

NK cells in HIV-1 infection

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CÉLULAS NK EN LA INFECCIÓN VIH-1

RESUMEN

La inmunodeficiencia producida por el virus de la inmunodeficiencia humana (VIH-1), puede ser una consecuencia de la estrategia que el virus lleva a cabo para sobrevivir en el huésped. La infección por VIH-1 no sólo afecta a las células CD4+ sino también a otros componentes de la respuesta adaptativa e innata. Cada día mayor número de trabajos apoyan la idea de que la función de las células NK, que son un componente esencial del sistema inmunitario innato y también del adaptativo, están dañadas durante la infección por VIH-1. Sin embargo, no se conoce ni la magnitud del deterioro de estas células ni su contribución al progreso de la enfermedad. En nuestro laboratorio, estamos analizando las bases celulares y moleculares del defecto funcional de las células NK de individuos infectados por VIH-1. Consideramos que el conocimiento del papel de las células NK en la infección VIH-1 contribuirá a un mejor entendimiento de la patogénesis del síndrome de inmunodeficiencia adquirida (SIDA).

PALABRAS CLAVE: Células NK/ Infección por VIH-1.

ABSTRACT

The induction of immune deficiency may be an important consequence of the survival strategy of the human immunodeficiency virus (HIV-1). HIV-1 infection not only affects CD4⁺ cells but also several other components of the immune system, including the innate and adaptive components. A growing body of data supports the hypothesis that the function of NK cells, which are the linkage between innate and adaptive immune systems, is impaired in HIV-1 infection. However, neither the extent nor the contribution of this impairment to the progress of the disease is well understood. We are currently analyzing the cellular and molecular basis of the impaired function of NK cells in HIV-1-infected patients. The data presented here update the possible significance of NK cells in HIV-1 infection and will contribute to better understand the pathogenesis of acquired immunodeficiency syndrome (AIDS).

KEY WORDS: NK cells/ HIV-1 infection.

INTRODUCTION

Human immunodeficiency virus type 1 (HIV-1) infection is associated with severe and progressive loss of the immune function in infected persons, leading to high risk of opportunistic infections and malignancies. HIV-1-related immune deficiency is characterized by a decrease in the number of circulating CD4⁺ T cells and also by qualitative changes in cytotoxic T lymphocytes (CTL), B lymphocytes and natural killer (NK) cells. Diminished proliferative cytokine T-cell response, defects on cytokine

and chemokine production, disorders in the lymphocyte receptor repertoire with expansion of some families and depletion of others, have been also observed in HIV seropositive individuals (1,2).

On the basis of recent observations, it is suggested that more attention should be paid to analyze the innate immune response in HIV-1 infection (3,4), and in particular to NK cells, which are one of the major components of this type of response (5). Here we update the current knowledge related to the NK cells in HIV-1 infection, including the analysis of the NK cell dysfunction and its possible causes.



NK CELL IMMUNOSURVEILLANCE AGAINST HIV-1

NK cells represent the third major lymphocyte subpopulation and are distinguishable from B and T lymphocytes by their phenotype. NK are CD3-lymphocytes that express either CD56 cells or CD16 or both (6-9). They have the ability to recognize and lyse target cells through two basic mechanisms: *natural cytotoxicity and antibody dependent cell cytotoxicity* (ADCC). NK cells are a component of the innate system that participate actively in the surveillance and killing of tumor cells, parasite-infected and virus-infected cells (10), rejection of MHC mismatched bone marrow grafts and pathogenesis of certain autoimmune diseases. The function of NK cells is mediated not only by their direct cytolytic activity but also by producing immunoregulatory molecules, such as certain cytokines and chemokines (11). The cytolytic function of NK cells is regulated by receptors, some of which are activators and some inhibitors, resulting the final effect as an equilibrium between them (12-16) (see latter).

It is known that NK cells contribute to eliminate HIV-infected cells (10) and inhibit HIV-1 infection (11). These cells also are a source of several cytokines and chemokines relevant to HIV infection (11), and are beneficial in clinical infection (17). A strong NK cell function has been associated with a healthy clinical state (18). On the other hand it is also known that NK cell function is impaired in HIV-infected individuals (19,20) and the degree of dysfunction is associated in many cases with rapid progress of the disease and lack of resistance to opportunistic infections (21).

