CD38 EXPRESSION ON CD8+ T CELLS IN HUMAN IMMUNODEFICIENCY VIRUS 1-POSITIVE ADULTS TREATED WITH HAART

O. BERAN[1*], M. HOLUB[1], J. ŠPÁLA[1], J. KALANIN[2], M. STAŇKOVÁ[1]

[1]3rd Department of Infectious and Tropical Diseases, First Faculty of Medicine, Charles University, Budínova 2, Prague 8, CZ-180 81, Czech Republic;
[2]Department of Immunology, Institute of Clinical and Experimental Medicine; Prague, Czech Republic

Received February 14, 2003; accepted March 13, 2003

Summary. – The aim of this study was to assess whether the density of CD38 antigen expression on CD8+ T cells can be used as a marker of activation of the immune system in Human immunodeficiency virus 1 (HIV-1)-positive patients treated with highly active antiretroviral therapy (HAART). T cell subsets, expression of CD38 antigen on CD8+ T cells, HIV-1 viral load and stage of the disease were analyzed at baseline and after 12 months of HAART in 24 HIV-1-infected patients. Our data showed that the use of HAART is effective in reducing plasma viral load and in achieving a stable CD4+ count and percentage of CD8+/CD38+ cells. The percentages of CD8+/CD38+ cells in HIV-1-infected patients at baseline and after 12 months of HAART were significantly higher than those of controls. Analysis of the density of CD38 expression revealed that it was due to CD8+/CD38+ subsets with low and medium density of antigen expression. Absolute number of CD4+ T cells correlated negatively with the percentage of CD8+/CD38+ cells at baseline of the study. Persistent up-regulation of the CD38 expression on CD8+ T cells and its correlation with the decreased CD4+ count despite the reduction of plasma viral load may reflect residual replication of HIV-1 in reservoirs. Thus, this immunological parameter can serve as a biological marker of HIV-1 infection and might have utility in clinical management of HIV-1-infected persons.

Key words: HIV-1; CD38; CD8+; HAART; flow cytometry

HIV infection is associated with chronic immune activation including increased proportion of CD8+ cells expressing the CD38 antigen. These cells contain anti HIV-1-directed cytotoxic T lymphocytes, which seem to contribute to control of viral replication in the HIV-1-infected host even though they fail to eradicate the infection (Ferbas, 1998). CD38 is an ectoenzyme that hydrolyzes nicotinamide adenine dinucleotide to nicotinamide and ADP-ribose. An increase of CD38 expression on lymphocytes is a marker of cellular activation (Savarino *et al*., 2000). Furthermore, earlier studies have reported a high percentage of CD8+/CD38+ cells as a predictor of HIV-1 disease progression, which may correlate with a failure of HAART (Liu *et al*., 1996). In the late phase of HIV-1 infection the CD38 expression level has been described to be more predictive than the relatively weaker CD4+ count (Giorgi *et al*., 2002). However, less is known about the density of expression of this activation antigen and the role of CD8+ T lymphocyte subsets with different level of expression of CD38 molecule. The aim of our study was to analyze the density of CD38 expression on CD8+ T lymphocytes in HIV-1 associated disease treated with HAART and to assess whether the level of CD38 expression correlates with the CD4+ T cell count, HIV-1 viral load and stage of the disease.

In a longitudinal prospective study a group of 24 patients with HIV-1 infection (sex ratio M/F was 21/3, age range

*Corresponding author. E-mail: berano@fnb.cz; fax: +420-283840504

Abbreviations: HAART = highly active antiretroviral therapy; HIV-1 = Human immunodeficiency virus 1; MAb = monoclonal antibody; MFI = mean fluorescent intensity

Table 1. Changes in HIV-1 RNA plasma load, percentages and absolute numbers of CD4⁺ T cells, mean fluorescence intensity of the CD38 antigen on CD8⁺ cells and percentage of CD8⁺/CD38⁺ in HIV-1-infected patients subsets at baseline and after 12 months of HAART

