacteria, salmonella	95
IL-21 receptor deficiency	<i>IL21R</i>	Cytotoxicity	Unclear	Multiple infections	96
X-linked immunodeficiency with Mg <sup>2+</sup> defect	<i>MAGT1</i>	Phenotype	Unclear	EBV, multiple infections	97
Rett syndrome–like MECP2 duplication	<i>MECP2</i>	Low numbers	Abnormal T-bet function	Fungi, pneumonia	98
CD25 deficiency	<i>IL2RA</i>	Low numbers	Unclear	CMV	99
Ataxia telangiectasia	<i>ATM</i>	Cytokine production	Unclear	Multiple infections	100

*ALPS*, Autoimmune lymphoproliferative syndrome; *CRAC*, Ca<sup>2+</sup> release–activated Ca<sup>2+</sup>; *FHL*, familial hemophagocytic lymphohistiocytosis; *IPEX*, immunodeficiency, polyendocrinopathy, enteropathy, X-linked; *NEMO*, NF- $\kappa$ B essential modulator; *NF- $\kappa$ B*, nuclear factor  $\kappa$  light chain enhancer of activated B cells; *PKC*, protein kinase C; *PLC*, phospholipase C; *SCID*, severe combined immunodeficiency; *STAT1*, signal transducer and activator of transcription 1; *XLP*, X-linked lymphoproliferative syndrome.

\*The gene names listed are those of the approved nomenclature of the Human Genome Organization Gene Nomenclature Committee, as confirmed on July 6, 2013, through <http://www.genenames.org>. The reader is referred to the reference cited in this table or to the Web site to find alternative names used or those more commonly applied in clinical immunology.

†Mechanism that specifically underlies the NK cell abnormality. The listing of “unclear” signifies that not enough information relative to NK cells is available to directly define or firmly infer the underlying mechanism.

‡For most diseases, there are multiple references that document the NKD or NK cell abnormality. In some cases there are also additional references that define the defective mechanism experimentally. Because of space constraints, a single reference was selected. Where possible, it is one that is particularly mechanistically illustrative or the most recent.

## Future of FNKD

Although there are numerous anecdotal reports of patients with susceptibility to infection and abnormal NK cell function, it is imperative that patients suspected of FNKD be rigorously considered and methodically evaluated (Fig 2). The complexity in approaching FNKD lies in the fact that NK cell functions can be negatively affected by physiologic stress, as well as certain therapeutic agents.<sup>56,57</sup> Thus great caution must be applied in considering FNKD. That said, it is predicted that other specific receptor or signaling molecule abnormalities will be discovered that impair specific NK cell subsets, NK cell functions, or both in isolation of other major immunologic effects. Enhanced NK cell subset and functional analyses in concert with careful evaluation of compelling patients will likely result in the significant growth of the FNKD diagnosis.

## KNOWN PIDs ASSOCIATED WITH AN NK CELL ABNORMALITY

Although CNKD and FNKD represent a specific subset of PIDs, there are at least 46 known genetically defined PIDs that include an effect on NK cell numbers, function, or both. They can be divided into diseases that (1) impair NK cell development or survival, (2) impair the mechanics of cytotoxicity, (3) impair signaling for cytotoxicity, and (4) impair some other mechanism. They are listed in Table III,<sup>52,58–100</sup> which provides an overview of the NK cell defect and mechanism, as well as a single key reference for each. By definition, these are not CNKD or FNKD because the NK cell component of the immunodeficiency represents a minority portion of the overall immunodeficiency. These diseases have been reviewed 4 times previously, and the reader is referred to those sources for a more comprehensive consideration of each condition and its underlying mechanistic effect on NK cells.<sup>17–20</sup> It is beyond

the scope of this review to cover each of these diseases in Table III in detail. However, an overarching theme continues to be a preponderance of viral susceptibilities to which the NK cell abnormalities might contribute.

Since the publication of previous reviews summarizing these diseases with regard to NK cells, there have been new insights into the known associations of NK cell defects with PIDs, previously identified PIDs that have now been defined to include an NK cell defect, and entirely new PIDs that include an NK cell defect. An example of a new insight into a known association is leukocyte adhesion deficiency type I. Here it was known that NK cells with the *ITGB2* mutation did not adhere effectively to their target cells because of the absence of the leukocyte function–associated antigen 1 integrin. However, recent studies have shown that an absent leukocyte function–associated antigen 1 signal in NK cells from patients with leukocyte adhesion deficiency type 1 also prevents effective lytic granule organization in the subset of patients' NK cells that can adhere to a target cell.<sup>58</sup>

An example of a previously known PID that has newly been associated with an NK cell abnormality is that of autosomal recessive hyper-IgE syndrome caused by dedicator of cytokinesis 8 (*DOCK8*) gene mutation. These patients' NK cells can bind to their target cell, but do not accumulate actin filaments at the lytic immunologic synapse,<sup>59</sup> which is required for effective cytotoxicity. This is especially interesting because patients with *DOCK8* mutations have a high incidence of warts, the defense against which can be contributed to by NK cells.