DECREASED NUMBER OF NK CELLS IN HIV-1 INFECTION

Accumulated evidence indicates that NK cell alterations observed in HIV-infected individuals are related to a decrease in their number. In fact, it has been shown that both the proportion and absolute number of CD56⁺CD16⁺ NK cells are significantly reduced to about half of that found in healthy individuals (22). Low levels are found at an early stage of HIV infection, even in individuals without clinical symptoms (23). In particular, CD3-CD16⁺CD8⁺ is the most significantly and consistently reduced subset of NK cells (23,24) indicating that pathogenesis of HIV disease includes both, alterations in the total number of NK cells as well as in certain subpopulations. Indeed, lower concentrations of CD16⁺CD56⁺ and CD16⁺CD56⁻ cells have been reported in individuals whose infection is progressing rapidly (20). Recently, we have found a selective reduction of CD3⁺CD56⁻ in seropositive individuals which

mainly affect the CD3⁺CD56^{dim} but not CD3⁺CD56^{bright} cell subpopulations (Tarazona et al, submitted).

FUNCTIONAL DEFECTS OF NK CELLS IN HIV INFECTION

The impairment of NK cells in HIV-1 infection also affects their functional capacity (25). NK cell dysfunction in AIDS patients is related to both cytotoxicity (natural or ADCC) and the production of cytokines and chemokines (Table I). In fact, NK cells from HIV-infected individuals show a decreased cytotoxic capacity against the classic target K562 cell line (26). This defect is related to defects at pre-binding and post-binding levels (27), including a dysfunction of CD16 receptors, cytoskeleton and certain NK receptors specific for HLA class I molecules.

Table I
Nature of NK cell impairment in HIV-1 infection

	References
Decrease in cell number	(20,23,47,87)
Cell dysfunctions	
Inability to release cytotoxic factors	(88)
Defective rearrangement of microtubular cytoskeleton and tubulin distribution	(40,89)
Progressive loss of CD16 surface receptor	(35)
Increased levels of certain KIRs	(46)
Increased levels of CD94	(48)
Blockade of L- type calcium channels	(76)
Decreased expression of CD3 chain	(39)
Decreased responsiveness of NK cells to certain cytokines	(4,27,63,90)

Alterations in the CD16 receptors

The phenomenon of *antibody dependent cell cytotoxicity* is mediated by CD16 which is the specific receptor for the Fc portion of IgG while *natural cytotoxicity* is regulated by a large variety of receptors, including the natural cytotoxicity receptors (NCR): NKp46, NKp44 and NKp30, (28). Other receptors are CD2 (29,30), CD26 (31) (32), CD69 (33) and 1 integrins (34).

The expression of CD16 receptor is markedly reduced on NK cells from HIV-infected individuals (35). This explains the impairment in both, ADCC (36) and CD16-dependent redirected



cytotoxicity (37). This observation is of particular relevance as it is known that NK cells eliminate HIV-infected cells directly or through ADCC (10), and ADCC may be a determinant element in the disease progression (17) including patients who are receiving retroviral therapy (38). Also decreased expression of molecules has been observed in NK cells from HIV infected individuals (39).

Alteration in the cytoskeleton

The NK cell dysfunction observed in HIV infection may also result from an inability to rearrange the microtubular (MT) cytoskeleton system, and to release the natural killer cytotoxic factor (NKCF), as well as from a defective distribution of tubulin (40). These data suggest that tubulin rearrangement deficiency might underlie the inability of NK cells to kill target cells in AIDS patients. In this regard, we have previously reported that NK cell cytotoxicity may be inhibited by HLA molecule-derived peptides that bind tubulin, probably interfering with cytoskeleton reorganization or its chaperone activity (41).

Alteration in the NK receptors specific for HLA class I (molecules)

A group of NK receptors with capacity to regulate the function of these cells are the *NK receptors specific for HLA molecules class I*. These receptors are present on NK cells, and a very minor subpopulation of T cells (42). In humans, they include members of two major families of molecules: the immunoglobulin (Ig) superfamily to which belong the killer cell Ig-like receptors (KIR) (43) and the C-type lectin heterodimers composed of CD94 molecule covalently associated with members of the NKG2 family of molecules to form the CD94/NKG2 complex (44,45).

The expression of KIR has been studied in the peripheral blood lymphocytes of HIV-infected patients in order to determine the relationship of these receptors to the progression of the disease (46,47). The levels of certain KIR such as KIR2D and KIR3D, were measured in leucocytes from HIV-infected individuals and were found increased not only in NK cells but also in T lymphocytes (46).

