

CD4⁺ CD38⁺ HLA-DR⁺ level as biomarker for evolution of HIV infection regardless of antiretroviral therapy

Niveau des CD4⁺ CD38⁺ HLA-DR⁺ comme évolution des biomarqueurs de l'infection par le VIH indépendamment de la thérapie antirétrovirale

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DOI : <https://doi.org/10.48087/BJMSoa.2017.4206>

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ABSTRACT

Introduction. HIV infection is characterized by an enhanced synthesis of cytokines and chemokines, a quantitative T cells disequilibrium and increased Turn over that signs a chronic activation of the immune system. Phenotype change in CD4⁺ T cells by over-expression of activation antigens CD38 and HLA-DR are suggested as markers of this process. **Materials and methods.** We investigated by four-color flow cytometry the expression of both activation markers on peripheral CD4⁺ T cells in 106 HIV-1 Algerian infected patients and 34 uninfected controls. Percentage's expression of CD4⁺CD38⁺HLA-DR⁺ cells was compared with clinical stages, viral load and anti-retroviral treatment (ARV). **Results.** The proportion of CD4⁺ T cells coexpressing HLA-DR and CD38 was higher in infected patients than in controls (respectively, 14.2 % ± 3.6 vs. 5.8 % ± 4.1, P=0.01), in symptomatic HIV patients than asymptomatic (13 % ± 3 vs. 15.9 % ± 4.6, P=0.01) and followed viral load kinetics. In matched treated and untreated patients, activated CD4⁺T proportion does not show any statical difference (respectively, 13 % and 14 %, p=0.09). **Conclusion.** In our cohort, CD4⁺ T cells expressing CD38 and HLA-DR were associated with HIV infection and correlated with disease progression, regardless of ARV treatment. As CD4⁺ count and viral load, this lymphocyte subset may be an interesting disease evolution marker; its value remains to be determined in prognostic or as therapy response indicator.

Keywords. CD4⁺ T cell, CD38, HLA-DR, HIV-1, immunophenotyping.

RÉSUMÉ

Introduction. L'infection par le VIH se caractérise par une synthèse accrue de cytokines et de chimiokines, un déséquilibre quantitatif des lymphocytes T et une augmentation du *turn over* qui signe une activation chronique du système immunitaire. Les changements de phénotype dans les lymphocytes T CD4⁺ par surexpression des antigènes d'activation CD38 et HLA-DR sont suggérés comme marqueurs de ce processus. **Matériels et méthodes.** Nous avons étudié par cytométrie de flux en quadrichromie l'expression des deux marqueurs d'activation sur les cellules T CD4⁺ périphériques chez 106 patients infectés par le VIH-1 algériens et 34 témoins non infectés. Les pourcentages d'expression des cellules CD4⁺ CD38⁺ HLA-DR⁺ ont été comparés aux stades cliniques, à la charge virale et au traitement antirétroviral (ARV). **Résultats.** La proportion de cellules T CD4⁺ coexprimant HLA-DR et CD38 était plus élevée chez les patients infectés que chez les témoins (respectivement 14,2 % ± 3,6 vs 5,8 % ± 4,1 ; P = 0,01) chez les patients VIH symptomatiques que chez les patients asymptomatiques (13 % ± 3 contre 15,9 % ± 4,6, P = 0,01) et suivi la cinétique de la charge virale. Chez les patients traités et non traités appariés, la proportion de CD4⁺ T activée ne montre aucune différence statistiquement significative (respectivement 13 % et 14 %, p = 0,09). **Conclusion.** Dans notre cohorte, les lymphocytes T CD4⁺ exprimant CD38 et HLA-DR étaient associées à l'infection par le VIH et corrélées à la progression de la maladie, quel que soit le traitement antirétroviral. Comme le nombre de CD4⁺ et la charge virale, ce sous-ensemble de lymphocytes peut être un marqueur intéressant de l'évolution de la maladie ; sa valeur reste à déterminer dans le pronostic ou comme indicateur de réponse thérapeutique.

Mots clés. Lymphocyte T CD4⁺, CD38, HLA-DR, VIH-1, immunophénotypage.

