Research Paper

Low Circulating Natural Killer Cell Counts are Associated With Severe Disease in Patients With Common Variable Immunodeficiency

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A R T I C L E   I N F O

Article history:
Received 22 December 2015
Received in revised form 12 February 2016
Accepted 15 February 2016
Available online 2 March 2016

Keywords:
Natural Killer cells (NK cells)
Innate immunity
Common variable immunodeficiency (CVID)
Redundancy of immune system
Granuloma
Invasive bacterial infections

A B S T R A C T

Natural Killer (NK) cells have been shown to exert antiviral and antitumoural activities. Nevertheless most available data are derived from mouse models and functions of these cells in human remain unclear. To evaluate the impact of low circulating NK cell counts and to provide some clues to the role of NK cells in natural conditions, we studied a large cohort of patients with common variable immunodeficiency (CVID) included in a multicenter cohort of patients with primary hypogammaglobulinaemia. Patients were classified into three groups on the basis of their NK cell counts: severe and mild NK cell lymphopenia (≤50–50 and 50–99 × 10⁶/L respectively), and normal NK cell counts (>100 × 10⁶/L). Clinical events were analyzed and compared between these three groups of patients. During study period, 457 CVID patients were included: 99 (21.7%) with severe NK cell lymphopenia, 118 (25.8%) with mild NK cell lymphopenia and 240 (52.5%) with normal NK cell counts. Non-infectious complications (57% vs. 36% and 35%), and particularly, granulomatous complications (25.3% vs. 13.6% and 8.8%), were more frequent in patients with severe NK cell lymphopenia than in other groups. Invasive infections (68.7% vs. 60.2% and 48.8%), including bacteraemia (22.2% vs. 5.9% and 8.3%) and infectious pneumonia (63.6% vs. 59.3% and 44.2%), were also more frequent in this population. However, no difference was observed for viral infections and neoplasms. Low circulating NK cell counts are associated with more severe phenotypes of CVID, which may indicate a protective role of these immune cells against severe bacterial infections and other complications and non-redundant immune functions when the adaptive immune response is not optimal.

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1. Introduction

Natural Killer (NK) cells are cytolytic innate lymphoid cells (ILCs) that secrete an array of cytokines and chemokines (Spits et al., 2013; Vivier et al., 2011). Our knowledge of the biology of these cells has greatly increased over the last two decades, but the exact functions of human NK cells in vivo remain elusive. Indeed, the principal known functions of NK cells, such as the control of viral infections and cancers, were identified in studies on mice, and few data are available for humans (Vivier et al., 2011). One major obstacle to be overcome in studies of the role of NK cells in humans is the rarity of selective and well-defined NK cell deficiencies (Jouanguy et al., 2013). Isolated NK cell deficiencies are exceptional, and full clinical phenotypes are not always available for the rare affected patients (Gineau et al., 2012; Hughes et al., 2012; Casey et al., 2012). It has been suggested that isolated NK cell lymphopenia is not associated with a particular clinical phenotype. This is the case in children with severe combined immunodeficiencies (SCID) with an NK − phenotype before hematopoietic stem cell transplantation (HSCT). The NK cell compartment is not successfully reconstituted after HSCT in a significant proportion of these patients (low NK cell count < 50 × 10⁶/L), and the incidence of clinical events in these patients is similar to that in patients with normal NK cell counts (Neven et al., 2009). This may reflect redundancy between NK cell responses and other types of immune response, as such redundancy is frequently observed in immunity (Nish and Medzhitov, 2011). We therefore reasoned that the impact of an NK cell deficiency might be revealed by studies of patients presenting other immune defects. We explored the clinical phenotypes of patients with severe NK cell lymphopenia and compared them with those of patients with mild or normal NK cell counts, in a large cohort of patients with common variable immunodeficiency (CVID) from the French DEFI study.

2. Materials and Methods

DEFI is a French national study set up in 2004 to collect clinical data and biological specimens from adult patients with primary hypogammaglobulinaemia (Oksenhendler et al., 2008). The inclusion criteria in the DEFI study were adult patients with primary hypogammaglobulinaemia (serum IgG concentration < 5 g/L, or a serum IgA concentration < 0.7 g/L, or a serum IgM concentration < 0.4 g/L, or IgG subclass deficiency). The exclusion criteria were secondary hypogammaglobulinaemia (SCID) and X-linked agammaglobulinaemia, respectively, and AICDA (Activating kinase, was performed in male patients in the DEFI study, to exclude patients with CVID. The diagnostic criteria used for CVID diagnosis were consis-...
The DEFI study was funded by a national program for clinical research (PHRC 2005) and by the French National Centre for Hereditary Immune Deficiencies (CEREDIH), the Laboratoire Français du fractionnement et des Biotechnologies (LFB) and Baxter Bioscience.

