To the editor:

Human NK cells can control CMV infection in the absence of T cells

A 3-month-old girl, the first child of consanguineous parents with a family history of unexplained febrile episodes including a death from infection of the eldest brother of the father at 8 months of age, was admitted to our hospital for gastroenteritis. She had a mild leukocytosis (27.4×10^9/L) that was due mainly to an increased number of lymphocytes. After oral rehydration, the child recovered rapidly, and she was discharged after the fever had disappeared. We subsequently learned from the laboratory data that the clinical symptoms had been caused by cytomegalovirus (CMV). In addition, we found that the lymphocyte subsets causing the leukocytosis were extremely unbalanced; 90% were $CD56^{dim}$ natural killer (NK) cells, 10% were B cells, while T cells were virtually absent (0.07×10^9/L). Genetic analysis showed early stop codons in the patient's genes encoding the α-chain of the interleukin-7 (IL-7) receptor, a defect known to be associated with this particular $T^-B^+NK^+$ SCID-phenotype.[1,2]

The correlation between viral load, number of NK cells, and cytokine serum-levels during the follow up was very suggestive of a causal relation (Figure 1A-C). The extremely high number of NK cells (23.8×10^9/L) present at the peak of viremia (4.1×10^4 copies CMV-DNA/mL of serum) remained elevated for approximately 2 weeks. At that moment, NK cell–derived[3] cytokines granulocyte macrophage–colony stimulating factor (GM-CSF) and interferon-γ (IFN-γ) and the ensuing IFN-γ inducible protein-10 were at very high levels (Figure 1B,C). The viral load, already reduced by one log, decreased further to 150 copies/mL at 4 to 5 weeks and could not be detected anymore after the 6th week. By then, the number of NK cells had dropped to 2×10^9/L and the cytokines had returned to normal levels (Figure 1B,C, gray bars).

The phenotype of the NK cells expanded at the peak of infection had several interesting features (Figure 1D). Half the population expressed the low-affinity Fcγ-receptor type 3 (CD16) only at low density, a phenomenon characteristic of activated $CD56^{dim}$ NK cells.[4] All cells expressed CD94 of which most was dimerized with NKG2C. NK cells expressing activating CD94-NKG2C receptors, which are rare ($< 1\%$) in CMV^- individuals,[5] may be triggered directly by CMV-infected fibroblasts.[6] Furthermore, the killer cell immunoglobulin-like receptors (KIR) repertoire appeared to be biased as more than 80% of NK cells were stained by a monoclonal antibody specific for KIR2DL2/2DS2/2DL3, while the other KIR, all present in the patient's genome, were expressed by less than 10% of NK cells (not shown).

To what extent NK cells contribute to human antiviral responses is unknown. Patients with diminished NK-cell functions may suffer from severe viral infections,[7-10] but this may also be due to lack of guidance of the adaptive immune system by NK cell–derived cytokines. Here, we report a $T^-B^+NK^+$ SCID patient who recovered from CMV disease without antiviral therapy after a significant expansion of IFN-γ–producing $CD16^+CD94$-$NKG2C^+$ NK cells had occurred. To our knowledge, this case provides the first direct evidence that human NK cells can effectively control CMV infection in the absence of T cells.