On the other hand the CD94/NKG2 family receptors, were also measured in HIV-infected individuals. Interestingly, we found that the percentages of T lymphocytes and NK cells expressing the C-lectin receptor, CD94, are significantly higher in HIV-infected individuals (48). These results were also confirmed by Andre et al (47). There is direct evidence that inhibitory HLA receptor expression on CD8⁺ CTL plays an important regulatory role in the

immune response to HIV-1 (49), as mAb-masking killer inhibitory receptors reverse the CTL function against HIV-1-infected cells (50,51).

Other mechanisms

Additional mechanisms have been shown to contribute to NK dysfunction in HIV infection, including a decrease in the expression of HLA-A and HLA-B without affecting HLA-C and HLA-E in the cells infected by HIV-1 (52,53). This phenomenon could mean that the HIV downregulate the expression of histocompatibility molecules in the infected cells in order to protect them from NK cells and CTL which express HLA inhibitory receptors able to recognize the HLA molecules. Similarly, it has been described a decrease of HLA molecules and an increase of MICA antigen expression during cytomegalovirus infection, likely protecting infected cells from the attack of NK and other cells which express the appropriate inhibitory receptors (54).

Recently in our laboratory we have observed that the molecule HLA-G which is normally expressed in trophoblast (55), appears in the majority of peripheral monocytes and in certain lymphocytes obtained from HIV+ individuals (56). This could be interpreted as the cells infected by HIV are protected from the killing by NK cells and monocytes, which express the inhibitory receptors able to recognize HLA-G molecule (Fig. 1). These receptors are ILT2 (LIR1) expressed in B, T, NK and dendritic cells, ILT4 (LIR2) expressed on monocytes and dendritic cells (57), and KIR2DL4 expressed on NK and T cells (58).

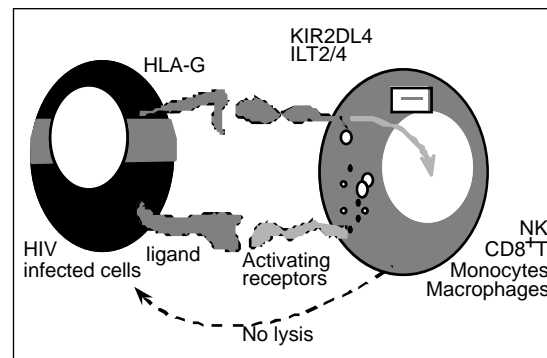


Figure 1. HIV-1 infected cells that express HLA-G could be protected from killing by effector cells expressing inhibitory receptors able to recognize HLA-G. The effector cells that can express HLA-G specific receptors are NK, CD8⁺ T cells, monocytes or macrophages.

Another aspect of interest in the relationship between NK function and HIV infection, is the finding that HLA class I alleles B*35 and Cw*04 consistently associate with rapid development to AIDS (59). The presence of these alleles has been associated with low NK cell activity (60).



Finally, we have found increased expression of CD43-hexasaccharide isoform on peripheral T lymphocytes of HIV-1 infected individuals, correlating with progression of the disease (61). Although we do not know the clinical relevance of this finding, the correlation of CD43 isoform expression with progression of the HIV-infection may indicate its linkage with the pathogenesis of the disease. This may be done by affecting NK cells, since we have also demonstrated that CD43 plays a relevant role in NK cells function (62).

CAUSES OF NK CELL ALTERATIONS IN HIV-1 INFECTION

The quantitative and qualitative changes in NK cells in HIV-infected individuals reported above, could be linked to several factors: a particular pattern of cytokines, release of peptides from HIV-1, extensive cellular death and others. Indeed, peripheral blood mononuclear cells (PBMC) from HIV-1⁺ individuals have low capacity to produce IL-12 (63) and IL-2 (64), which are essential to maintain the NK cell function. IL-2 and IL-12 restore *in vitro* certain depressed immunological functions, suggesting that a defect in the levels of IL-2 and IL-12 production may play a pathogenic role in the immunodeficiency of HIV-infected individuals (65). On the other hand, it has been demonstrated that the levels of several cytokines increase in HIV infection (66), including IL-4, IL-5, IL-6 (67), IL-10 (68), IL-15 (69) and TGF- β . Some of these cytokines have important modifying effects on the levels of HLA-specific receptors on NK and CTL. Thus, IL-15 upregulates the expression of CD94 on both NK and CTL (70,71), while IL-10 induces the expression of CD94 on long-term activated T-cells (72). TGF- β has also been shown to induce the expression not only of CD94 but also of NKG2A on CD8⁺ cells responding to toxic shock syndrome toxin 1 or to other staphylococcal superantigens (73). Recently, a novel cytokine, IL-21, produced by CD4⁺ cells and involved in NK cell maturation, has been described (74). Based on this, an additional cause of NK cells dysfunction in HIV positive individuals could be impaired NK cell maturation due to a possible defective production of IL-21 by the declining CD4⁺ cells in the HIV infection, as suggested (Tarazona et al, submitted) (Table II).