3. Results

3.1. Patient Population, Clinical Events and Treatments

Within the DEFI study, 466 patients were diagnosed with CVID, and NK cell counts at inclusion were available for 457 of these patients. We found that 99 of these 457 patients (21.7%) had severe NK cell lymphopenia (\( \leq 50 \times 10^6/L \)), 118 (25.8%) had mild NK cell lymphopenia (50–99 \( \times 10^6/L \)) and 240 (52.5%) had normal NK cell counts (\( \geq 100 \times 10^6/L \)) (Fig. 1). The demographic and diagnostic characteristics of these three groups of patients are presented in Table 1. Sex ratio, median age at onset, at diagnosis and at evaluation, and the proportion of patients diagnosed before the age of 15 years were similar in these three groups. A trend towards a higher prevalence of consanguineous families was noted in the severe NK cell lymphopenia group (8.3% versus 4.4% and 5%, respectively).

Among these 457 patients, 275 patients (60.2%) presented infections only, whereas 182 patients (39.8%) presented both infections and other disease-related complications. The principal clinical events in CVID patients, according to their NK cell counts, are presented in Table 2. The proportion of patients with different types of infectious events was significantly higher in the group with severe NK cell lymphopenia than in the other two groups (20.2%, 22.9% and 17.1%, respectively, \( P_c = 0.05 \); Fig. 2). Pneumonia (63.6% vs. 59.3% and 44.2%; \( P_c = 0.02 \)) and septicemia (22.2% vs. 5.9% and 8.3%; \( P_c < 0.001 \)) were more frequent in patients with severe NK cell lymphopenia than in patients from the other two groups. *Streptococcus pneumoniae* was documented in 28 patients from the severe NK cell lymphopenia group, 26 patients from the mild NK cell lymphopenia group, and 43 patients from the group with normal NK cell counts (22.4%, 19% and 16.4%, respectively, of the pneumonia episodes in each group). *Haemophilus influenzae* was documented in 12 patients from the severe NK cell lymphopenia group, 10 patients from the mild NK cell lymphopenia group, and nine patients from the group of patients with normal NK cell counts (9.6%, 14.3% and 3.4%, respectively, of the pneumonia episodes in each group). Other pathogens documented during pneumonia episodes were less frequent and are detailed in Supplemental Table S3. The pathogens identified in cases of septicemia were gram-negative bacilli in 7 (35% of septicemia episodes in the severe NK cell lymphopenia group), 3 (42.9% of septicemia episodes in the mild NK cell lymphopenia group) and 5 (26.3% of septicemia episodes in the group of patients with normal NK cell counts) patients, and gram-positive cocci were identified in 13 (65% of septicemia episodes), 4 (57.1%) and 14 (73.7%) patients from the severe NK cell lymphopenia, mild NK cell lymphopenia and normal NK cell count groups, respectively.

Immunoglobulin (Ig) replacement therapy at inclusion in the DEFI study was administered to 86.9% of patients with severe NK cell lymphopenia, 75.2% of patients with mild NK cell lymphopenia and 65.4% of patients with normal NK cell counts (\( P_c < 0.001 \)). Nine patients (9.1%) in the group with severe NK cell lymphopenia, 5 patients

![Flow chart of the "NK-DEFI" study.](image-url)
(4.3%) in the group with mild NK cell lymphopenia and 24 patients (10%) in the group with normal NK cell counts underwent splenectomy. Corticosteroids had been used in 15 patients (15.2%) with severe NK cell lymphopenia, 13 patients (11.4%) with mild NK cell lymphopenia and 21 patients (8.9%) with normal NK cell counts. Other immunosuppressive treatments were less frequently prescribed and were found in only two patients from the severe NK cell lymphopenia group (2%), three patients from the mild NK cell lymphopenia group (2.5%) and six patients (2.5%) in the normal NK cell count group (Table 1).

### 3.2. Immunologic Evaluation

The results of the analyses of the principal lymphocyte subsets at inclusion, serum immunoglobulin levels at diagnosis, and comparisons between patients with severe NK cell deficiency and patients with mild or no NK cell lymphopenia are provided in Table 3.

