Retrovirology

BioMed Central

Short report

Open Access

Downregulation of CD94/NKG2A inhibitory receptors on CD8+ T cells in HIV infection is more pronounced in subjects with detected viral load than in their aviraemic counterparts

Mustapha Zeddou[1], Souad Rahmouni*[1], Arnaud Vandamme[1], Nathalie Jacobs[2], Frédéric Frippiat[3], Philippe Leonard[3], Nicole Schaaf-Lafontaine[4], Dolores Vaira[3], Jacques Boniver[2] and Michel Moutschen[1,3]

Address: [1]University of Liège, Laboratory of Immunology and Infectious Diseases, GIGA-R, Liège, Belgium, [2]University of Liège, Department of Pathology, CRCE-GIGA, Liège, Belgium, [3]University of Liège, Department of Infectious Diseases, CHU, Liège, Belgium and [4]University of Liège, Department of Biological Haematology, CHU, Liège, Belgium

Email: Mustapha Zeddou - mzeddou@ulg.ac.be; Souad Rahmouni* - srahmouni@ulg.ac.be; Arnaud Vandamme - Arnaud.Vandamme@student.ulg.ac.be; Nathalie Jacobs - n.jacobs@ulg.ac.be; Frédéric Frippiat - f.frippiat@chu.ulg.ac.be; Philippe Leonard - philippe.leonard@chu.ulg.ac.be; Nicole Schaaf-Lafontaine - nicole.schaaf@chu.ulg.ac.be; Dolores Vaira - dvaira@chu.ulg.ac.be; Jacques Boniver - J.Boniver@ulg.ac.be; Michel Moutschen - michel.moutschen@ulg.ac.be

* Corresponding author

Published: 10 October 2007

Retrovirology 2007, **4**:72 doi:10.1186/1742-4690-4-72

Received: 2 April 2007
Accepted: 10 October 2007

This article is available from: http://www.retrovirology.com/content/4/1/72

Abstract

The CD94/NKG2A heterodimer is a natural killer receptor (NKR), which inhibits cell-mediated cytotoxicity upon interaction with MHC class I gene products. It is expressed by NK cells and by a small fraction of activated CD8+ T lymphocytes. Abnormal upregulation of the CD94/NKG2A inhibitory NKR on cytotoxic T cells (CTLs) could be responsible for a failure of immunosurveillance in cancer or HIV infection. In this study, CD94/NKG2A receptor expression on CD8+ T lymphocytes and NK cells was assessed in 46 HIV-1-infected patients (24 viraemic, 22 aviraemic) and 10 healthy volunteers. The percentage of CD8+ T lymphocytes expressing the CD94/NKG2A inhibitory heterodimer was very significantly decreased in HIV-1-infected patients in comparison with non-infected controls. Within the HIV infected patients, the proportion of CD8+ T lymphocytes and NK cells expressing CD94/NKG2A was higher in subjects with undetectable viral loads in comparison with their viraemic counterparts. No significant difference was detected in the proportion of CD8+ T lymphocytes expressing the activatory CD94/NKG2C heterodimer between the HIV-1 infected patients and the healthy donors, nor between the vireamic and avireamic HIV-1 infected patients. In conclusion, chronic stimulation with HIV antigens in viraemic patients leads to a decreased rather than increased CD94/NKG2A expression on CD8+ T lymphocytes and NK cells.

Findings

The CD94/NKG2 heterodimer is a C-type lectin receptor, formed by the covalent association of CD94, a protein with a short non-signaling intracytoplasmic tail [1], and one of the NKG2 molecules. To generate a functional receptor, CD94 is disulfide linked with a member of the NKG2 family, namely NKG2A, -B, -C or -E [2,3]. In humans, CD94/NKG2A interacts with complexes of non-classical HLA-E molecules [4,5]. The intracellular domain of NKG2A contains immunoreceptor tyrosine-based inhibition motifs (ITIMs), responsible for transducing inhibitory signals [6]. The other NKG2 members lack ITIMs and are linked to transmembrane proteins, such as DAP10 and DAP12 which contain immunoreceptor tyrosine-based activating motifs and transduce activating signals [7]. CD94/NKG2A is normally expressed on most NK cells and on a small fraction of $CD8^+$ T lymphocytes. The proportion of NK cells bearing the CD94/NKG2A inhibitory receptor decreases in advanced HIV infection [8], in contrast with other inhibitory receptors of the KIR family which are upregulated. It is presently unknown if HIV infection has similar effects on the expression of the CD94/NKG2A inhibitory receptor by $CD8^+$ T cells. A few studies have shown that CD94 expression by $CD8^+$ T cells is increased during HIV infection [9-11] and have led to postulate that increased expression of the CD94/NKG2A inhibitory receptors is one of the mechanisms rendering HIV-specific $CD8^+$ T lymphocytes unable to control HIV-1 infection [12]. Nevertheless, the simultaneous expression of both subunits of the inhibitory receptor on $CD8^+$ T cells has hardly been studied in HIV infection. Costa *et al*. using two-color FACS analysis to study $CD3^+$ $NKG2A^+$ T cells, showed no difference between uninfected controls, long term non progressors or aviraemic subjects under HAART. A slight increase was noted in subjects with active viral replication [13], in contradiction with the downregulation previously observed on NK cells from infected subjects.

