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CD11b-bearing mononuclear leucocytes and IgA levels in the staging of human immunodeficiency virus infection

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Abstract. Certain immunological parameters (i.e. low CD4+ T cell numbers, high serum soluble CD8) have been described as prognostic factors for the progression of human immunodeficiency virus (HIV) infection to later clinical stages. In the present study we have found in one hundred HIV-infected Spanish patients (81% drug abusers, 7% homosexuals, 6% heterosexuals, and 6% other or unknown risk groups) that CD11b+ peripheral blood mononuclear cells are increased in those with persistent lymphadenopathy as compared to other clinical stages (asymptomatic, AIDS-related complex and AIDS). Serum IgA was significantly increased in AIDS patients, and in patients at any other clinical stage who had concomitant infections (mainly mycobacterial and fungal). CD11b (an integrin with complement receptor functions) may thus be of clinical interest for the staging of HIV-infected patients, and reflect stage-selective immunological changes in mononuclear cell biology during HIV infection. High IgA on the other hand, would be a marker of concomitant infection as well as of disease progression. The results concern mostly drug addicts (the main risk group in Spain), but may apply to the other risk groups because no significant differences were detected between drug addicts ($n = 81$) and non-drug addicts ($n = 19$) for the studied variables ($p > 0.05$).
Key words. Monocytes; natural immunity; IgA; CD11b; human immunodeficiency virus.

In order to understand the pathogenesis and natural history of infection with the human immunodeficiency virus (HIV) it is important to identify factors that correlate with, and possibly contribute to, the outcome of the infection. Several epidemiological studies have confirmed the crucial role of reductions in CD4+ T cells and of increases in serologic markers of lymphocyte activation (soluble CD8, CD25 and β2microglobulin) in the progression of HIV infection[1, 2]. Phenotypic and functional changes in other immune cells, like monocytes, may also be of interest, particularly since this cell type can also be a target of HIV infection, as has indeed been previously reported[3]. We therefore studied one hundred HIV-infected Spanish patients to determine the changes

in subpopulations of peripheral blood mononuclear cells (PBMC), including lymphocyte and monocyte subsets, and immunoglobulin (Ig) levels, in different stages of HIV infection.

Methods

Subjects. HIV antibodies were detected by commercial ELISA (Abbott, Irving, TX) and Western blot (Dupont, Dreieich, Germany) techniques. One hundred HIV-infected patients were classified according to clinical criteria and risk group as previously described[4]. In brief, the patients were placed in one of the following clinical groups: (AS) asymptomatic, (PLA) persistent generalized lymphadenopathy, (ARC) AIDS-related complex (lasting high fever, weight loss, severe diarrhoea, oral moniliasis, multidermatomal Herpes zoster, hairy leucoplakia, persistent cough and/or breathing difficulties), or (AIDS) fully-developed acquired immunodeficiency syndrome (Kaposi's sarcoma, or B-cell neoplasia and opportunistic infections, or both). The following risk groups were recognized: drug addicts, homosexuals, heterosexual transmission, post-transfusion seroconversions, and others. Normal values for the immunological and cytological determinations were calculated in 200 HIV-uninfected individuals, and values outside the 5th and the 95th percentile were considered abnormal. In addition, a small sample of uninfected drug addicts (n = 20) was also studied.

Laboratory variables. Absolute lymphocyte and monocyte counts from venous blood were measured in a flow cytometer (Coulter, Hialeah, FL). Peripheral blood mononuclear cells (PBMC) were isolated using appropriate gradients (Lymphoprep: Nyegaard, Oslo, Norway) and stained with a fluorescein-conjugated F(ab')2 goat anti-mouse IgG (Kallestad, Austin, TX) after pretreatment with mouse monoclonal antibodies against CD3, CD4, CD8, CD11b or CD57 epitopes (OKT3, OKT4, OKT8, OKM1 and Leu7, respectively). OK-series monoclonals were purchased from Ortho Pharmaceuticals (Raritan, NJ), and Leu7 from Becton Dickinson (Mountain View, CA). Fluorescent cell percentages were counted in an optical epifluorescent microscope and absolute numbers calculated. This method does not allow the distinction between monocytes and CD11b+ lymphocytes. Cytospin samples were stained in parallel with Wright's stain to exclude granulocyte contamination of the PBMC cell samples (< 5%). Serum Ig levels were measured by rate nephelometry (Beckman, Brea, CA). Infection with *M. tuberculosis* and *C. albicans* was identified in secretions, biopsies or blood samples by standard microbiological techniques (microscopy, acid-fast and gram stains and/or specific culture procedures) or by automatic devices (Bactec: Becton Dickinson, Mountain View, CA).

Statistical analysis. Comparison of quantitative variables between the different clinical groups was performed with the unpaired Student's t-test and the Mann-Whitney test. Similar results were found with both tests for significance. Two-tailed tests were used, and p values less than 0.05 were regarded as significant. When percentages were compared, a χ^2 test was used.

Results

The patient distribution by risk groups in the studied sample was as follows: 81% drug addicts, 7% homosexuals, 6% heterosexuals, 4% post-transfusion seroconversions and 2% unknown. The distribution by clinical stage was the following: 27% asymptomatic (AS), 27% persistent lymphadenopathy (PLA), 26% AIDS-related complex (ARC) and 20% acquired immunodeficiency syndrome (AIDS).

