Development of the adaptive NK cell response to human cytomegalovirus in the context of aging

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Abstract

Human Cytomegalovirus (HCMV) establishes a highly prevalent life-long latent infection. Though generally subclinical, HCMV infection may have severe consequences during fetal development and in immunocompromised individuals. Based on epidemiological studies HCMV(+) serology has been associated with the development of atherosclerosis, immune senescence and an increase mortality rate in elderly people. Such long-term detrimental effects of the viral infection presumably result from an inefficient immune control of the pathogen, depending on the quality and evolution of the individual host-pathogen relationship. Together with antigen-specific T lymphocytes, NK cells play an important role in anti-viral immune defense. HCMV promotes in some individuals the differentiation and persistent steady state expansion of an NK cell subset bearing the CD94/NKG2C activating receptor. The relationship between this adaptive NK cell response to HCMV and aging is overviewed.

Keywords: immunity; age; Natural Killer cells; cytomegalovirus; NKG2C

Human Cytomegalovirus (HCMV) infection in immunocompetent individuals is generally asymptomatic and followed by the establishment of latency. Viral reactivation is generally subclinical and allows an effective propagation through secretions, reaching a high prevalence variable depending on socio-economic factors. HCMV infection constitutes the major infectious cause of congenital sensorineural disorders (1) and may have a severe clinical impact in immunocompromised patients, being related with reduced host survival in transplantation (2). Based on epidemiological studies, HCMV(+) serology has been associated with atherosclerosis, immune senescence and an increased mortality rate in elderly individuals (3), as well as with a reduced risk of Multiple Sclerosis (MS) (4), pointing out the potential multifaceted implications of the life-long host-pathogen interaction.

Adaptive NK cell response to HCMV infection

T and NK cells, together with specific antibodies, contribute to immune defense against HCMV. NK cells mediate cytotoxicity and cytokine secretion, favoring the development of the T cell dependent adaptive immune response (5). The importance of both lymphoid lineages is illustrated by the reciprocal development of viral immune evasion mechanisms specifically targeting their interactions with infected cells (6,7).

HCMV promotes the differentiation and expansion of a mature NK cell subset displaying high surface levels of the CD94/NKG2C activating lectin-like receptor (7,8). NKG2C^{bright} cells may represent >50% of the NK cell compartment in some HCMV(+) healthy blood donors, whereas others display low proportions of NKG2C^{dim} NK cells and are indistinguishable from HCMV(-) individuals, thus revealing the existence of distinct host-pathogen interaction profiles. Remarkably, the NK cell subset redistribution persists under steady state conditions, and is reminiscent of the development of murine “memory” NK cells expressing the Ly49H activating receptor specific for the m157 viral glycoprotein (9).

Among a number of distinctive features, adaptive NKG2C+ NK cells maintain the expression of CD16 and NKG2D, display inhibitory receptors for HLA class I molecules i.e. KIR and ILT2 (LILRB1 or LIR1), partially downregulate some activating receptors (e.g. NKp46, NKp30), and acquire the CD57 marker shared by cytotoxic T cells and other NK cell subsets (7,8,10). Epigenetic down-regulation of signaling molecules (e.g. FcεR-I γ, Syk, Eat-2) and transcription factors (e.g. PZLF1), together with upregulation of *IFNG* expression have been recently reported (11,12). The variability of these features in NKG2C^{bright} cells from different individuals is consistent with a progressive acquisition along their differentiation. Moreover, a similar phenotypic profile has been

observed in NKG2C- NK cells, indicating the existence of alternative adaptive NK cell subsets (13,14).

The adaptive expansion of NKG2C+ cells has been related with HCMV in healthy adults and children (15), being already detected early after birth in infants suffering congenital or early postnatal infection (16,17). NKG2C+ NK cell expansions in the course of other viral infections (e.g. HIV-1, Hepatitis B and C, Chikungunya, Hantavirus) have been invariably associated to HCMV co-infection (18-20). The effect appeared particularly prominent in a primary T cell immunodeficiency (21) and immunosuppressed transplant recipients (22), suggesting an inverse relationship between T cell-mediated control of HCMV and the magnitude of adaptive NK cell development. HCMV infection after hematopoietic stem cell transplantation (HSCT) promoted an adaptive NK cell response encompassed by the maturation of the NK cell compartment (23,24).

A frequent deletion of *NKG2C* (officially termed *KLRC2)* results in three distinct genotypes (i.e. *NKG2C*$^{+/+}$, *NKG2C*$^{+/del}$ and *NKG2C*$^{del/del}$), found in populations of different ethnic origins (25-28). *NKG2C* copy number has been related with CD94/NKG2C expression levels and function in response to receptor engagement, as well as with steady state numbers of circulating adaptive NKG2C+ NK cells, which tend to be greater among *NKG2C*$^{+/+}$ than hemizygous HCMV(+) individuals (27). Detection of the *NKG2C*$^{del/del}$ genotype and the absence of adaptive NKG2C+ NK cells in healthy HCMV(+) blood donors unequivocally indicate that they are dispensable for controlling the pathogen. Yet, persistent high titers of anti-HCMV IgG in NKG2C$^{del/del}$ children suggested a qualitative influence of the *NKG2C* genetic profile on the anti-viral response (28).

In summary, adaptive NKG2C+ NK cells appear reminiscent of memory cytotoxic T lymphocytes, being programmed for survival and activation through a restricted set of NKR (e.g. NKG2C, CD16, NKG2D) which efficiently trigger effector functions and induce cytokine-dependent proliferation encompassed by late differentiation events (29) .