Total lymphocyte counts were lower in patients with severe NK cell lymphopenia (median: 967 × 10^6/L) than in patients with mild NK cell lymphopenia (median: 1331 × 10^6/L) or normal NK cell counts (median: 1629 × 10^6/L) \((P_c = 0.003)\). Within the T-cell population, absolute numbers of CD4^+ T cells were lower in patients with severe NK cell deficiency (median: 415 × 10^6/L) than in the other two groups (median: 568 × 10^6/L and 691 × 10^6/L, respectively) \((P_c = 0.003)\). The counts of naive CD4^+ T cells (defined as CD4^+ CD45RA^+ CCR7^+ cells) were also lower in patients with severe NK cell deficiency (median: 63 × 10^6/L) than in the other groups (135 × 10^6/L and 183 × 10^6/L, respectively) \((P_c = 0.003)\). Severe CD4^+ T-cell deficiency (CD4^+ T cells < 20 × 10^6/L) affected 8-11% of the patients with severe NK cell deficiency, and severe naive CD4^+ T-cell deficiency (naive CD4^+ T cells < 20 × 10^6/L) affected 5.9% (6/118) of patients. Patients who developed severe CD4^+ T-cell deficiency (late-onset combined immunodeficiency or “OCID” patients according to Malphettes and colleagues [Malphettes et al., 2009]) were found in 25.3% of the patients with severe NK cell deficiency (12.0% and 9.7% respectively in the 2 other groups) \((P_c = 0.025)\). Total B cell counts were also lower in patients with severe NK cell lymphopenia than in patients from the other two groups (median: 59 × 10^6/L vs. 99 × 10^6/L and 122 × 10^6/L) \((P_c = 0.003)\). Furthermore, 16.5% of patients with severe NK cell lymphopenia had a CD19^+ B cell count lower than 100 × 10^6/L (late-onset CD19^+ B cell deficiency).

### Table 1

| Demographic, diagnostic and therapeutic characteristics | NK < 50 (n = 99) | NK 50–99 (n = 118) | NK ≥ 100 (n = 240) |
|---------------------------------------------------------|------------------|-------------------|-------------------|
| Sex, n (%) male patients                                | 49/99 (49.5)     | 46/118 (39.0)     | 100/240 (41.7)    |
| Patients with known consanguinity, n (%)               | 8/93 (8.6)       | 5/110 (4.5)       | 12/229 (5.2)      |
| Age at onset (years)                                    | 19.8 [11.7–34.8] | 22.5 [11.5–36.0]  | 21.5 [10.4–38.1]  |
| Age at diagnosis (years)                                | 34.9 [24.0–46.1] | 34.3 [21.6–49.1]  | 36.8 [25.0–52.6]  |
| Age at evaluation (years)                               | 44.1 [29.6–55.9] | 40.5 [27.7–53.3]  | 44.8 [33.8–57.8]  |
| Patients with diagnosis before the age of 15 years, n (%) | 8/99 (8.1)       | 14/118 (11.9)     | 24/240 (10.0)     |
| Time from onset to diagnosis (years)                    | 9.3 [2.4–17.8]   | 6.0 [1.2–15.1]    | 6.5 [1.1–18.5]    |
| Ig replacement, n (%)                                   | 86/99 (86.9)     | 88/117 (75.2)     | 157/240 (65.4)    |
| Splenectomy, n (%)                                      | 9/99 (9.1)       | 5/116 (4.3)       | 24/240 (10.0)     |
| Corticosteroid use during disease course, n (%)         | 15/99 (15.2)     | 13/113 (11.5)     | 21/235 (8.9)      |
| Immunosuppressive drug use during disease course, n (%) | 2/99 (2.0)       | 3/115 (2.6)       | 6/237 (2.5)       |

Note. All P values were adjusted by Benjamini–Hochberg procedure \((P_c)\); ns = not statistically significant.

