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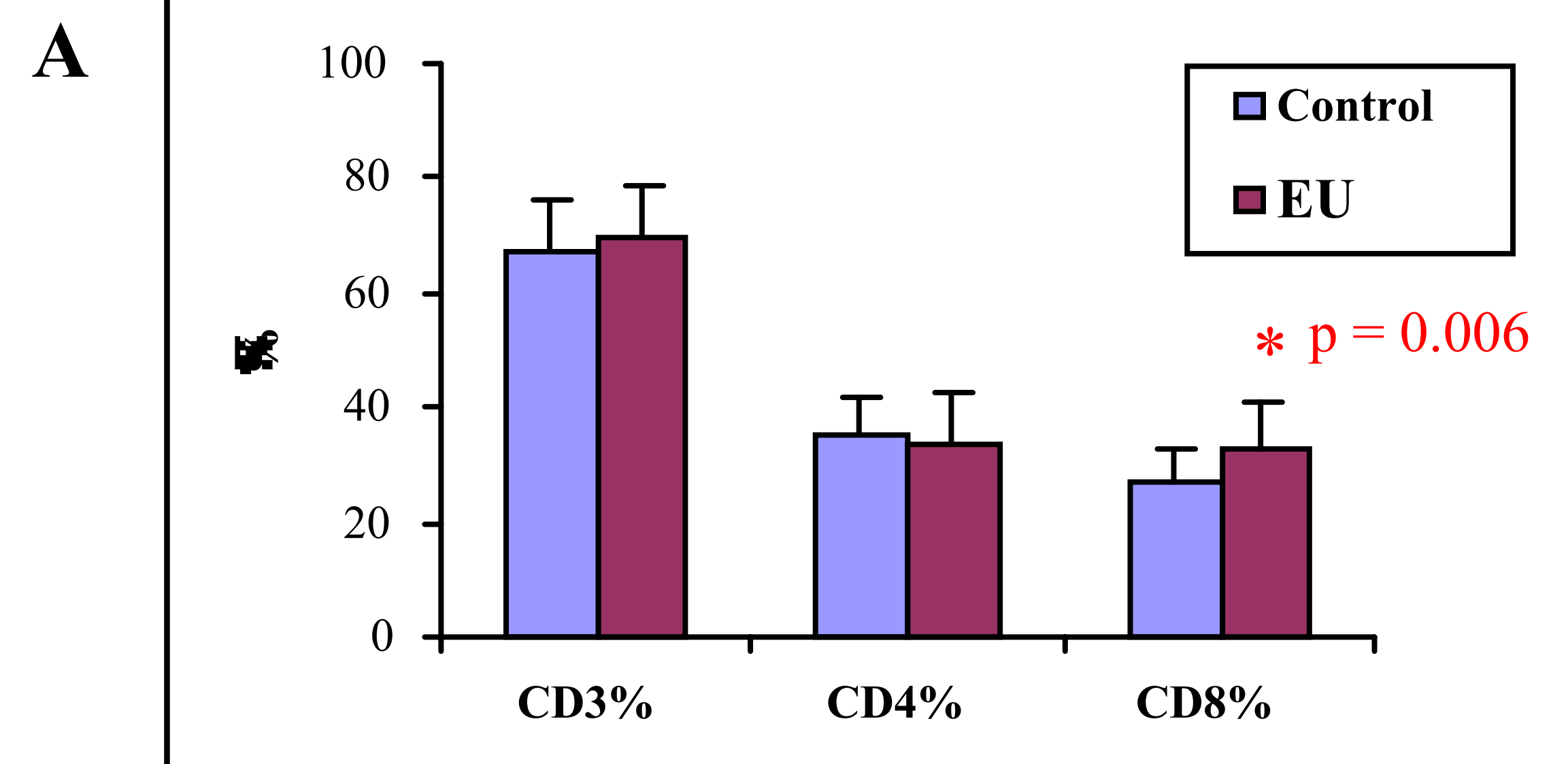
Background : Most studies on HIV-1 exposed seronegative individuals are focusing on HIV-specific immune responses in sexually exposed populations. This study aimed to analyse immune factors of protection against HIV-1 in intravenous drug-users who are at high risk of infection by systemic route not only for HIV but also for other blood transmitted pathogens. These individuals, therefore, are susceptible to have a persistent activation of their immune system. We identified a population of HIV-exposed uninfected individuals (EUs) among intravascular drug users (IVDUs) in Ho Chi Minh City (Vietnam). A very high prevalence (near to 100%) of other viral infections, such as HBV, HCV, HTLV-1 was found in both uninfected or infected IVDUs. None of the IVDUs were presenting the $\Delta 32$ mutation of the HIV co-receptor CCR5.