Thus far, the molecular and cellular mechanisms underlying the development and steady state expansion of adaptive NKG2C^{bright} NK cells remain uncertain (7). Based on in vitro observations co-culturing PBMCs with HCMV-infected fibroblasts, it has been hypothesized that the process involves an instructive phase driven by a cognate interaction of the CD94/NKG2C receptor with a ligand induced by HCMV infection (30,31). Paradoxically, no evidence supporting an active role of the CD94/NKG2C receptor in triggering NK cell effector functions against HCMV-infected cells has been reported (32). By contrast, antibody-dependent stimulation via CD16 (FcγR-IIIA)

vigorously activates adaptive NKG2C+ NK cells to mediate specific cytotoxicity, cytokine production and proliferation in response to HCMV-infected cells (33). These results support that adaptive NKG2C+ NK cells in combination with HCMV-specific IgG may play an important role in the control of the viral infection, contributing as well to antibody-dependent defense against other pathogens, which might promote in that way their activation independently of HCMV (12).

Adaptive NK cells and aging

Marked oligoclonal expansions of HCMV-specific CD8+ T cells are detected in some elderly individuals. Such “inflation” of the anti-viral response is associated to immune senescence, being part of the so called “immune risk profile” which, according to epidemiological studies, has been proposed to be related with a higher mortality rate in aged people (3,34). The detrimental effects of HCMV infection presumably result from a defective control of viral reactivation, determined by host-virus interaction features not perceived through standard serological tests.

In this regard, some studies have directly addressed the relation of the adaptive NKG2C+ NK cell response with aging. No correlation between expansions of NKG2C+ CD57+ NK cells and CD8+ T cells were found in a well-defined cohort of elderly people, concluding that both events were in general non-overlapping and independent (35). On the other hand, an inverse relationship between NK and T-cell responses to HCMV was detected in younger blood donors (36). In this study, individuals with low HCMV-specific IgG titers, considered to reflect an effective control of the infection, displayed increased proportions of either CD8+ CD28- CD27- T cells or NKG2C+ CD57+ NK cells; moreover, the latter appeared associated to reduced numbers of T cells specific for the IE-1 antigen. No relation of age with the proportions of NKG2C+ or NKG2A- KIR+ NK cells (comprising the NKG2C+ subset) was found by other reports (37,38), in line with our own experience (unpublished results) indicating that the frequency of HCMV(+) individuals displaying adaptive NKG2C+ cell expansions and their average magnitude did not increase among elderly populations. On the other hand, expansions of NKG2C+ CD57+ NK cells and terminally differentiated oligoclonal CD8+ T cells, encompassed by a loss of naïve T cells, were detected in a cohort of HCMV(+) patients who had suffered a complete thymectomy early after birth during cardiovascular surgery (39), further supporting the inverse relationship between T and adaptive NK cell mediated responses observed in immunocompromised individuals. It is of note that cross-sectional studies do not provide information about the dynamics of the HCMV-induced changes in the immune system; this limitation is particularly

relevant to interpret the relation of adaptive NK cell expansions with aging, as they might be established early after birth in some individuals, as suggested by studies in infants and young blood donors.

Altogether, these observations are consistent with the hypothesis that the imprint of HCMV on the NK cell repertoire in healthy individuals is generally fixed at the time of primary infection, rather than resulting from a cumulative process secondary to HCMV reactivation events along the life-long host-pathogen co-existence. The steady state magnitude of the effect presumably depends on host and virus genetics, as well as on circumstantial factors (e.g. age at primary infection and viral load). Yet, an inefficient control of the latent infection associated to immune senescence may boost the expansion of pre-differentiated adaptive NK cells, as observed in immunosuppressed transplant recipients.

Systematic epidemiological and clinical studies are warranted to assess the putative relation between the adaptive NKG2C+ NK cell response to HCMV and prevalent pathologies in aged people (i.e. cancer, vascular disease, neurodegenerative disorders and infections). In line with the “infectious burden” hypothesis, acute myocardial infarction and carotid intima-media thickness in healthy individuals appeared associated to increased numbers of LILRB1+ NK and T cells, but not of NKG2C+ cells (40); by contrast, increased adaptive NK cells were associated with high-risk carotid atherosclerotic plaques (41). The detection of increased NKG2C+ cells in a small cohort of patients with CD4+ Vβ 13.1+ T-LGL lymphocytosis, specific for a peptide from the gB HCMV antigen presented by HLA-DR*0701, supported a role of HCMV in the pathogenesis of this lymphoproliferative disorder (42). Whether the adaptive NK cell response to HCMV may be related with the incidence and/or evolution of cancer in immunocompetent individuals deserves attention. Expansions of NKG2C+ NK cells have been related with the incidence of some epithelial tumors (e.g. head-neck and colorectal carcinomas) in liver transplant recipients (43). Moreover, adaptive NK cells have been related with the reduced AML relapse rate in allogeneic HSCT recipients suffering HCMV reactivation (44). On the other hand, the adaptive NKG2C+ NK-cell response to HCMV infection has been associated with a decreased risk of disability progression in MS patients (45), in line with previous epidemiological studies relating HCMV seropositivity with a reduced incidence of this autoimmune disease. It is conceivable that HCMV “priming” of the immune system in infancy may exert a broader influence on the immune response to other pathogens, and eventually on the risk of autoimmunity, as predicted according to the “hygiene hypothesis”.

In conclusion, further attention is warranted to unravel the mechanisms which underlie the development of adaptive NK cells in response to HCMV, the basis for the individual

variability of this effect, their role in immune defense and the relation with the development of antigen-specific B and T cells. Integrating this information with suitable clinical and epidemiological studies should provide further insights on the implications of the host-HCMV interaction pattern in health.

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