In the present study, we used four-colour FACS to investigate the expression of CD94/NKG2A and CD94/NKG2C on $CD3^+$ $CD8^+$ T lymphocytes and NK cells from HIV-1 infected patients, and its relationship with HIV-1 viraemia. Immunostaining was performed with fluorochrome-conjugated antibodies in 100 μl of peripheral blood from HIV-1 infected patients. Participants included 46 HIV-1 infected patients (23 viraemic and 23 aviraemic) and 10 healthy age-matched controls. The cells were analyzed on FACSvantage with CellQuest software (BD Biosciences). Flow Cytometry analysis was performed using fluorescence-conjugated antibodies to CD3, CD8, CD56, CD94, NKG2A and NKG2C. The Mann-Whitney test was used to compare the proportion of cells expressing each heterodimer between the three different groups of subjects (i.e. HIV infected viraemic, aviraemic and non infected controls). Figure 1 (1A, 1B and 1C) shows a representative dot plot of the CD94/NKG2A expression by $CD8^+$ T lymphocytes and NK cells from healthy and HIV-1 infected controls (viraemic and aviraemic). There was a dramatic decline in the proportion of $CD8^+$ T cells expressing the CD94/NKG2A heterodimer in HIV-infected patients in comparison with uninfected controls (mean ± SEM, 4.91 ± 0.49% n = 46 vs. 17.93 ± 3.26% n = 10 $p < 0.0001$) (Figure 1D). Interestingly, the decrease of CD94/NKG2A expression was more pronounced in patients with detected viral load than in patients with less than 50 copies/ml (mean ± SEM, 4.15 ± 0.65% n = 23 vs. 5.68 ± 0.7190 n = 23; p = 0.0379). Similarly, the proportion of $CD8^+$ T cells expressing the CD94/NKG2C was lower in HIV infected patients than in controls but the difference was not statistically significant (mean ± SEM, 1.73 ± 0.59% n = 16 vs. 5.45 ± 2.25 % n = 10, p = 0.1626) (data not shown).

In HIV infected patients, there was a weak but significant correlation between the proportion of $CD8^+$ T lymphocytes and NK cells expressing the CD94/NKG2A heterodimer ($r^2 = 0{,}09184$; p = 0.0406) and the proportion of NK cells expressing the inhibitory receptor tended to be lower in viraemic patients than in subjects with less than 50 copies/ml. (mean ± SEM, 43.11 ± 5.67% vs. 56.05 ± 4.67%; p = 0.019). There was no correlation of the expression of the inhibitory receptor with absolute or relative CD4 counts (data not shown).

In summary, we observed a downregulation of CD94/NKG2A on $CD8^+$ T cells in HIV infection, in accordance with what was previously described for NK cells. The mechanisms linking viral replication with downregulation of the inhibitory CD94/NKG2A receptor remains obscure. Upregulation of CD94/NKG2A has previously been observed in various animal models of viral and bacterial infections [14] and in chronic antigenic stimulation [15]. Loss of CD94/NKG2A might correspond to the terminal differentiation which occurs in a large fraction of $CD8^+$ T cells during HIV infection. Indeed, recent observations made in an experimental model of persistent polyoma virus infections suggest that CD94/NKG2A $CD8^+$ T lymphocytes might constitute a less differentiated subset of $CD8^+$ T cells and maintain a higher proliferative potential and capacity to secrete IL-2 [16]. Whatever is the mechanism involved, the loss of CD94/NKG2A in HIV infection could also contribute to the polyclonal activation which characterizes HIV infection.