### Table 2

| Clinical events | NK < 50 (n = 99) | NK 50–99 (n = 118) | NK ≥ 100 (n = 240) | P_c |
|-----------------|------------------|-------------------|-------------------|-----|
| Infections only, % (n) | 43.4 (43/99) | 63.6 (75/118) | 65.4 (157/240) | 0.02 |
| Other disease-related complications | | | | |
| Tumours, % (n) (solid tumours and lymphoma) | 19.2 (19/99) | 16.9 (20/118) | 14.2 (34/240) | ns |
| Lymphoid hyperplasia, % (n) | 35.3 (35/99) | 24.6 (29/118) | 19.2 (46/240) | ns |
| Granuloma, % (n) | 25.3 (25/99) | 13.6 (16/118) | 8.8 (21/240) | <10^-3 |
| Autoimmune disease, % (n) | 13.1 (13/99) | 17.8 (21/118) | 16.7 (40/240) | ns |
| Autoimmune cytopenia, % (n) | 25.3 (25/99) | 12.7 (15/118) | 14.6 (35/240) | ns |
| Enteropathy, % (n) | 41.4 (41/99) | 280 (33/118) | 242 (58/240) | ns |
| Infectious manifestations | | | | |
| Unusual infections, % (n) | 20.9 (18/86) | 27.4 (31/113) | 22 (52/236) | ns |
| Opportunistic infections, % (n) | 10.1 (10/99) | 3.4 (4/118) | 5.8 (14/240) | ns |
| Viral infections, % (n) | 18.2 (18/99) | 22.9 (27/118) | 16.7 (40/240) | ns |
| Digestive infections, % (n) | 29.3 (29/99) | 19.5 (23/118) | 18.3 (44/240) | ns |
| Invasive infections, % (n) | 68.7 (68/99) | 60.2 (71/118) | 48.8 (117/240) | 0.05 |
| (pneumonia + sepsicaemia + meningitis) | | | | |
| Infectious pneumonia, % (n) | 63.6 (63/99) | 59.3 (70/118) | 44.2 (106/240) | 0.02 |
| Sepsis, % (n) | 22.2 (22/99) | 5.9 (7/118) | 8.3 (20/240) | <10^-3 |
| Meningitis, % (n) | 8.1 (8/99) | 5.1 (6/118) | 7.3 (18/240) | ns |

Note. All P values were adjusted by Benjamini–Hochberg procedure \((P_c)\); ns = not statistically significant.

- Lymphoid hyperplasia: any follicular hyperplasia or polyomorph lymphoid infiltrate (without argument for lymphoma) occurring within lymphoid organ (lymphadenopathy, spleen, tonsils, cawyn) or within extra-nodal/extra-lymphoid organ;
- Autoimmune disease: autoimmune diseases excluding autoimmune cytopenia (rheumatoid arthritis, systemic lupus erythematosus, Sjögren syndrome, autoimmune thyroiditis, Biermer disease, type 1 diabetes, inflammatory myositis...);
- Enteropathy: any cause of recurrent acute diarrhea or chronic diarrhea, including celiac-like villous atrophy and Crohn’s-like inflammatory bowel disease. Documented granuloma, lymphoid hyperplasia or lymphoma in the gut were not included in this category but in the corresponding ones (respectively “Granuloma”, “Lymphoid hyperplasia” and “Lymphoma”).
count corresponding to ≤1% of the total lymphocyte count (B—group in the EuroClass classification (Wehr et al., 2008)), versus only 12.3% of patients with mild NK cell lymphopenia and 7.7% of patients with normal NK cell counts. Within the B-cell compartment, the median percentage of IgD−CD27+ switched memory B cells was 1%, 2% and 3% of B cells in patients with severe, mild and no NK cell deficiency, respectively. Expansion of CD21low B cells has been associated with lymphoproliferative and autoimmune manifestations during CVID. Number of CD21low B cells was available in n = 209 patients of our study and a correlation between CD21low B cells and NK cells numbers was observed (r = 0.13, P = 0.05).

Little is known about the stability of peripheral circulating NK cell counts in humans. We therefore checked the stability of NK cell counts over time in 35 patients from the principal center participating in the DEFI study (Saint-Louis Hospital, Paris). These patients underwent several phenotypic investigations, all carried out at the same reference laboratory, during follow-up. Ten of these 35 patients presented severe NK cell lymphopenia (<50 × 10^6/L) at inclusion and, for 80% of these patients, NK cell counts remained below 100 × 10^6/L over time. For the 15 patients with NK cell counts >100 × 10^6/L at inclusion, NK cell counts remained above 100 × 10^6/L over time in 73% (data not shown). Thus, NK cell lymphopenia and normal NK cell counts appeared to remain stable over time in most of these patients. Furthermore no correlation was observed between age at evaluation or age at diagnosis and NK cell count in our patients (data not shown).

Median serum immunoglobulin (Ig) G levels at diagnosis were lower in patients with severe and mild NK cell lymphopenia than in patients with normal NK cell counts (Pc = 0.003). IgA and IgM levels at diagnosis were also lower in patients with severe NK cell lymphopenia than in the other groups (Pc = 0.003 and Pc = 0.005 respectively).