DÉFINITION

HIV infection is marked by CD4⁺ T cell loss and progression to AIDS and death subsequent to chronic immune activation [1, 2] contrasting with SIV infection of primate [3-5]. Persistent activation can be followed by cytokines and chemokines levels [6-8], B cell subset quantitative and phenotypic deregulation [9] and imbalance in T cell equilibrium [10, 11]. Expression of activation markers CD38 and HLA-DR by T cells is another disease progression marker [12, 13], relative proportion of CD4⁺ T cells expressing HLA-DR and CD38 is increased in HIV-seropositives, the relative expression of both markers is higher in symptomatic and AIDS patients [13], however the causes

of this activation are incompletely understood. In this study we explore, for the first time for patients in our region, the phenotypic expression of CD38 and HLA-DR antigens on T CD4⁺ lymphocytes in Algerian HIV+ patients and healthy controls. Activated T cell proportion was compared with progression markers such as viral load (VL), the use of antiretroviral treatment (ARV) and the stage of infection.

PATIENTS AND METHODS

Study subjects and clinical specimens.

One hundred and six Algerian HIV+ patients confirmed with Western blot were recruited from Infectious disease department of

To cite this article:

Meddour Y, Zerrouk I, SOUID FZ, et al. CD4⁺ CD38⁺ HLA-DR⁺ level as biomarker evolution of HIV infection regardless of antiretroviral therapy. *Batna J Med Sci* 2017;4(2):155-158. <https://doi.org/10.48087/BJMSoa.2017.4206>

Central Hospital of Army (CHA). Thirty four healthy blood donor HIV-negative controls were enrolled from transfusion center (CHA). HIV⁺ subjects were clinically classified according to CDC (Center of Disease Control, Atlanta 1993) classification system[14] (Table1). Twenty-five patients were asymptomatic or presented with lymphadenopathy (CDC stage A), 53 were symptomatic non-AIDS patients (CDC stage B), and 28 were AIDS patients (CDC stage C). The study protocol was approved by the hospital ethics committee and written informed consent was obtained from patients and controls.

Table1. Patients and Controls characteristics

Characteristics	Patients n=106 (%)	Controls n=34
Age ^a (yr)	38 ±13	31 ± 6
Sex ratio ^b	5,5	4,6
CDC stages	A =25 (24)	
	B =53 (50)	
	C =28 (26)	
ARV treatment	A = 5/25 (22)	
	B = 30/53 C (58)	
	= 28/28 (100)	
Infection duration ^c (yr)	3 [1 - 9]	
VL ^d (copies/ml)	76.088 [$<50 - 1,750000$]	

^aMean ± SD, ^bMale/Female, ^cMean [Min-Max], ^dVL at inclusion Mean [Min-Max].

CD38 and HLA-DR flow cytometry analyses of CD4 lymphocyte

Whole blood cells (100µl) were incubated for 30 min at room temperature with 10µl of staining antibody cocktail: CD3 *PerCP*, CD4 *FITC*, CD38 *PE* and HLA-DR *APC* (BD Biosciences, San Jose, CA). Red blood cells were lysed with diluted 10x Lysis solution (BD Biosciences). Data acquisition was performed on four colors FACS Calibur (BD Biosciences) using Cell Quest Pro software (BD Biosciences) (Figure1).

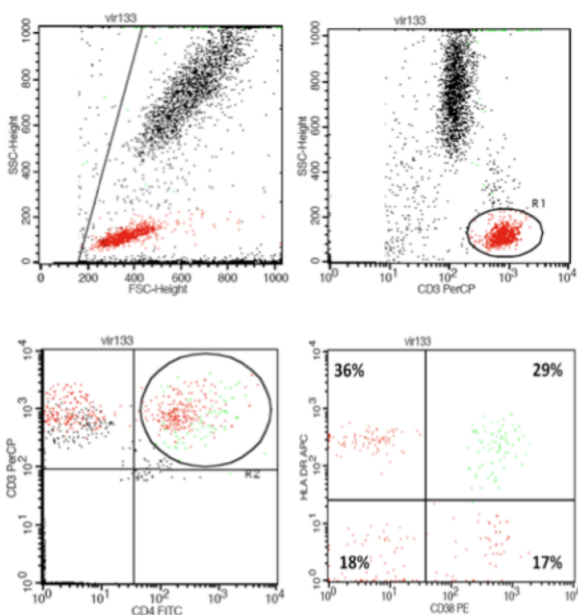


Figure1. Four-color immunophenotyping gating strategy.

Statistical analyzing

Data were calculated using SPSS V 16.0 software program (Chicago, SPSS Inc.) and GraphPad Prism version 6.00 (San Diego California USA). Differences between groups were tested using the nonparametric Mann-Whitney U-test to determine the significance of the two samples. *P* values were corrected applying the *Bonferroni* adjustment for multiple comparisons.