The previously-described lymphopenia with low CD4+ T cells and high CD8+ T cells (reflected in the table by the low CD4/CD8 ratio), associated with AIDS, was also observed in our advanced disease subset (ARC and AIDS, table). In addition, a rise in monocyte counts, CD11b+ and CD57+ cells was found in PLA patients as compared to all other groups. These differences are not simply side effects of drug addiction, but changes due to the progress of HIV infection, since a control group of seronegative drug addicts tested for all the above-mentioned parameters differed from healthy individuals only in their higher IgG levels (1600 ± 800 mg% vs 1040 ± 623 in controls, $p < 0.01$) and CD8+ cell percentages (41% vs 30% in controls, $p < 0.01$. Lastly, *M. tuberculosis* and *C. albicans* infection were frequently di-

Subpopulations of peripheral blood mononuclear cells and immunoglobulin levels in HIV infection*

Number of patients	Asymptomatic 27	Persistent lymphadenopathy 27	AIDS-related complex 26	AIDS 20
Lymphocytes/µl	2082 ± 664	2211 ± 750	1586 ± 1338	1147 ± 897
Monocytes/µl	331 ± 219[a]	510 ± 245	377 ± 254	280 ± 110[b]
CD3 PBMC/µl	1954 ± 538	2067 ± 570	1413 ± 963	856 ± 538
CD4 PBMC/µl	1040 ± 307	949 ± 354	664 ± 162	189 ± 212
CD4/CD8 ratio	1.2 ± 0.5	1.09 ± 0.47	0.65 ± 0.39	0.23 ± 0.13
CD11b PBMC/µl	282 ± 186[b]	594 ± 309	389 ± 292[c]	369 ± 180[a]
CD57 PBMC/µl	181 ± 63	376 ± 215[e]	162 ± 112	143 ± 91
IgG (mg%)	2092 ± 944	2254 ± 648	2357 ± 1131	2197 ± 877
IgA (mg%)	263 ± 160	218 ± 108	265 ± 196	583 ± 323[d]
IgM (mg%)	234 ± 103	259 ± 13	273 ± 181	247 ± 141

*Mean values ± standard deviation. Statistical significance is only indicated for the monocyte count, CD11b, CD57 and Ig variables. [a] $p < 0.01$ vs PLA. [b] $p < 0.001$ vs PLA. [c] $p < 0.05$ vs PLA. [d] $p < 0.001$ vs all other groups. [e] $p < 0.01$ vs all other groups.

agnosed in AIDS patients (in 40% and 75% of the cases, respectively).

As shown in the table, all patients had high IgG serum levels (> 994 mg%), but only AIDS patients showed significantly higher IgA levels than other risk groups; indeed, 75% of AIDS patients had elevated IgA levels (> 310 mg%) vs 18% in all other groups ($\chi^2 = 22.16$, $p < 0.001$).

Discussion

Most (81%) of our patients were drug addicts, thus our results and their interpretation concern mainly this particular risk group. The size of each of the other risk groups was too small (<7 patients/group) to make meaningful statistical comparisons, but, taken together, they did not differ significantly for any of the studied variables from drug addicts ($p > 0.05$, see below); they may therefore show similar immunological features.

CD11b epitopes are expressed on the Mac-1/complement receptor 3 integrin carried by granulocytes, monocytes, large granular lymphocytes (including natural killer – NK – cells) and null cells, which mediate the so-called 'natural immunity'. Our isolation method excluded granulocyte contamination, as confirmed by Wright's stain of cytospin PBMC samples. Thus, our results suggest that mononuclear CD11b+ cells may be increased and/or activated in the PLA stage of HIV infection, perhaps due to a non-specific early immune response to avoid HIV replication. High numbers of monocytes were found in PLA patients and probably account in part for the rise in CD11b-bearing cells, since our immunofluorescence technique did not allow the differentiation of monocytes and CD11b+ lymphocytes. Also, NK cells were found to be increased in our PLA patients (as defined by the Leu7/HNK-1 monoclonal antibody), contributing in part to the CD11b+ cell rise. Other authors found an increase in NK cells (as defined by monoclonal antibodies HNK-1, B.73.1 and N.901) in PLA in Italian drug addicts[5], but CD11b expression was not analyzed.

Our results suggest a difference in the pattern of immune response to HIV infection between the AS and the PLA groups. Although longitudinal studies are needed to assess this point, the results may indicate that monocytes and NK cells (CD11b+ PBMC) are increased in numbers or are activated as infection progresses from the AS to the PLA stage. Indeed, CD11b+ monocytes were found by others to be decreased in the AS stage, particularly within the HIV antigen+ subset, as compared to seronegative controls[3]. However, CD11b+ PBMC counts dropped in our ARC and AIDS patients, and this may reflect an immune response failure and the progression of disease from the PLA stage onwards. In contrast, no CD11b expression changes have been observed in neutrophils of HIV-infected drug abusers[6], suggesting that the CD11b effect may be selective for mononuclear cells. In summary, CD11b expression dynamics (low in AS, high in PLA and dropping later in ARC and AIDS) may reflect profound and still largely unknown stage-selective immunological changes in PBMC biology as HIV infection progresses. Measurements of CD11b cells may thus be useful for staging purposes.

We think that the raised IgA levels in AIDS patients may be secondary to the frequent concomitant mycobacterial and fungal infections. Indeed, IgA levels correlated in the whole sample with the presence of concomitant infections (401 ± 292 mg% in infected vs 228 ± 126 mg% in non-infected patients, $p < 0.01$). Thus, a rise in IgA levels may be useful as a marker of disease progression and also alert the clinician to a possible concomitant infection in HIV-infected patients. The level of IgA is considered to be of prognostic value in HIV infection[2], and high serum IgA has also been found by others in both homosexual and drug addict AIDS patients[7]. The correlation of IgA levels with concomitant infections, however, has not been previously recognized.

Further studies with similar immunological markers are in progress in Spanish homosexuals to confirm the existence of the HIV infection features described here in this particular risk group.

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