Parameters	Controls (n = 19)	HIV-1-positive patients (n = 24)	
		Baseline	After 12 months of HAART
Viral load [log (copies/ml)]	–	2.7 ± 0.8	2.6 ± 0.6 [a]
$CD4^+$ T cells (%)	41.8 ± 10.4	23.9 ± 66 [b]	25.4 ± 1.4 [b]
$CD4^+$ T cells/μl	1007 ± 95.0	580 ± 66 [b]	597 ± 48 [b]
MFI	33.9 ± 2.0	45.4 ± 4.2	48.0 ± 6.6
$CD8^+/CD38^-$ (%)	44.4 ± 2.6	28.3 ± 3.2 [b]	29.7 ± 3.0 [b]
$CD8^+/CD38^+$ (%)	55.4 ± 2.6	72.0 ± 3.2 [b]	70.7 ± 3.0 [b]
$CD8^+/CD38^{low}$ cells (%)	46.6 ± 2.1	55.0 ± 2.0 [b]	52.1 ± 2.1 [b]
$CD8^+/CD38^{med}$ cells (%)	8.1 ± 0.7	15.6 ± 1.9 [b]	16.7 ± 2.9 [b]
$CD8^+/CD38^{high}$ cells (%)	0.38 ± 0.1	0.7 ± 0.2	0.9 ± 0.3

[a] $p < 0.05$. Significance of the difference between the baseline values and the values obtained after 12 months of HAART (Student's *t*-test)
[b] $p < 0.05$. Significance of the difference between patients and control group. One-way Anova test by the Dunn's method.
MFI = mean fluorescence intensity.

20–61 years) treated with HAART was followed for a period of 12 months. A group of HIV-negative healthy individuals, matched for age and gender to HIV-1-positive patients was chosen at random to obtain comparison values. T-cell subsets were analyzed by two-color flow cytometry (FACSCalibur, Becton Dickinson, San José, USA) using the SimulSET™ and CellQuest™ softwares for acquisition and analysis. For standard immunophenotyping, 4 double-stained leukocyte suspensions were prepared for each blood sample. Conjugates against surface molecules were used as follows: CD45(FITC)/CD14 (PE), isotype control, CD3(FITC)/CD4(PE), CD3(FITC)/CD8 (PE), (all BD, Heidelberg, Germany). CD38 expression on $CD8^+$ cells was analyzed using an anti-CD38 (PE) MAb in combination with gating on cells stained with an anti-CD8 (PerCP) MAb to select $CD8^{high}$ cells. Consequently, a mean fluorescent intensity (MFI) was calculated for each sample with four selected markers: negative density, low density, medium density, and high density. Plasma HIV-1 RNA load was detected by PCR (Roche Diagnostic Systems, Rotkreuz, Switzerland). Statistical analysis was done using the SigmaStat software version 2.0 (Jandel Scientific, San Rafael, USA). Correlation between parameters was analyzed using the Spearman and Pearson tests, comparison of parameters between groups was done using the One-Way Anova test. Follow-up data obtained from patients were compared by Student's *t* test. Values are given as means ± SD.

Baseline characteristics of $CD4^+$ and $CD8^+$ counts, the percentages of $CD8^+/CD38^+$ cell subsets and their changes after 1 year of follow-up are summarized in Table 1. We observed a significant decrease in plasma viral load after 12 months of HAART. Percentages and absolute numbers of $CD4^+$ T cells were significantly lower at baseline and after 12 months of HAART in HIV-1-infected patients compared to the control group. The level of expression of the CD38 antigen on $CD8^+$ T cells was significantly higher both at baseline and after 12 months of HAART in HIV-1-positive subjects compared to controls. Additionally, percentages of $CD8^+/CD38^{low}$ and $CD8^+/CD38^{med}$ cell subsets were significantly higher in HIV-1-positive patients than in controls (Fig.1). However, there was no difference in $CD8^+/CD38^+$ subsets in HIV-1-positive patients at baseline and after 12 months of follow-up. At baseline, the stage of the illness and absolute numbers of $CD4^+$ T cells correlated with percentages of $CD8^+/CD38^+$ cells, but only for the $CD4^+$ count significantly ($p = 0.02$; $r = -0.41$). By contrast, no significant correlation between these parameters was found at 12 months of HAART.