Methods:

► Forty-five EUs and fifty low risk uninfected voluntary blood donors, as a control group, were included in our study.
 ► Peripheral blood lymphocyte subsets and activation markers were analyzed by flow cytometry on whole blood.
 ► Peripheral blood mononuclear cells (PBMC) or CD4+ cell susceptibility to HIV infection was evaluated by infecting PHA-activated cells with a Vietnamese IVDU primary HIV-1 isolate (132W) or with X4 (NL-4.3) or R5 (BaL) HIV-1 strains.
 ► Homologous CD8+ cells, activated with anti-CD3 and anti-CD28 antibodies, were added to infected CD4+ cells at various CD4:CD8 ratios to evaluate their role in virus suppression.

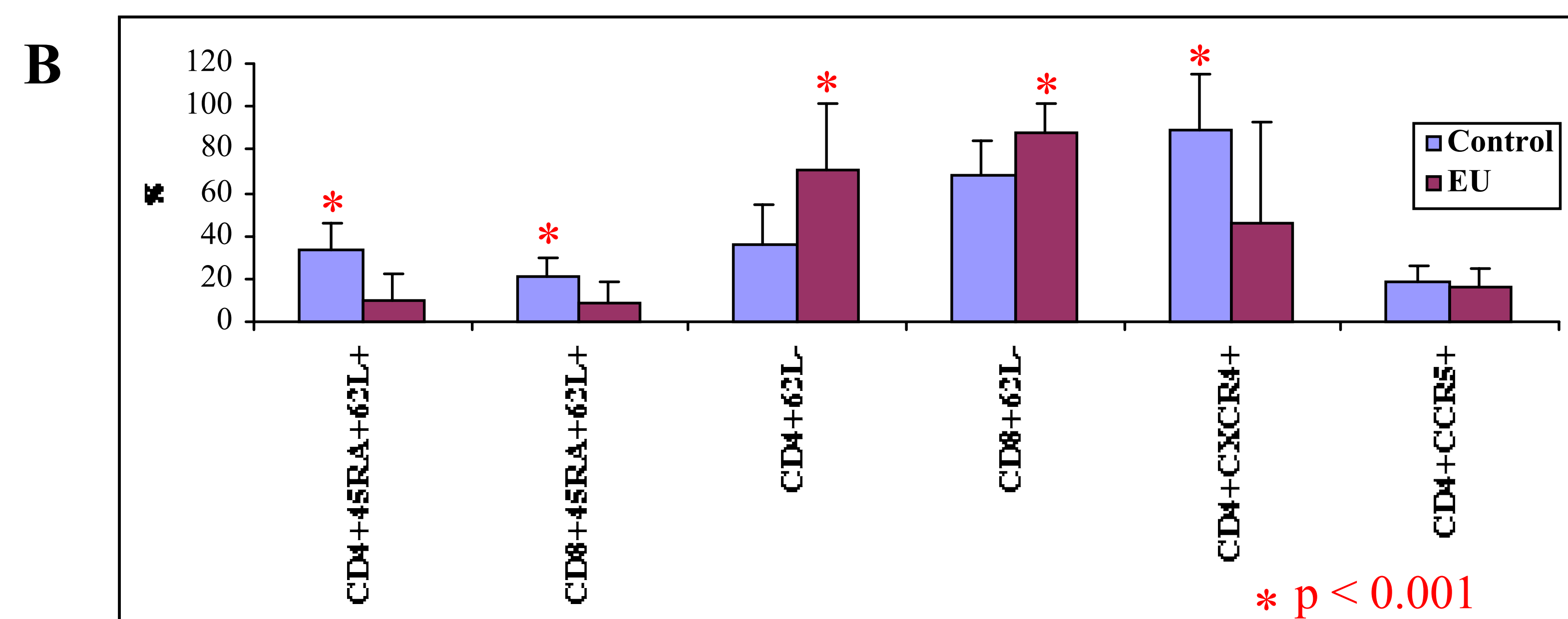
RESULTS

> Higher T CD8 cell proportion and T CD8 and T CD4 memory phenotypes in EUs



A. Total lymphocyte count and CD3+ cell proportion were similar in EUs and controls. T CD8+ cell proportion was higher in EUs.