Finally, an example of a new PID that includes an NK cell defect is that of phospholipase C (PLC)- $\gamma$ -associated immunodeficiency in which defective NK cell degranulation caused by aberrant activation-induced calcium flux has been documented.<sup>60</sup> The list of these diseases will inevitably continue to grow and represents a unique mechanistic contribution to the field of NK cell biology. Importantly, the link to NK cell defense might

provide some further clues as to the full range of clinical phenotype in affected patients.

## TREATMENT OF NKDs

A variety of treatments are reported as having been applied to patients with CNKD and FNKD. That said, there has never been an organized clinical trial of any therapy in these patients. Most therapeutic approaches have focused on the susceptibility to herpesviruses and the application of prophylactic antiviral drugs. Anecdotal cases have described perceived success, with the most common being the use of acyclovir, ganciclovir, and related agents.<sup>22,27,47,50</sup> Breakthrough infections might require treatment with higher doses or parenteral forms. Therapies for papillomaviruses have also been described with more limited success, including topical agents, physical approaches, and immunostimulants. Given the susceptibility to HPV, all patients given a diagnosis of NKD should be considered for HPV vaccination.

Systemic administration of cytokine therapies has also been described in NKD, either for antiviral effect or even for some potential effect on the NK cells themselves. A recently reported example is that of IFN- $\alpha$  in patients with CNKD1,<sup>33</sup> which potentially induced some NK cell cytotoxic function. It has also been used for this purpose in patients with FNKD.<sup>53</sup> Theoretically, any therapeutic NK cell stimulatory cytokine has the potential to be of value, but this topic requires more specific evaluation.

For patients whose deficiency is perceived as more immediately life-threatening, hematopoietic stem cell transplantation might be an option. This has been successfully applied in patients with CNKD1,<sup>37</sup> as well as in those with otherwise undefined CNKDs.<sup>29</sup> Overall therapeutic approaches to patients with CNKD and FNKD require further clarification and need to be considered on a case-by-case basis.

## CONCLUSION

A growing number of patients have been recognized who have immunodeficiency that affects NK cells as the majority immune defect. A larger number of broader PIDs also include an NK cell abnormality, which has been mechanistically informative and potentially clinically useful. However, detailed clinical and phenotypic evaluation of patients with NKD has allowed paradigms to emerge, which include susceptibility to herpesviruses and HPV, as well as patients who lack NK cells (CNKD) or their functions (FNKD). Genetic advances have enabled the identification of 3 genes that can cause these conditions, *GATA2*, *MCM4*, and *FCGR3A*, and further investigation is likely to uncover additional genetic mechanisms. The insight that these patients provide into NK cell biology is in its infancy, as are the clinical and diagnostic approaches to patients. However, consideration of NKD represents a first step in appropriately linking patients to a diagnosis. Collaborative efforts around patients with such a diagnosis are likely to provide clearer paths to effective patient management and treatment.

I would like to acknowledge the inspiration and encouragement derived from patients with NKD, as well as their collaboration in investigating these conditions further. I also acknowledge quality collaborations with referring physicians and NK cell biologists who have made this work possible. Finally, I apologize to the authors of relevant works that could not be cited herein because of bibliography limitations.

## What do we know?

- NKD is rare but results in susceptibility to herpesvirus and papillomavirus infections.
- NKD types include those of number and function (CNKD) or just function (FNKD).
- Two genes for CNKD (*GATA2* and *MCM4*) and 1 for FNKD (*FCGR3A*) have been identified.
- At least some types of CNKD include effects on NK cell development or NK cell developmental intermediates.
- The mechanism underlying FNKD1 is abnormal interaction between mutant CD16 and an NK cell costimulatory receptor.
- At least 46 known single-gene PIDs include an NK cell defect.

## What is still unknown?

- Prevalence estimates for NKD
- Why the CNKD genes *GATA2* and *MCM4* can specifically affect NK cells
- What genes underlie other CNKD and FNKD subtypes
- Truly effective treatment options for patients with NKD
- The mechanism by which all of the PID genes that affect NK cells result in abnormalities

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