Suppression of HIV-1 replication following HAART results in clear clinical and immunological benefits in most patients. However, immune reconstitution is only partial due to severe damage to the immune system, especially in patients with advanced HIV-1 infection (Plana *et al*., 2000). Introduction of new immunological markers as predictors of the disease progression is needed because of routine use of effective antiretroviral drugs, which are responsible for reduction of plasma viral load and stable $CD4^+$ count. Although it has been shown that the CD38 antigen expression responds to effective suppression of HIV-1 replication (Gea-Banacloche and Clifford Lane, 1999), there is still a query about changes in the density of expression of this marker and its relation to the clinical course of the disease. In agreement with earlier studies on HIV-1-positive adults (Bouscarat *et al*., 1998) we demonstrated higher expression of the CD38 antigen on $CD8^+$ T cells than in healthy controls. The negative correlation between $CD4^+$ count and percentage of $CD8^+/CD38^+$ cells observed throughout the study supports the hypothesis that $CD8^+$ T cells expressing CD38 play an important role in elimination of HIV-1-infected $CD4^+$ T cells (Giorgi *et al*., 1993; Ho *et al*., 1993). Also, we demonstrated that the increase in the $CD8^+/CD38^+$ percentage in HIV-1-positive persons was due to the subsets with low and medium expression of CD38 antigen. Interestingly, HIV-1-positive patients showed a stable density of CD38 expression throughout the study. This finding may be due to residual viral replication in reservoirs (e.g. lymph nodes) where the bio-availability of HAART is reduced (Wong *et al*., 1997). Moreover, the persistent up-regulation of CD38 expression could also reflect an enhanced immune activation caused by opportunistic infections complicating the clinical course of HIV-1 infection (Bergamini *et al*., 1999).

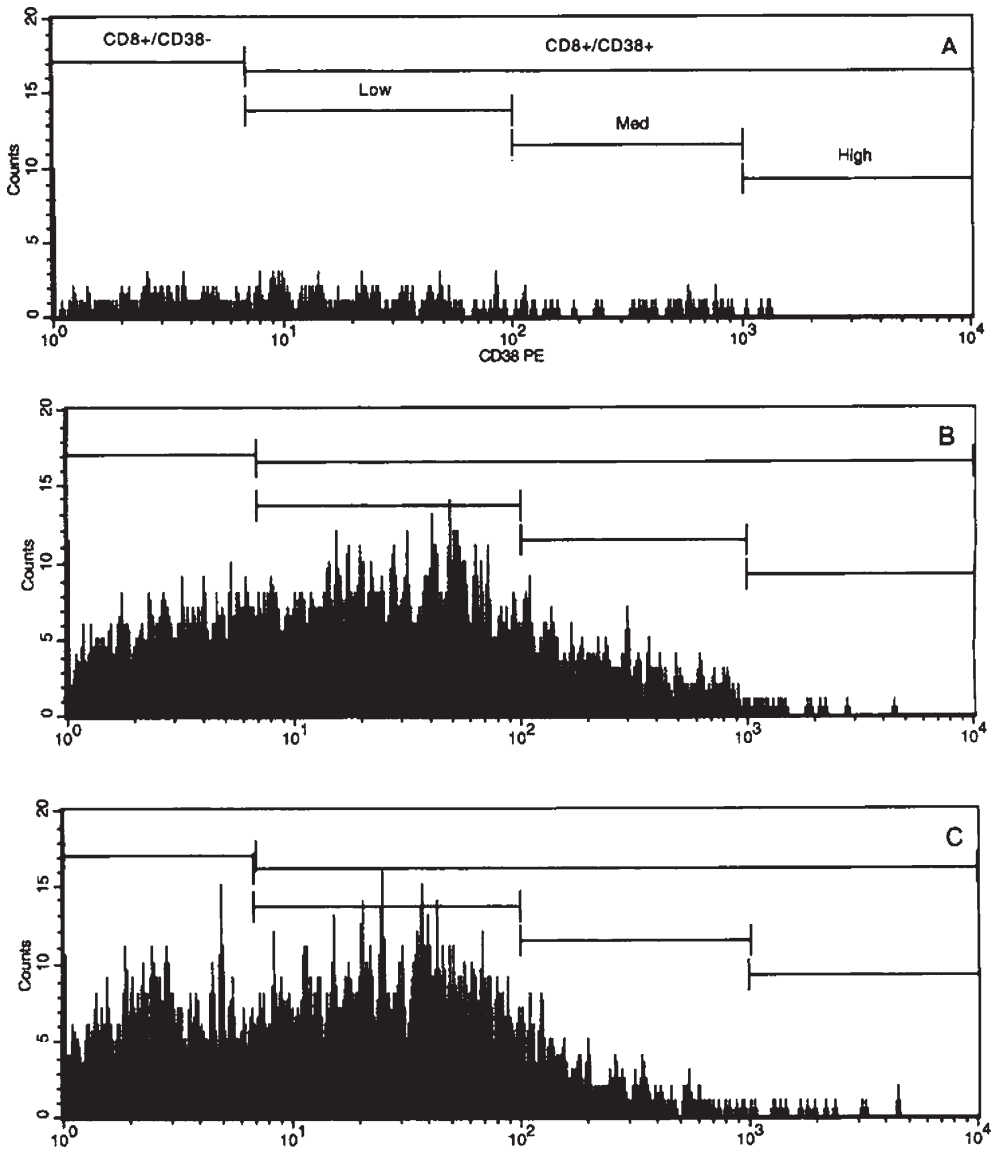

Fig. 1
Density plots of CD38 expression in CD8⁺ cells detected in control subject (A) and HIV-1-positive patients classified in CDC stage A1 (B) and C2 (C)