B. T CD8+ and T CD4+ cell phenotypes indicate a greater proportion of memory cell sub-populations in EUs than in the control group



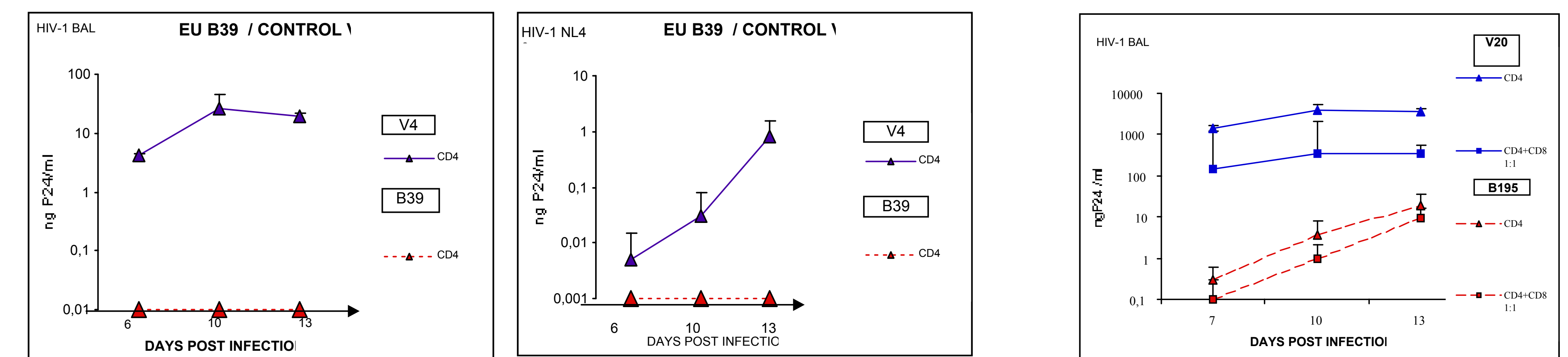
> Relative resistance of EUs PBMC to HIV-1 infection

In vitro infectivity assays showed a reduced susceptibility to HIV-1 infection in EUs PBMC compared to controls PBMC. PBMC from 21 out of 45 EUs (46.66%) showed a reduction of at least 1 log in p24 production compared to a reference stock of PBMC after infection with at least one of the 3 viruses tested. In contrast, only 5 out of 50 control PBMC (10%) showed a similar resistance to HIV-1 infection. Eight EUs and 1 control PBMC were resistant to 2 or 3 viruses.

> Different mechanisms are involved in PBMC resistance to HIV-1 infection

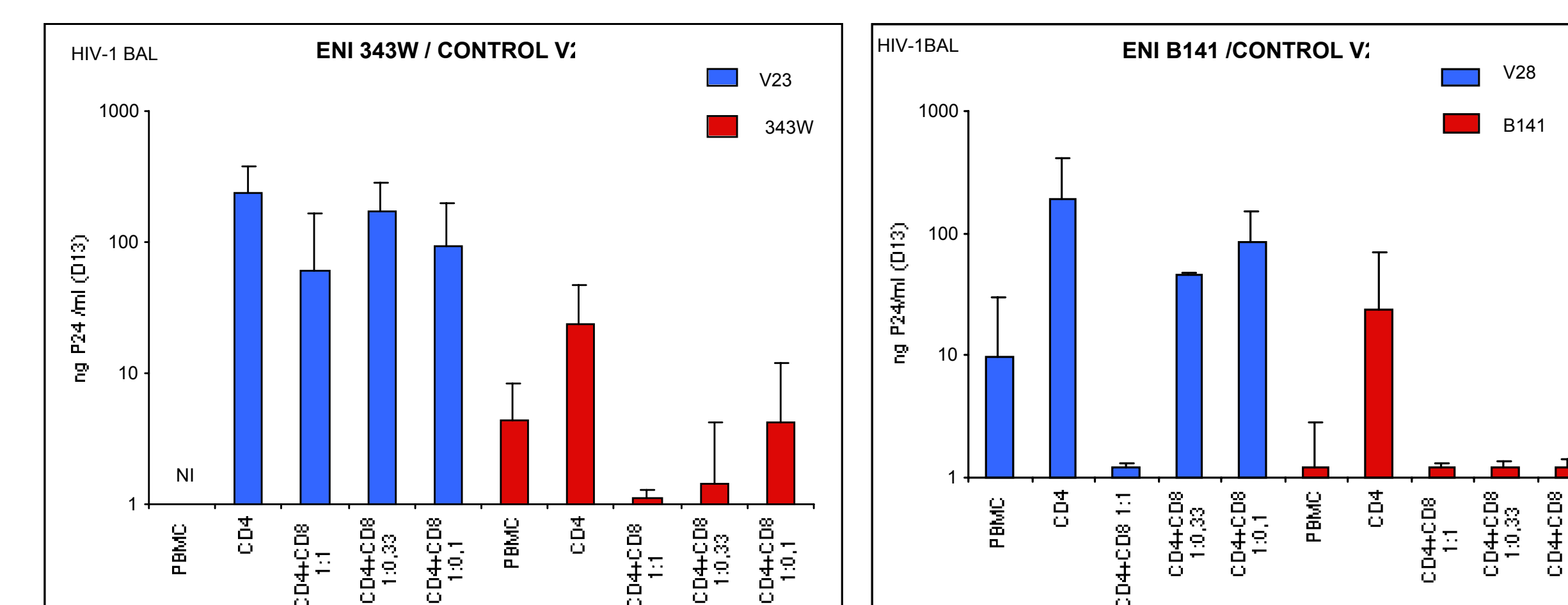
The role of CD4+ and CD8 cells in *in vitro* resistance to HIV-1 infection was analyzed in 13 resistant EUs PBMC.

