RESEARCH NOTE

CD4 T Helper Lymphocytes and Antigen Presenting Cells in the Physiopathology of AIDS

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ANTIGEN PRESENTING CELL INFECTION AND PHENOTYPE IN HIV INFECTION

CD4+ T lymphocytes have long been shown to be the site of HIV replication (D Klatzmann 1984 *Nature 312*: 767-768), but monocyte/macrophages and dendritic cells, which express the CD4 receptor, were susceptible to harbor the virus as well. It was important to quantitate their infection, because they were thought to be a reservoir of HIV, and dendritic cells confer infection to CD4+ T lymphocytes optimally *in vitro*, particularly when they activate them during antigen presentation (M Pope 1995 *J Exp Med 182*: 2045-2056, D Weissman 1996 *J Exp Med 183*: 687-692), but their isolation is not as straightforward as that of lymphocytes. Quantitation of infected dendritic cells in the blood yielded conflicting results, probably due to differences in isolation methods (SE Macatonia 1990 *Immunology 71*: 38-45, PE Cameron 1992 *Clin Exp Immunol 88*: 226-236, E Karhumäki 1993 *Clin Exp Immunol 91*: 482-488). Interaction between immune cells as well as HIV replication are very active in secondary lymphoid organs rather than in blood (K Tenner-Racz 1986 *Am J Path 123*: 9-15, J Embretson 1993 *Nature 362*: 359-362, G Pantaleo 1993 *Nature 362*: 355-358). Therefore we chose to quantify infected cells from the spleen, which is a major secondary lymphoid organ.

Six patients with advanced HIV disease (CD4 numbers <250/mm^3) were studied (D McIlroy 1995 *J Virol 69*: 4737-4745, D McIlroy 1996 *Res Virol 147*: 115-121). They had been splenectomized for therapy of idiopathic thrombopenic purpura, Castleman's syndrome or macrophage activation syndrome (Pr JP Clauvel, Dr E Oksenhendler, Hospital St Louis, Paris, France). Dendritic cells freshly isolated from the spleen express low levels of CD4; most of them do not express activation markers like CD83 or CD86, nor CD1a, which is a marker of Langerhans cells in the epithelia. Their isolation was based on negative selection of the other lineages. Pure populations were obtained after a final sorting by fluorescence activated cell sorter, on the basis of a HLA-DR+++, CD2, 20, 16 and 14-negative phenotype. CD4+ T lymphocytes and CD14+ monocyte/macrophages were purified by positive magnetic bead cell sorting. The frequency of HIV infected cells was quantified by limiting dilution PCR: purified cells were diluted into non-infected cells, ten replicates of each dilution were amplified using a sensitive nested PCR specific for HIV *env* DNA, and the proportion of infected cells in each purified cell population was determined using the Poisson law. Spleen dendritic cells were indeed infected by HIV, but with a low frequency (1/3000) compared to that of CD4+ T lymphocytes (1/60). Monocyte/macrophages were not a quantitatively important reservoir, since only 1/37000 CD14+ cells and 1/2100 adherent cells (which contained approximately 70% monocyte/macrophages) carried HIV DNA (McIlroy 1995 *loc. cit.*).

Despite this low infection frequency, a defect in antigen presentation was suggested in HIV-infected patients. Particularly, a defect in Langerhans cell-mediated allogeneic antigen presentation was found in identical twins discordant for HIV infection (A Blauvelt 1995 *J Immunol 154:* 3506-3515). A dendritic cell depletion or a defect in the expression of HLA or costimulatory molecules at their surface could be responsible. A rare event analysis of dendritic cells from the spleen was set up in the laboratory using three-color flow cytometry. Dendritic cells were not depleted, since they made up 0.6±0.4% (n=6) of mononuclear cells in HIV+ patients vs. 0.8±0.5 (n=20) in normal organ donors (spleen samples provided by Dr B Barrou, Transplantation Unit, Pitié-Salpêtrière Hospital). They were not more activated in HIV patients than in controls: most of them were CD80 negative and expressed only low levels of CD86 or CD40. They retained the ability to mature in culture, i.e. to express very high levels of CD80, 86, CD40 and MHC class II molecules. However, CD83, which