In conclusion, our data support the utility of analysis of CD38⁺ antigen expression on CD8⁺ T lymphocytes as a marker of chronic immune activation in HIV-1-positive patients treated with HAART and its potential importance in the clinical management of HIV-1 infection.

Acknowledgement. This work was supported by the grant No. 6303 of the Internal Grant Agency of the Ministry of Health of the Czech Republic. The authors thank to Dr. J. Kasten-Jolly, Wadsworth Center, Albany, USA for critical reading of the manuscript.

References

Bergamini A, Faggioli E, Bolacchi F, Gessani S, Cappannoli L, Uccella I, Demin F, Capozzi M, Cicconi R, Placido R, Vendetti S, Colizzi GM, Rocchi G (1999): Enhanced production of tumor necrosis factor-alpha and interleukin-6 due to prolonged response to lipopolysaccharide in human macrophages infected in vitro with human immunodeficiency virus type 1. *J. Infect. Dis.* **179**, 832–842.

Bouscarat F, Levacher M, Landman R, Muffat-Joly M, Girard PM, Saimot AG, Brun-Vezinet F, Sinet M (1998): Changes in

blood CD8+ lymphocyte activation status and plasma HIV RNA levels during antiretroviral therapy. *AIDS* **12**, 1267–1273.

Ferbas J (1998): Perspectives on the role of CD8+ cell suppressor factors and cytotoxic T lymphocytes during HIV infection. *AIDS Res. Hum. Retroviruses* **14** (Suppl. 2), S153–S160.

Gea-Banacloche JC, Clifford Lane H (1999): Immune reconstitution in HIV infection. *AIDS* **13** (Suppl. A), S25-S38.

Giorgi JV, Liu Z, Hultin LE, Cumberland WG, Hennessey K, Detels R (1993): Elevated levels of CD38+ CD8+ T cells in HIV infection add to the prognostic value of low CD4+ T cell levels: results of 6 years of follow-up. The Los Angeles Center, Multicenter AIDS Cohort Study. *J. Acquir. Immune Defic. Syndr.* **6**, 904–912.

Giorgi JV, Lyles RH, Matud JL, Yamashita TE, Mellors JW, Hultin LE, Jamieson BD, Margolick JB, Rinaldo CR Jr, Phair JP, Detels R (2002): Predictive value of immunologic and virologic markers after long or short duration of HIV-1 infection. *J. Acquir. Immune Defic. Syndr.* **29**, 346–355.

Ho HN, Hultin LE, Mitsuyasu RT, Matud JL, Hausner MA, Bockstoce D, Chou CC, O'Rourke S, Taylor JM, Giorgi JV (1993): Circulating HIV-specific CD8+ cytotoxic T cells express CD38 and HLA-DR antigens. *J. Immunol.* **150**, 3070–3079.

Liu Z, Hultin LE, Cumberland WG, Hultin P, Schmid I, Matud JL, Detels R, Giorgi JV (1996): Elevated relative fluorescence intensity of CD38 antigen expression on CD8+ T cells is a marker of poor prognosis in HIV infection: results of 6 years of follow-up. *Cytometry* **26**, 1–7.

Plana M, Garcia F, Gallart T, Tortajada C, Soriano A, Palou E, Maleno MJ, Barcelo JJ, Vidal, C, Cruceta A, Miro JM, Gatell JM (2000): Immunological benefits of antiretroviral therapy in very early stages of asymptomatic chronic HIV-1 infection. *AIDS* **14**, 1921–1933.

Savarino A, Bottarel F, Malavasi F, Dianzani U (2000): Role of CD38 in HIV-1 infection: an epiphenomenon of T-cell activation or an active player in virus/host interactions? *AIDS* **14**, 1079–1089.

Wong JK, Gunthard HF, Havlir DV, Zhang ZQ, Haase AT, Ignacio CC, Kwok S, Emini E, Richman DD (1997): Reduction of HIV-1 in blood and lymph nodes following potent antiretroviral therapy and the virologic correlates of treatment failure. *Proc. Natl. Acad. Sci. USA* **94**, 12574–12579.