3.3. Survival and Analysis of Subgroups Defined on the Basis of NK and CD4+ T Cell Counts

Survival five years after inclusion in the DEFI study was 86.7% for the severe NK cell lymphopenia group, 92.6% for the mild NK cell

Fig. 2. Proportion of CVID patients with “other disease-related complications”, i.e. non-infectious complications, according to NK cell count. Percentage of patients with A. other disease-related complications, B. granuloma, C. tumours, D. lymphoid hyperplasia, E. enteropathy and F. autoimmune cytopenia. In black, patients with severe NK cell deficiency; in grey, patients with mild NK cell lymphopenia; and in white, patients with normal NK cell counts. The statistical tests were two-tailed and the P values were then adjusted for multiple testing, by Benjamini–Hochberg procedure, for each type of complications (B to F).

Fig. 3. Proportion of CVID patients with different types of infection, as a function of NK cell count. Percentage of patients with A. viral infections, B. opportunistic infections, C. invasive infections, D. sepsis, E. infectious pneumonia. In black, patients with severe NK cell deficiency; in grey, with mild NK cell lymphopenia; and in white, patients with normal NK cell counts. The statistical tests were all two-tailed and the P values were adjusted for multiple testing, by Benjamini-Hochberg procedure.
4. Discussion

Large cohorts for studying the correlation between NK cell deficiency and clinical events in humans are extremely rare. The study by Imai and colleagues (Imai et al., 2000) reporting correlation between low NK cell cytotoxicity and the incidence of cancer in a cohort of 3625 patients is the only such study published to date. Our study is original in addressing the role of NK cells in a large cohort of immunodeficient patients through exploration of the phenotype of patients with quantitative NK cell deficiency. 

Lymphopenia group and 92.4% for the group of patients with normal NK cell counts at inclusion (P = 0.16). Kaplan–Meier curves were plotted (Fig. 4A).

Low CD4+ T cell count is known to be associated with higher rates of mortality and clinical events in patients with CVID (Malphettes et al., 2009), and patients with severe NK-cell deficiency also have lower CD4+ T cell counts in our study. The cut-off of 200 × 10^6/L CD4+ T cells was based on the results of a previous work showing that a particular phenotype of “LOCID” (Late Onset Combined ImmunoDeficiency) was observed in CVID patients with CD4+ T cells below this value and on the basis of 2014 ESID definition of CVID patients with profound T-cell defect (Ameratunga et al., 2014). We studied survival and clinical events in four subgroups of patients defined on the basis of NK and CD4+ T cell counts (Supplemental Fig. S2): a “T–NK−” group (8 patients with both CD4+ T cell counts < 200 × 10^6/L and NK cell counts < 50 × 10^6/L), a “T− NK−” group (18 patients with CD4+ T cells < 200 × 10^6/L and NK cells ≤ 50 × 10^6/L), a “T+ NK−” group (91 patients with CD4+ T cells ≥ 200 × 10^6/L and NK cells < 5 × 10^6/L) and a “T+ NK+” group (351 patients with both CD4+ T cells ≥ 200 × 10^6/L and NK cells ≤ 50 × 10^6/L). Survival in these four subgroups of CVID patients is presented in Fig. 4B. Mortality was particularly high in the subgroup of patients with both NK cell and CD4+ T cell deficiencies (the “T− NK−” group), as survival at 5 years was 93.2%, 89.5%, 71.6% and 58.3% for the “T+ NK+,” “T+ NK−,” “T− NK+” and “T− NK−” subgroups, respectively (P < 0.0001). The prevalence of septicemia (Fig. 5) and pneumonia was higher in the “T− NK−” (50% of patients for septicemia and 87.5% of patients for pneumonia) and “T+ NK−” (19.8% of patients for septicemia and 61.5% of patients for pneumonia) subgroups, and lower in the “T+ NK+” (5.6% and 61.1%, respectively) and “T+ NK−” (7.4% and 48.7% respectively) subgroups. Other disease-related complications (non-infectious clinical events) were observed in respectively 4/8 patients in the “T− NK−” group (50.0%), 11/18 patients in the “T− NK+” group (61.1%), 52/91 patients in the “T+ NK−” group (57.1%) and 120/351 patients in the “T+ NK+” group (34.2%). 

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![Fig. 4](image-url)
Our decision to study a cohort of patients with immunodeficiency is justified by the conclusion of a probable redundant role of these cells in humans reported by some studies in patients with “selective” NK cell deficiency. Studies of NK cell deficiency in patients with concomitant defect of one or several components of adaptive immunity, as in CVID patients, could thus reveal redundant in vivo functions of NK cells in humans.