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is not expressed on most dendritic cells *ex vivo*, but is upregulated after *in vitro* maturation, was significantly less upregulated in HIV patients. CD83 is a member of the immunoglobulin superfamily which is preferentially expressed by dendritic cells. It has a potential role in antigen presentation which needs to be confirmed (RJ Armitage 1996 *Tissue Antigens 48*: 453). Macrophages and B lymphocytes were normal in HIV patients. Our results (D McIlroy 1998 *AIDS Res Hum Retrovir 14*: 505) imply that a potential defect in antigen presentation during the course of HIV infection would not be due to a defect in the expression of CD40, 80, 86 and MHC class II molecules on antigen presenting cells.

RECOVERY OF CD4+ T CELL FUNCTION AFTER HIGHLY ACTIVE ANTIRETROVIRAL THERAPY (HAART)

Higly effective combined antiretroviral therapies have now proven able to block any detectable HIV replication. CD4 T cell absolute cell numbers increased after HAART, but the first question was to know whether they were functionnally active against pathogens, especially HIV. Eight previously untreated patients with a mean CD4 cell count of 164±86 /mm^3 were treated with ritonavir, AZT and DDC (B Autran 1997 *Science 154*: 1408-1417). Their mean plasma viral load (HIV RNA copies/ml) decrease was 1.9±0.6 log. Their CD4 cell counts increased to 327±74/mm^3 at day 15, and they steadily increased for the next 12 months with a mean linear slope of +10%, and a three phase pattern: (1) an early rise of memory cells (CD45RO+, CD45RA-) during the first four months of the study, with a recovery of competent CD4+CD28+ cells; (2) a decrease in expression of T cell activation markers (HLA-DR, CD25, the IL-2 receptor a chain, and CD38); (3) between months 4 and 12 after initiation of treatment, a two-fold increase of the absolute number of functional naive CD4 cells, characterized by the expression of CD45 RA and CD62L: the proportion of CD4+45RA+ cells expressing CD69L was 23±25% before treatment, and 62±25% after 12 months of treatment, compared to normal values of 96±3% in normal donors. On the other hand, a subpopulation of terminally differentiated CD4 cells, which do not express CD7 and was found to be increased and activated in HIV patients (B Autran 1995 *J Immunol 277*: 112-116), was decreased after HAART. This subpopulation is interesting because it has a TH0/TH2 cytokine secretion profile, i.e. preferential secretion of IL-4 and IL-10, low secretion of IL-2 and lower proliferation as compared with CD4+CD7+ cells, and therefore it might participate in the impaired cytokine secretion profile observed during infection (Autran 1995 *loc. cit.*).

In a second study in six non-pretreated patients with similar viral loads and CD4 cell counts, the function of CD4 cells was assessed after treatment with indinavir and AZT or D4T + 3TC (B Autran 1997 *Science 154*: 1408-1417). At initiation of treatment, no proliferative responses against recall antigens from two major opportunistic agents, CMV and PPD, were found. After six months of treatment, proliferative responses gradually appeared against CMV and PPD proteins, reaching normal ranges (mean normal indexes: 50±40) for three of the six patients. Recovery seemed to correlate with a viral load decrease of at least two logs and a gain in CD4+28+ immunocompetent cells.

In conclusion, these results point to the central role of HIV replication in the pathogenesis of the immune deficiency, rather than to indirect, non-viral mechanisms that were invoked before HAART allowed to generate data on the dynamics of HIV infection (X Wei 1995 *Nature 373:* 117-122, DD Ho 1995 *Nature 373*: 123-126). CD4+ T lymphocytes are definitely the most frequently infected cells. However, many questions remain to be addressed, particularly on the potential recovery of HIV-specific responses even in advanced disease, and on the role of the rarely infected dendritic cells and macrophages as relays of T cell infection upon antigen presentation, during the first hours and during the course of the disease.

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