Taco W. Kuijpers, Paul A. Baars, Carole Dantin, Mirjam van den Burg, Rene A. W. van Lier, and Eddy Roosnek
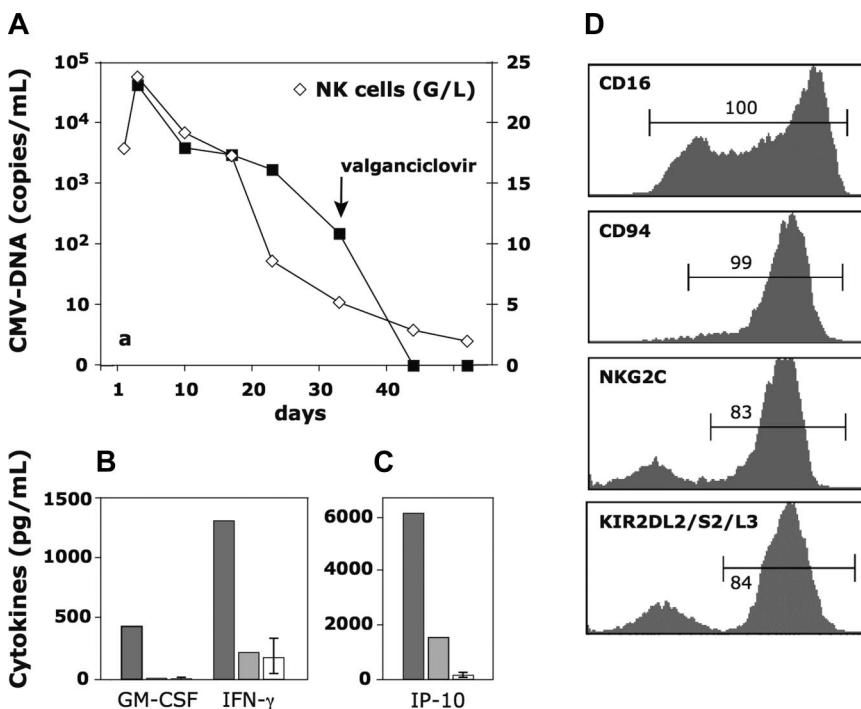
Figure 1. Kinetics of NK-cell expansion and cytokine serum-levels in relation to viral load, and phenotype of the expanded NK cells. (A) Number of CMV-DNA copies (■-■) and number of NK cells (◇-◇). The number of NK in the last sample (day 50) is at the upper limit of that in normal age-matched controls. → indicates the first day of oral valganciclovir given to maintain CMV suppression. (B,C) Serum cytokine-levels during viremia (■, day 9 in panel A) and in a late CMV-DNA free sample (■, day 43 in panel A). □ depicts the mean value plus or minus SD in 10 healthy controls. (D) FACS analysis of the respective marker with gates on CD3-CD56+ lymphocytes.

Correspondence: Taco W. Kuijpers, MD, PhD, Emma Children's Hospital, Academic Medical Center (Room G8-205), Meibergdreef 9, 1105 AZ Amsterdam, The Netherlands; e-mail: t.w.kuijpers@amc.uva.nl.

References

1. Puel A, Ziegler SF, Buckley RH, Leonard WJ. Defective IL7R expression in T(-)B(+)NK(+) severe combined immunodeficiency. Nat Genet. 1998;20:394-397.

2. Roifman CM, Zhang J, Chitayat D, Sharfe N. A partial deficiency of interleukin-7R alpha is sufficient to abrogate T-cell development and cause severe combined immunodeficiency. Blood. 2000;96:2803-2807.

3. Biron CA, Nguyen KB, Pien GC, Cousens LP, Salazar-Mather TP. Natural killer cells in antiviral defense: function and regulation by innate cytokines. Annu Rev Immunol. 1999;17:189-220.

4. Loza MJ, Perussia B. The IL-12 signature: NK cell terminal CD56+high stage and effector functions. J Immunol. 2004;172:88-96.

5. Gumá M, Angulo A, Vilches C, Gomez-Lozano N, Malats N, Lopez-Botet M. Imprint of human cytomegalovirus infection on the NK cell receptor repertoire. Blood. 2004;104:3664.

6. Gumá M, Budt M, Saez A, et al. Expansion of CD94/NKG2C+ NK cells in response to human cytomegalovirus-infected fibroblasts. Blood. 2006;107:3624-3631.

7. Merino F, Henle W, Ramirez-Duque P. Chronic active Epstein-Barr virus infection in patients with Chediak-Higashi syndrome. J Clin Immunol. 1986;6:299-305.

8. Biron CA, Byron KS, Sullivan JL. Severe herpesvirus infections in an adolescent without natural killer cells. N Engl J Med. 1989;320:1731-1735.

9. Joncas J, Monczak Y, Ghibu F, et al. Brief report: killer cell defect and persistent immunological abnormalities in two patients with chronic active Epstein-Barr virus infection. J Med Virol. 1989;28:110-117.