Different classifications and subgroups of CVID patients have been proposed, defined on the basis of the quantitative abnormalities of B cells and T cells (Malphettes et al., 2009; Wehr et al., 2008), but data regarding the NK cell compartment of these patients and the phenotype of CVID patients with NK cell deficiency are rare. Few studies have already evaluated number of NK cells in CVID patients and observed a decreased number of NK cells in these patients (Aspalter et al., 2000; Berrón-Ruiz et al., 2014; Kutukculer et al., 2015). Nevertheless, our study is unique in reporting clinical manifestations and phenotype of patients with NK cell deficiency, especially in a cohort as large as more than 450 CVID patients. In 2000 Aspalter and colleagues reported a decrease in absolute and relative numbers of NK cells (defined as CD3−CD16+ cells) in 55 CVID patients compared to healthy controls, but without study of clinical manifestations associated with this NK cell deficiency (Aspalter et al., 2000). In 2015 Kutukculer and colleagues explored innate immunity in a small cohort of 20 pediatric CVID patients, with decreased percentage and increased cytotoxicity of NK cells in patients with severe disease (depending on the presence of splenomegaly, granuloma and/or bronchiectasis), but without detail about each of these clinical manifestations and other complications of the disease (Kutukculer et al., 2015). In the study of Aspalter et al., a decrease of invariant NK T cells has also been found in CVID patients, with again difficulties to conclude without available clinical data in this study (Aspalter et al., 2000). Finally, type 1 innate lymphoid cells (ILCs) can share phenotypical markers, such as CD56, with bona fide NK cells (Hazenberg and Spits, 2014) and a type 1 ILCs defect could potentially contribute to the observed phenotype of patients with NK cell deficiency.

Interestingly, although there is a large body of evidence concerning the role of NK cells in defense against viral pathogens (Lugli et al., 2014; Della Chiesa et al., 2014; Lee et al., 2007), the proportion of patients with viral infections did not differ between groups defined on the basis of their circulating NK cell counts in this large population of CVID patients. We also found no qualitative evidence for susceptibility to a particular type of virus in patients with severe NK cell lymphopenia. For example, Human papilloma virus (HPV) infections were equally frequent in all groups of patients, despite reports implicating NK cells in the control of infections with this pathogen (Kamili et al., 2014; Bere et al., 2014). Similarly, CMV infections did not appear to be more frequent in patients with severe NK cell deficiency than in patients with mild or no NK cell deficiency in our study. Difficult formal diagnosis of viral pathogens in clinical practice in these hypogammaglobulinemic patients, underestimation of benign viral episodes, or compensation by T cell compartment in this adult population could be as many explanations for this apparent discrepancy between earlier reports and our present study. In any event, these data highlight that the demonstration of a non-redundant role of NK cells in the control of viral infections has been obtained in the mouse, but not formally in humans.

The role of NK cells in anti-tumour responses has been extensively studied, for various types of tumour, in animal models and humans (Vivier et al., 2012; Shafer et al., 2013; Velardi et al., 2012). Imai and colleagues reported that low peripheral NK cell cytotoxicity was associated with a higher incidence of cancer (Imai et al., 2000). Surprisingly, in our study comparing CVID patients with different counts of peripheral NK cells, no differences between groups were observed for the incidence of neoplasms, including solid tumours and lymphomas. The median time from the onset of symptoms and evaluation in this study was 18 to 24.3 years in the groups studied, and the duration of follow-up would also be unlikely to account for this lack of association. The relatively young age at evaluation (median: from 40.5 to 44.8 years, depending on the group considered) in the DEFI study may account for the missing cancers, as many tumours occur after the age of 50 years (most of the events in the study by Imai and colleagues) (Imai et al., 2000).

