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Research Paper

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

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ABSTRACT

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 and 50–99 $\times 10^6$/L respectively), and normal NK cell counts (>100 $\times 10^6$/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 $< 50 \times 10^6$/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 and refusal to participate.

In total, 691 patients were included in the DEFI study. We present here the characteristics of 457 patients from 47 centers enrolled in the study between April 2004 and April 2013, all of whom were diagnosed with CVID. The diagnostic criteria used for CVID diagnosis were consistent with the European Society for Immune Deficiencies/Pan-American Group for Immunodeficiency criteria (Conley et al., 1999): (Spits et al., 2013) markedly low IgG levels (at least 2 SD below the mean for age) and markedly low levels of IgM, IgA, or both, (Vivier et al., 2011) a diagnosis of immunodeficiency after the age of two years, and (Jouanguy et al., 2013) no other known cause of hypogammaglobulinaemia. Screening of the genes encoding the CD40 ligand, the signalling lymphocyte activation molecule-associated protein, and Bruton's tyrosine kinase, was performed in male patients in the DEFI study, to exclude patients with X-linked hyper-IgM, X-linked lymphoproliferative disease, and X-linked agammaglobulinaemia, respectively, and AICDA (Activation-induced cytidine deaminase) was also sequenced to exclude patients with an autosomal recessive form of hyper-IgM from the CVID group.

The patients were enrolled in the study prospectively, and blood samples were collected, analyzed, and stored at inclusion. Clinical data were then collected retrospectively. The clinical file of each patient included a retrospective analysis of the patient's infectious, autoimmune, lymphoproliferative, and tumoural complications, and the patient's family medical history. A blood sample collected at inclusion was used for the determination of detailed T, B and NK cell phenotypes at the same centralized reference laboratory for all patients. All patients gave written informed consent for participation, including possible inclusion in genetic studies, and the study was approved by the local ethics committee. The clinical and biological database is centralized at the Department of Clinical Immunology of Saint-Louis Hospital in Paris. Clinicians were asked to inform the principal investigator of any significant clinical events during follow-up.

Patients were assigned to three groups on the basis of their NK cell counts (NK cells were defined as $CD3^- CD56^+ CD16^+$ cells) at inclusion. Less than 5% of healthy controls have NK cell counts below 50 x10^6/L (Supplemental data Fig. S1, obtained in 145 healthy adults in our specialized NK cell laboratory). In another independent cohort of 52 blood healthy donors, median NK cell count was 185×10^6/L (IQR, 115–270), with no case below 50×10^6/L (data not shown). This last population was the reference population used in the reference laboratory of the DEFI study. We therefore used a cut-off of 50 NK cells $\times 10^6$/L to define severe NK cell lymphopenia. Patients were also considered to have mild NK cell deficiency if they had NK cell counts between 50 and 99 cells $\times 10^6$/L, and normal counts if their NK cell counts were ≥100 cells $\times 10^6$/L at the time of evaluation. Clinical events and biological characteristics of patients with severe NK cell deficiency, mild NK cell deficiency and normal NK cell counts were analyzed and compared between groups.

Opportunistic infections (OIs) were consistent with the revised classification system of the manifestations of human immunodeficiency virus (HIV) infection and definition for AIDS from CDC (From the Centers for Disease Control and Prevention, 1993) and were referred to as OIs. Some atypical infections, such as pulmonary tuberculosis or recurrent herpes infections, are not included in these opportunistic infections and should be distinguished from "usual infections" during CVID (essentially ENT and respiratory bacterial infections by usual infectious agents). Instead, they were referred to as "unusual infections" (Supplemental data Table S1). Other clinical events were defined as previously described (Chapel et al., 2008, 2012). Lymphoid hyperplasia was defined as any follicular hyperplasia or polymorph lymphoid infiltrate (without argument for lymphoma) occurring within lymphoid organ (lymphadenopathy, spleen, tonsils, cavum) or within extra-nodal/extra-lymphoid organ. Autoimmune disease was defined as any autoimmune disease excluding autoimmune cytopenia (rheumatoid arthritis, systemic lupus erythematosus, Sjögren syndrome, autoimmune thyroiditis, Biermer disease, type 1 diabetes, inflammatory myositis…). Finally enteropathy was defined as 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").

Descriptive analysis used medians with interquartile range (IQR) values. Statistical comparisons between the 3 NK groups (<50, 50–99, ≥100) were based on the non-parametric Kruskal–Wallis test for continuous variables and were based on Pearson's chi-square test or Fisher's exact test when appropriate for comparison of proportions of patients in multiple groups. Given the large list of clinical and biological characteristics compared between the 3 NK groups, we report Benjamini–Hochberg adjusted P values (P_c), which maintains the false discovery rate at the nominal alpha 0.05 level (Benjamini–Hochberg, 1995). Overall survival (OS) was calculated from inclusion in DEFI study until the last visit or death from any cause. Follow-up ended in April 2014. Survival was estimated by the Kaplan–Meier product-limit method. For OS comparisons, the groups considered were severe, mild and no NK cell lymphopenia, and then a second analysis was performed in 4 groups, as a function of NK cell and/or $CD4^+$ T cell deficiency, and the log-rank test was used. Statistical analyses were performed with STATA Statistical Software version 11.1 (Stata Corp., College Station, TX, USA).