10. Orange JS. Human natural killer cell deficiencies. Curr Opin Allergy Clin Immunol. 2006;6:399-409.

To the editor:

Heparan sulfate proteoglycans, Fc receptors, and DC suppression

In their recent article in *Blood*,[1] You et al hypothesized on the mechanism by which decoy receptor 3 (DcR3), a member of the tumor necrosis factor receptor family (TNF-R), could promote tumor growth in patients with cancer. They reported that DcR3 induces apoptosis of dendritic cells (DCs) by binding to their heparan sulfate proteoglycans (HSPG). They argued that the ensuing immune suppression would explain why high levels of DcR3 are associated with reduced survival in cancer patients. This effect of DcR3 was shown by using a recombinant form of DcR3 fused to the Fc portion of human IgG1 (DcR3-Fc). However, it is known that the Ig moiety of fusion proteins bind Fc receptors (FcR).[2] Because DCs express high-affinity FcR[3] that can produce a strong signal into cells,[4] the results from You et al do not establish that, without this Ig-FcR interaction, the binding of DcR3 to HSPG is sufficient to induce DC apoptosis. As a consequence, it is not known whether the native form of DcR3 can be held responsible for reduced survival of cancer patients by inducing apoptosis in DCs or, alternatively, by its decoy activity on proapoptotic molecules such as Fas ligand.[5]

Using another member of the TNF-R family, the transmembrane activator, calcium modulator, and cyclophilin ligand interactor (TACI)–Fc, we observed a similar inhibition of DC generation from peripheral blood monocytes (Figure 1A top) as reported by You et al with DcR3-Fc.[1,6] It is noteworthy that TACI, like DcR3, has an HSPG-binding domain.[7] By contrast, B-cell maturation antigen (BCMA)–Fc that binds the same ligands as TACI-Fc but has no HSPG binding domain[7] and control IgG1 did not affect DC generation. Such inhibition of DC generation was also observed with a member of the TNF family, a proliferation-inducing ligand (APRIL) also bearing an HSPG binding domain[8] fused to the same Fc of IgG1 (Fc-APRIL; Figure 1A bottom). A mutant of the latter molecule without its HSPG binding domain, Fc-APRIL$_{H98}$,[8] or the wild-type molecule oligomerized by a non-Ig moiety, the collagen domain of adiponectin, ACRP-APRIL,[9] failed to inhibit DC generation in spite of binding to DC very efficiently (Figure 1B). Hence, binding to HSPG is essential, but an additional interaction with FcR is prerequisite to affect DCs.

The finding that simultaneous cross-linking of FcR and HSPG and/or bridging FcR and HSPG eliminates DCs and may cause immune suppression is of great interest. Indeed, any protein carrying an HSPG-binding domain fused to a Fc portion of IgG may achieve immunosuppression. Such immunosuppression is likely to constitute an advantage in the ongoing clinical trial with TACI-Fc in autoimmune disorders.[10]

Eddy Roosnek, Pascal Schneider, and Bertrand Huard

Contribution: E.R. designed the research and contributed to the writing; P.S. performed experiments; and B.H. designed research, performed experiments, and wrote the manuscript.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

Correspondence: Dr Bertrand Huard, Division of Hematology, Geneva University Hospital, 1 Rue Michel Servet, Geneva, Switzerland 1211; e-mail: bertrand.huard@medecine.unige.ch.
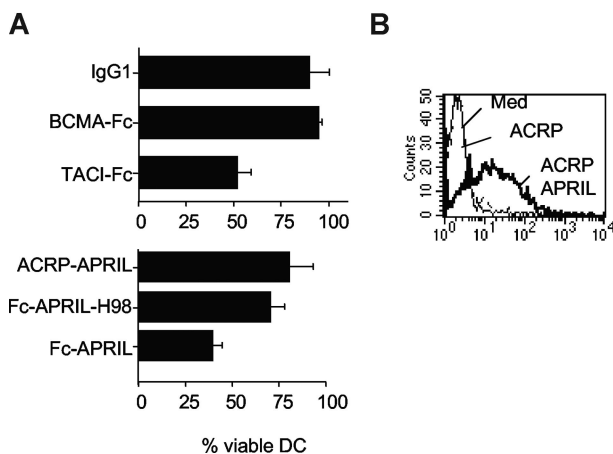
Figure 1. HSPG and FcR cross-linking impair DC generation. (A) Peripheral blood monocytes were cultured in GMCSF/IL-4 in the presence of 10 μg/mL of the indicated reagents. After 6 days, the number of viable DCs in the culture was assessed by dye exclusion; 100% was arbitrarily defined as the number of DCs recovered from culture with medium alone. Error bars represent SD. (B) Binding of 10 μg/mL ACRP-APRIL on monocyte-derived DCs. ACRP alone served as a negative control.