The main finding in this study was the high frequency of invasive bacterial infections in CVID patients with severe NK cell lymphopenia (68.7%). This frequency was higher than that in patients with mild (60.2%) or no NK cell lymphopenia (48.8%). This higher incidence of severe bacterial infections was accounted for by a higher incidence of pneumonia and sepsis/bacteremia in particular, with bacteremia being found in 22.2% of patients with severe NK cell lymphopenia (versus only 5.9% and 8.3% of the patients in the other two groups). This higher incidence of severe bacterial infection did not seem to be related to the proportion of splenectomised patients, similar in the 3 studied groups, or to be related to an associated CD4+ T cell deficiency, because it persisted in the NK−T+ group, particularly for sepsis/bacteremia. Several studies have reported protective or deleterious roles of NK cells in various models of bacterial infectious diseases. A protective role for NK cells was demonstrated for infections with Listeria monocytogenes, Mycobacterium avium, Mycobacterium tuberculosis and various strains of Salmonella (Katz et al., 1990; Harshan and Gangadharan, 1991; Bermudez et al., 1990; Wherry et al., 1991; Vankayalapati et al., 2005). Similarly, NK cells have been shown to play a critical protective role in septic arthritis and models of pulmonary infection with Staphylococcus aureus (Nilsson et al., 1999; Small et al., 2008). The mildly protective role of NK cells in these bacterial infections is thought to involve IFN-γ secretion, promoting macrophage phagocytic functions and/or the lysis of infected macrophages through the recognition of as yet unidentified activation ligands (Souza-Fonseca-Guimaraes et al., 2012). By contrast, deleterious effects of NK cell activation were reported after infection with gram-negative bacteria such as with Escherichia coli (Badgwell et al., 2002) or gram-positive bacteria such as with S. pneumoniae (Kerr et al., 2005). In these conditions, NK cells have been shown to contribute to septic shock (Barkhausen et al., 2008; Carson et al., 1999; Emoto et al., 2002; Heremans et al., 1994), but their depletion in S. pneumoniae-infected mice has also been reported to impair bacterial clearance (Elhaik-Goldman et al., 2011). The dual role of NK cells, which may be initially beneficial, helping to control bacterial load, but may then become deleterious in the acute phase of sepsis, mediating tissue damage, requires further investigation.

The prevalence of non-infectious disease-related complications, such as granuloma in particular, was also found to be higher in patients with severe NK cell lymphopenia than in patients with mild or no NK cell lymphopenia. In a description of 59 patients with granulomatous disease and CVID from the DEFI study, patients with granulomatous disease had lower NK cell counts (median: 60 × 10⁶/L) than patients without granulomatous disease (median: 109 × 10⁶/L) (Bouriquet et al., 2016).
NK cell deficiencies were not available in other series of patients with granulomatous disease and CVID (Ardeniz and Cunningham-Rundles, 2009; Bouvry et al., 2013; Mullighan et al., 1997). The etiology and mechanisms involved in granuloma in patients with CVID have yet to be elucidated. Granuloma may be a consequence of a persistent but undetected infection with a virus or another microorganism, leading to weaker cell-mediated, and possibly NK cell immunity. Consistent with this hypothesis, Bodemer and colleagues recently described the long-term persistence of live rubella virus vaccine as an antigenic trigger of cutaneous granulomas in patients with primary immunodeficiency (Bodemer et al., 2014). Advanced DNA sequencing technologies might be able to detect the presence in granuloma lesions of microbes not detected in conventional in vitro growth assays.

Although the first of its kind, our study has some limitations. Firstly, our results are based on clinical observations and measurements, with mostly retrospective data collection, and a statistical correlation cannot be interpreted as indicative of a causal relationship. Given the exploratory nature of this study, we carried out multiple hypothesis testing using the Benjamini–Hochberg method to control for false discovery rate. However these results should be interpreted with caution, and we cannot conclude that all significances are really cases, but most rejected hypotheses (e.g., around 95%) correspond to cases where the alternative hypothesis is true. The stability of NK cell counts in these patients over time is also a non-resolved issue.

Another key point relates to the lower counts of CD4+ T cells, naïve CD4+ T cells and B cells in the group of patients with severe NK cell lymphopenia. Indeed, severe CD4+ T cell deficiency and naïve CD4+ T cell deficiency have been associated with the “LOCID” phenotype, with a high frequency of non-infectious disease-related complications and opportunistic infections (Malphettes et al., 2009). Similarly, the “no-B” phenotype (B cells <1%) and the presence of dysregulated B-cell subpopulations (low proportions of switched memory B cells, high transitional and CD21low B cell counts) are associated with clinical complications, such as splenomegaly, lymphadenopathy and granuloma (Wehr et al., 2008). In this line, higher rates of invasive infections and non-infectious complications observed in patients with NK cell deficiency could be explained by global, T and B cell lymphopenia observed in these patients. Nevertheless, patients with the “no-B” phenotype, severe CD4+ T cell deficiencies (<200 × 10⁹/L) and very low naïve CD4+ T cell counts (<20 × 10⁹/L) accounted for “only” 16.5%, 8.1% and 25.3%, respectively, of the patients with severe NK cell deficiency, suggesting that these factors alone cannot account for the observed phenotype of patients with severe NK cell lymphopenia. The persistence of a higher frequency of pneumonia and bacteremia in the T+NK− subgroup also suggests that NK cells could contribute to this phenotype. Although the coexistence of T cell and B cell deficiencies associated in these patients introduce some difficulties in interpreting the clinical manifestations observed, we are convinced that it is in these “non-redundant” conditions of associated adaptive immune system deficiency that NK cell deficiency could be clinically relevant. Unfortunately, to show strictly and statistically this point, comparison should be performed between T−(B−)NK− and T−(B−)NK+ groups, but these groups are the smallest groups (most uncommon patients) within our large cohort of CVID patients, with only respectively 8 and 18 patients. These numbers do not enable us to make stringent statistical analysis on the independent role of NK cells. Finally, the coexistence of T, B and NK cell deficiencies in some patients with CVID raises questions about the possibility of an as yet unidentified genetic defects leading to global lymphopoietic deficiencies in at least some of these patients (Ochs, 2014).