RESULTS

CD4⁺ T cells in patients and controls

Patients and controls lymphocytes proportion, lymphocytes, CD3⁺ and CD4⁺ T cells are presented in Table 2.

Table2. Lymphocytes subsets percentage in patients and controls

Cells subsets	Patients n=106	Controls n=34
Lymphocytes % [range]	24.3 [10.1-53.5]	38.1 [22.3- 41]
% cell subsets from whole blood white cells		
CD3 ⁺ cell % [range]	16.1 [2.2-40.2]	29.3 [18.6 - 34]
CD4 ⁺ T cell % [range]	5.2 [0.4-7.7]	11.1 [6.3-13.9]

CD38 and HLA-DR expression on T CD4⁺ cells

Compared with controls, all HIV⁺ patients showed relative enhance of CD38 and HLA-DR expression on CD4⁺ T cells. CD38⁺ HLA-DR⁺ CD4⁺ cells were increased in patients than controls (respectively, 14.2 % ± 3.6 vs. 5.8 % ± 4.1 ; *p*=0.01). In controls, CD4⁺ CD38⁺ were more frequent than double negative CD38⁻ HLA-DR⁻ CD4⁺ T cells and HLA-DR⁺ CD4⁺ cells (respectively, 50.7 %, 40.5 % and 3 %). CD38⁺ HLA-DR⁺ CD4⁺ T cells represented 5.8 % (Figure 2).

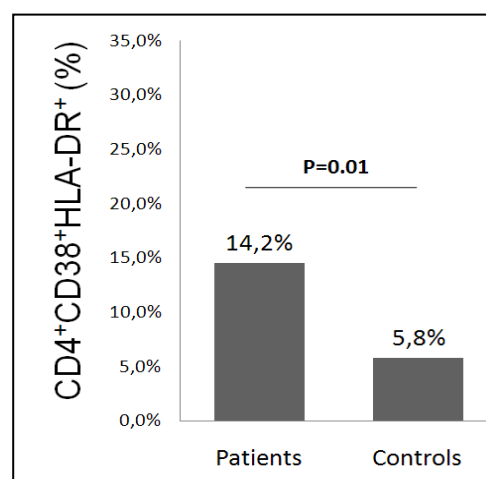


Figure2. CD4⁺CD38⁺HLA-DR⁺ cell levels in patients and controls

Correlation between activation markers and CDC stages

When analyzing according to CDC stages, it appeared that HLA-DR and CD38 expression increased with disease progression. This difference was statically significant only between stages A-C (13% ± 3 vs. 15.9 % ± 4.6, *P*=0.01)(Figure 3).

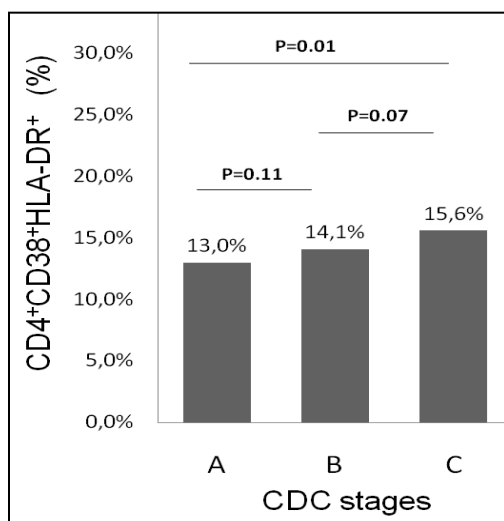


Figure 3. CD4⁺CD38⁺HLA-DR⁺ cell percentage and CDC stages

Correlation between markers of CD4⁺ and viral load

When patients were grouped into three groups according to viral load (Group1<50, Group2 [50-400], Group3>400 copies/ml) all HIV⁺ patients having more than 400 copies/ml VL (n1=38) showed higher levels of CD38⁺ HLA-DR⁺ CD4⁺ T cells (Figure 4). This difference was significant compared with HIV⁺ patients with less VL<50 copies/ml (n2=25). (Respectively, 13.2 % ± 2.1 vs. 15.4 % ± 3.8, P=0.01). Conversely, the expression did not differ significantly between closer VL levels.