Inhibition of NK cell activities in normal donors and AIDS patients by means of envelope-derived peptides from HIV-1 (75) has been described. In particular, HIV-1 Tat inhibit human NK cell function by blocking L-type calcium channels (76).

NK CELLS RECONSTITUTION BY HAART

The availability of highly active antiretroviral therapy (HAART) for suppressing the replication

Table II
Factors inducing NK cell dysfunction in HIV-1 infection

	References
Decreased levels of IL-2	(64,91-94)
Direct effect of HIV-1 Tat	(76)
Impaired IL-12 production	(63,95)
Increased levels of IL-15	(69)
Increased levels of IL-10	(68,96)
Increased levels of TGF-	(97)

of HIV-1 and revert some immune system functions (77, 78) have provided further insight into the story of the HIV-infection. Thus, AIDS has reduced its original challenge, once it has been initiated the new antiretroviral treatments (HAART). However, with these treatments the HIV is not eliminated completely and the virus replicates and expand once the treatment is interrupted. Because of this, new efforts are required to obtain a complete removal of the virus.

Specifically, we have studied the NK compartment after HAART and we have found that the number of peripheral blood CD3⁺CD16⁺ NK cells increases after 12 weeks of HAART in HIV-1-infected patients whose viral load was lower 200-copies/ml (79). This increase is likely related to the increased level of CD4⁺ cells observed during HAART treatment (80) and the subsequent IL-2 production (81), since a positive correlation between both CD4⁺ lymphocytes and NK enhancement levels was found (79). Also, it has been described that patients with persistent viral replication during HAART, have a significantly decreased NK cell activity when compared with patients which effectively control HIV-1 viremia (82). In other study it was reported that, as a consequence of HAART, the proportion of CD3⁺CD16⁺CD56⁺ NK cells increased while CD3⁺CD16⁺CD56⁻ NK cells fell (83).

NK cells have been also investigated in clinical studies where HAART was given in combination with various immune modulators, such as IL-2, IL-12 and G-CSF. The number and activity of NK cells increased significantly when the patients were treated with HAART plus IL-2 (84), IL-12 (85) or G-CSF (86) compared with HAART alone.

CONCLUDING REMARKS AND FUTURE PERSPECTIVES

The data summarized here indicate that both the number and the function of peripheral blood NK cells are significantly impaired in patients



which are not receiving HAART. This deterioration affects not only NK cell cytotoxicity but also their capacity to produce chemokines, probably contributing to the disease progression. The increased numbers of circulating NK cells after HAART may contribute to the clinical recovery of patients and support the relevance of immunomodulatory therapies such as IL-2 treatment supplementing HAART (Fig. 2). These data may be useful to better understand the pathogenesis of HIV infection and to improve clinical therapeutic approaches.

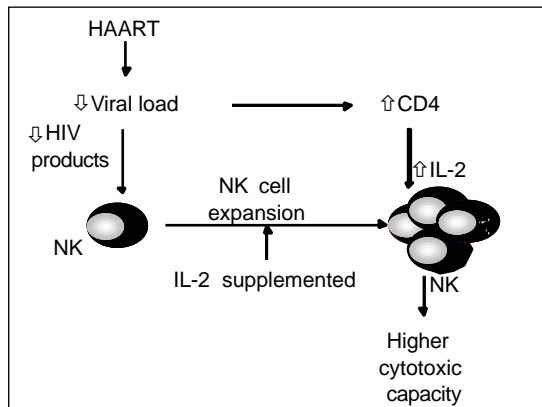


Figure 2. Putative model of the beneficial effects of HAART on NK cell function. Administration of HAART in HIV infected individuals decreases viral load and raises the number of peripheral CD4⁺ cells. These cells would produce a higher amount of IL-2, which would have a direct effect on the NK cell function. On the other hand, the reduced viral load decreases the presence of peripheral HIV products, thereby allowing NK cell expansion, which is enhanced in the presence of exogenous IL-2.

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