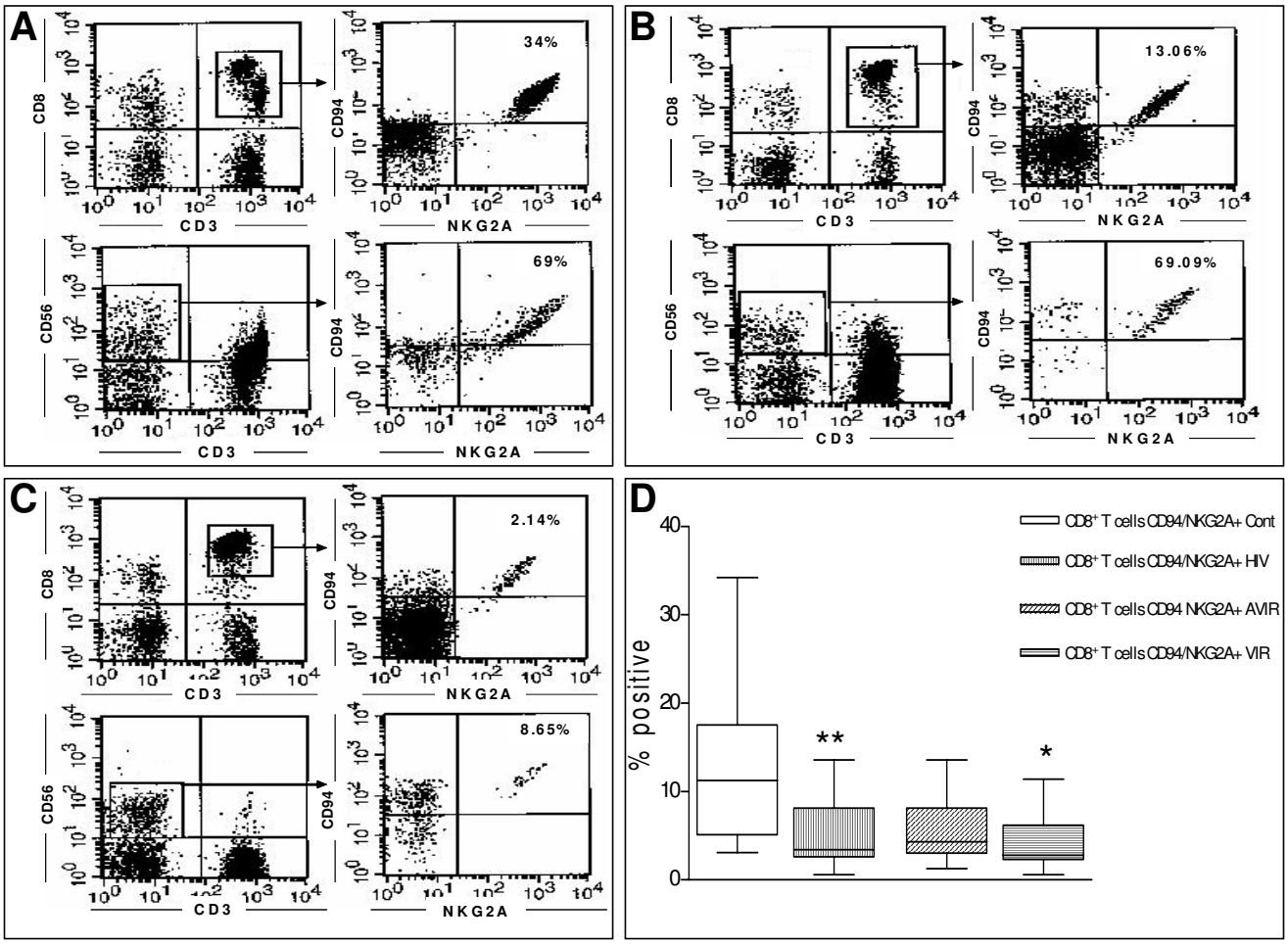

Figure 1

CD94/NKG2A expression in CD8+ T lymphocytes and NK cells from healthy donors and HIV-1 infected patients. Flow cytometry analysis was performed on 100 μl of peripheral blood from **A**: healthy controls, **B**: avireamic HIV-1 infected patients, **C:** vireamic HIV infected patient, using fluorescent conjugated antibodies to CD3, CD8, CD56, CD94, and NKG2A. Analysis was performed on gated cells. **D:** Comparison of CD94/NKG2A expression in CD8+ T lymphocytes from healthy controls (open bar; n = 10), HIV-1 infected patients (vertical hatched bar; n = 46), avireamic HIV-1 infected patients (oblique hatched bar; n = 23) and vireamic HIV-1 infected patients (horizontal hatched bar; n = 23). Data represent the mean ± SEM of each group. The Mann-Whitney test was used to calculate significant differences between the different groups. **p < 0,01 significance of difference of CD94/NKG2A expression in CD8+ T cells between healthy controls vs HIV-1 infected patients; *p < 0,05 significance of difference of CD94/NKG2A expression in CD8+ T cells between HIV-1 infected avireamic patients vs vireamic patients.