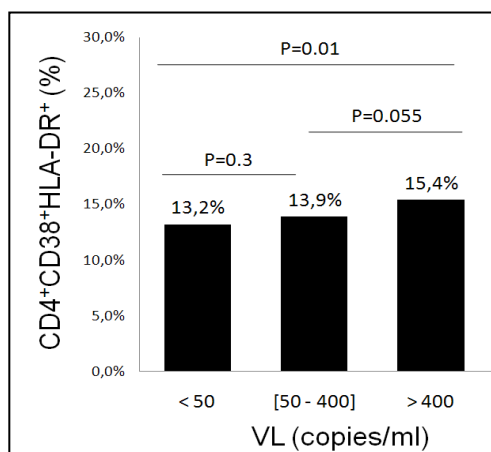


Figure4. CD4⁺CD38⁺HLA-DR⁺ T cell percentage according to viral load

Activated CD4⁺ T cells and ARV treatment

Two groups of patients, treated (n=23) and untreated (n=21), were selected on the basis of their percentage in CD4⁺ T cells (respectively, 6.1 % and 6.9 %, p=0.09) then we compared CD4⁺CD38⁺HLA-DR⁺ expression to understand the impact of treatment on CD4⁺ T cells phenotype. Treated and untreated patients showed similar levels expression of CD38⁺ and HLA-DR⁺ on CD4⁺ T (respectively, 13 % and 14 %, P=0.09).

DISCUSSION

HIV is a strong activator of the immune system. This chronic activation is a specific HIV immunopathology mechanism causing T-cell depletion and all associated complications [15-17]. In our study on Algerian infected and uninfected subjects, we highlighted the increase of CD38 and HLA-DR coexpression on CD4⁺ T cells in HIV⁺ patients than healthy subjects, enhance appeared linked with infection progression, higher in late stages infection than in asymptomatic patients. Our results are consistent with those of literature. Indeed, increase of CD38 and HLA-DR as activation markers of CD4⁺ T cells [11,13] is more frequent in patients with elevated in vivo turnover [18, 19] and increased apoptosis [20]. Markers expression on levels on CD4⁺ and CD8⁺ T cells in HIV⁺ patients are related to disease evolution [12, 13]. This chronic activation remains understood. Recent findings show this activation as cell type specific, mechanisms differ between T helper and T cytotoxic lymphocytes [21, 22].

Moreover, our results showed that proportion of CD4⁺ T cells with the CD38⁺HLA-DR⁺ phenotype did not differ significantly in subjects from closer VL levels (VL<50 vs. [50-200] and VL [50-200] vs. >200) but were significantly elevated in patients with high VL levels than less one. This is consistent with study of *Deeks et al*, where they conclude that T-cell activation was positively associated with viremia at entry of study and with CD8⁺ T-cell activation levels at plasma RNA levels more than 10,000 copies/mL [23].

When combining our data, we observed parallel increase of viremia and infection progression stage with CD4⁺ T cells loss and activation (data not shown). This supports previous hypothesis [17] but also offers a new progression markers (activated CD4⁺ T cells) for monitoring HIV infection progression. Indeed, reports showed that detection of CD38 and HLA-DR percentage expression on CD8⁺T cell may be available for prediction about plasma HIV load assay as substitute method to survey the disease progression [24]. It may be also true for CD4⁺ T cells [11, 25-27].

However, in disagreement with previous reports [28], ARV treatments did not show, in our study, effects on CD38 and HLA-DR expression levels on CD4⁺ T cells. This results were not so far discordant, recent findings show that new class of host directed therapies may be needed to reduce HLA-DR⁺ CD38⁺ CD4⁺ T cells number [29].

When the current trend of HIV infection shows a decline in sub-Saharan Africa with 40 % less HIV infection between 2001 and 2012, in our region (North Africa), estimated number of newly HIV infected has increased by more than 50 % [ONU SIDA 2013]. Our results raise the importance of biological monitoring of HIV infection by evaluating CD4⁺ T cells rate and viral load. If viremia can't be assessed, by lack of resources, measuring CD4⁺ and CD8⁺ T activation levels could be an interesting alternative as biological markers.

In conclusion, CD38 and HLA-DR expression within the CD4⁺ T cell increased notably in HIV infections patients and appeared to be linked to disease progression. These activation markers followed the same kinetics as the viral load. Assessing value of CD38 and HLA-DR on CD4⁺ T cells in monitoring disease activity could be interesting but remains to be determined in therapy response or as prognostic indicator.

Disclosure of interest: the authors declare no conflict of interest with regard to the article.

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