1. Restriction of virus replication in CD4+ cells



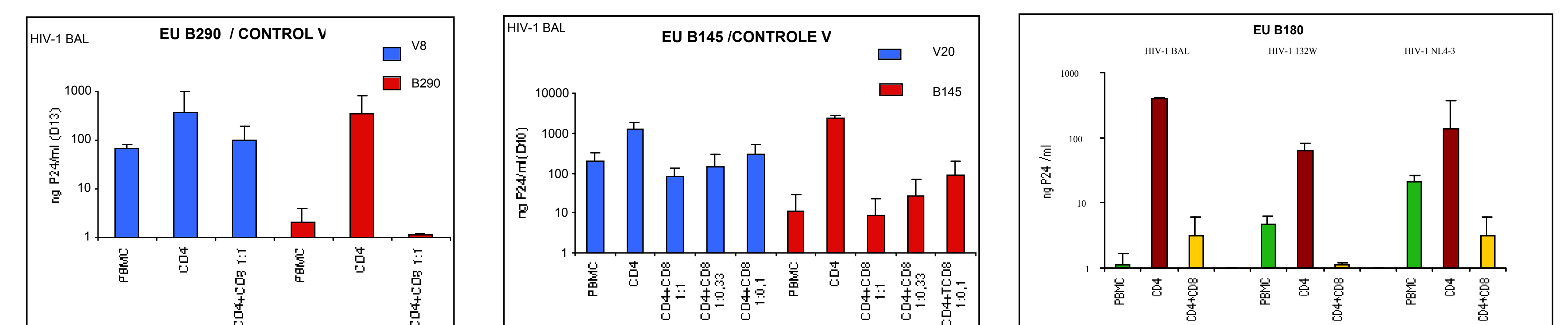
In three case, CD4+ cells were refractory (B39) or scarcely susceptible (B195) to HIV-1 infection

2. Concurrent CD4+ cell low susceptibility and CD8+ cell suppression of HIV-1



In two cases, both a low permissivity to infection of CD4+ cells and an inhibitory action of CD8+ cells appeared to contribute for PBMC resistance to HIV-1

3. CD8+ cell suppression of HIV-1 infection



- In most cases (8 of the 13 tested) the resistance of PBMC was related to a suppression of CD4+ cell infection by CD8 cells.
- In some cases (ex; B290 and B145) the inhibition was efficient only with HIV-1 R5 strains, suggesting a role of β -chemokines. Indeed, PBMC which showed a low susceptibility to BaL secreted more RANTES than Bal susceptible PBMC ($p = 0.038$).
- In other cases, such as B180, both R5 and X4 viruses were inhibited by CD8+ cells, suggesting a suppressive mechanism independent from virus entry.

CONCLUSIONS

- Our results indicate that resistance to HIV-1 infection in vietnamese EU IVDUs is associated with a reduced susceptibility of PBMC to HIV-1 infection. This *in vitro* resistance is related either to secreted anti-viral factors or to a refractory state of HIV-1 target cells.
- Taken together, data from lymphocyte phenotypic analysis and infectivity assays suggest that multiple mechanisms may account for the natural protection against HIV-1 in these individuals. These mechanisms include a CD4+ cell refractory state to HIV-1 or anti-viral defenses associated to the activation of compartments of the immune system as suggested by the expansion of CD8+ memory cell population in ENI.