Acknowledgements

This work was supported by the Fond National de la Recherche Scientifique (FNRS), the Centre anticancéreux près l'Université de Liège (CAC) and a grant from the Walloon Region.

References

1. Chang CA, Rodriguez M, Carretero M, Lopez-Botet JH, Phillips , Lanier LL: **Molecular characterization of human CD94: a type II membrane glycoprotein related to the C-type lectin superfamily.** *Eur J Immunol* 1995, **25:**2433-2437.
2. Brooks AG, Posch PE, Scorzelli CJ, Borrego F, Coligan JE: **NKG2A complexed with CD94 defines a novel inhibitory natural killer cell receptor.** *J Exp Med* 1997, **185:**795-800.
3. Carretero M, Cantoni C, Bellon T, Bottino C, Biassoni R, Rodriguez A, Perez-Villar JJ, Moretta L, Moretta A, Lopez-Botet M: **The CD94 and NKG2-A C-type lectins covalently assemble to form a natural killer cell inhibitory receptor for HLA class I molecules.** *Eur J Immunol* 1997, **27:**563-567.
4. Brooks AG, Borrego F, Posch PE, Patamawenu A, Scorzelli CJ, Ulbrecht M, Weiss EH, Coligan JE: **Specific recognition of HLA-E, but not classical, HLA class I molecules by soluble CD94/NKG2A and NK cells.** *J Immunol* 1999, **162(1):**305-313.
5. Lee N, Llano M, Carretero M, Ishitani A, Navarro F, Lopez-Botet M, Geraghty DE: **HLA-E is a major ligand for the natural killer inhibitory receptor CD94/NKG2A.** *Proc Natl Acad Sci USA* 1998, **95:**5199-5204.
6. Kabat J, Borrego F, Brooks A, Coligan JE: **Role that each NKG2A immunoreceptor tyrosine-based inhibitory motif plays in**

mediating the human CD94/NKG2A inhibitory signal. *J Immunol* 2002, **169:**1948-1958.
7. Lanier LL, Corliss B, Wu J, Phillips JH: **Association of DAP12 with activating CD94/NKG2C NK cell receptors.** *Immunity* 1998, **8:**693-701.
8. Mavilio D, Benjamin J, Daucher M, Lombardo G, Kottilil S, Planta MA, Marcenaro E, Bottino C, Moretta L, Moretta A, Fauci AS: **Natural killer cells in HIV-1 infection: dichotomous effects of viremia on inhibitory and activating receptors and their functional correlates.** *Proc Natl Acad Sci USA* 2003, **100:**15011-15016.
9. Galiani MD, Aguado E, Tarazona R, Romero P, Molina I, Santamaria M, Solana R, Pena J: **Expression of killer inhibitory receptors on cytotoxic cells from HIV-1-infected individuals.** *Clin Exp Immunol* 1999, **115:**472-476.
10. Tarazona R, DelaRosa O, Casado JG, Torre-Cisneros J, Villanueva JL, Galiani MD, Pena J, Solana R: **NK-associated receptors on CD8 T cells from treatment-naive HIV-infected individuals: defective expression of CD56.** *AIDS* 2002, **16:**197-200.
11. Wesch D, Kabelitz D: **Differential expression of natural killer receptors on Vdelta1 gammadelta T cells in HIV-1-infected individuals.** *J Acquir Immune Defic Syndr* 2003, **33:**420-425.
12. Moser JM, Byers AM, Lukacher AE: **NK cell receptors in antiviral immunity.** *Curr Opin Immunol* 2002, **14:**509-516.
13. Costa P, Rusconi S, Fogli M, Mavilio D, Murdaca G, Puppo F, Mingari MC, Galli M, Moretta L, De MA: **Low expression of inhibitory natural killer receptors in CD8 cytotoxic T lymphocytes in long-term non-progressor HIV-1-infected patients.** *AIDS* 2003, **17:**257-260.
14. McMahon CW, Zajac AJ, Jamieson AM, Corral L, Hammer GE, Ahmed R, Raulet DH: **Viral and bacterial infections induce expression of multiple NK cell receptors in responding CD8(+) T cells.** *J Immunol* 2002, **169:**1444-1452.
15. Thimme R, Appay V, Koschella M, Panther E, Roth E, Hislop AD, Rickinson AB, Rowland-Jones SL, Blum HE, Pircher H: **Increased expression of the NK cell receptor KLRG1 by virus-specific CD8 T cells during persistent antigen stimulation.** *J Virol* 2005, **79:**12112-12116.
16. Byers AM, Andrews NP, Lukacher AE: **CD94/NKG2A expression is associated with proliferative potential of CD8 T cells during persistent polyoma virus infection.** *J Immunol* 2006, **176:**6121-6129.