Mortality was not significantly higher in patients with severe NK cell deficiency than in patients with mild or no NK cell deficiency in our study. Nevertheless, mortality was particularly high (41.7% at 5 years) in the subgroup of patients with both severe NK cell lymphopenia and a severe CD4+ T cell deficiency. This suggests that NK cells could play a non-redundant role when the adaptive immune system, and the CD4 compartment in particular, is not optimal. Another recent study in 40 patients with idiopathic CD4 lymphopenia reported similar conclusions, with a correlation between low peripheral NK cell counts, and a high frequency of infections and low survival in these patients (Régent et al., 2014). In the same line, a recent study in five unrelated children with inherited DOCK2 deficiency, leading to T-cell, B-cell, and NK-cell defective responses, found early-onset invasive bacterial infections in these patients (Dobbs et al., 2015).

In conclusion, this large cohort study of CVID patients, despite some limitations, provides new insight into the functions of NK cells in vivo. Patients with CVID and NK cell deficiency seem to have a particular phenotype, with high frequencies of severe bacterial infections (pneumonia and bacteremia), and non-infectious disease-related complications, such as granuloma in particular. These data suggest that we should reconsider the role of human NK cells in controlling non-viral infections, such as extracellular bacterial infections in particular. The high frequency of these pathological events in this group of patients, and the high mortality of the T−NK− subgroup suggest that NK cells may have non-redundant immune functions in humans when the adaptive immune response is not optimal, and that reciprocally NK cells exert redundant functions in natura in normal individuals, at least in adults. This observation and the absence of a specific phenotype in SCID patients displaying a failure to reconstitute the NK compartment after HSCT suggest that there may be cooperation between T cells and NK cells, with NK cells helping to protect against pathogenic bacteria in the absence of T cells but becoming redundant if T cells are present. Several advantages are associated with redundant mechanisms. First, they can provide robust fail-safe mechanisms that ensure adequate protection (Nish and Medzhitov, 2011). Second, they can also limit the risk of immunopathologies, by ensuring the limited activation of each lymphocyte subset on challenge (Nish and Medzhitov, 2011). Our results thus pave the way for dissecting in more detail the interplay between NK cells, T cells and B cells in the control of bacterial infections in natura.

Authors’ Contributions

M. E., L. G., F. V., S. C., C. F., E. O. analyzed the data; L. G. and S. C. performed the statistical analysis; N. V. and M. M. performed the genetic sequencing; L. G. and S. C. performed the figures; M. E., E. O., N. S. and E. V. devised the concept, designed the research, supervised the study, and wrote the manuscript, with the assistance of the other coauthors.

Declaration of Interests

E.V. is the cofounder of and a shareholder in Innate Pharma. The other authors have no conflicting financial interests to declare.

Ethics Committee Approval

All patients gave written informed consent for participation, including possible inclusion in genetic studies, and the DEFI study was approved by the local ethics committee.

Acknowledgements

The EV laboratory is supported by the European Research Council (THINK Advanced Grant), the Ligue Nationale contre le Cancer (Equipe Labellisée) and by institutional grants from INSERM, CNRS and Aix-Marseille University awarded to CIML. E.V. is a scholar of the Institut Universitaire de France.

SCa is partly funded by ANR grant no. ANR-11-DPBS-0002.

The DEFI study was supported by a national program for clinical research (PHRC 2005) (AOM5018) and by the National Centre for
Hereditary Immune Deficiencies (CEREDIH), the Laboratoire Français du fractionnement et des Biotechnologies (LFB) and Baxter BioScience.

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.ebiom.2016.02.025.

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