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Presence of HIV-1 Nef specific CD4 T cell response is associated with non-progression in HIV-1 infection

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Abstract

The *ex vivo* response to three HLA-DR-restricted Nef peptides (Nef 66–97, Nef 133–159, Nef 180–202) and one HLA-DQ-restricted Nef peptide (Nef 56–68) was evaluated in 28 HIV-seropositive patients and 6 Long-term Non-Progressors (LTNPs). Analyzing specific proliferative response and IFN-γ secretion, patients were identified as high responders, medium responders and non-responders to peptides. As high responder patients, LTNP patients showed strong proliferative response to all the Nef-peptides as strong IFN-γ secretion. Twenty-four months later, all high responder patients were always without antiretroviral treatment whereas 50% of medium responders and at least 66% of low responder patients followed bi-therapy. CDC classification confirmed also unfavourable evolution for these two last groups. All high responder patients conserved stable CD4 counts, proliferative response to Nef peptides as strong IFN-γ secretion during this 24-month period. So, early good T CD4 response to peptides of the Nef protein could thus be regarded as a factor of good prognosis in HIV infection and a tool of importance in the decision to put or not a patient under treatment.

Keywords: CD4 epitope; Peptide; Protection

1. Introduction

Immunotherapeutic strategies for human immunodeficiency virus type 1 (HIV-1) infection that can delay or ultimately prevent disease progression to acquired immunodeficiency syndrome (AIDS) represent a critical development towards the future management of HIV-1 infection. It is now clear that helper activities of antigen-specific $CD4^+$ T cells appear critical in maintaining HIV-1 suppression [1]. So, the lack of $CD4^+$ T cell responses observed in most HIV-infected individuals could then explain the decline of cytotoxic T lymphocyte (CTL) response seen over time and the progression of the disease [2]. Numerous studies show that in Long-term Non-Progressor patients (LTNPs) and in patients treated with highly active antiretroviral therapy (HAART) early during primary infection, enhanced $CD4^+$ T helper (Th) cell response to HIV-1 is associated with high levels of HIV-1-specific CTL and with lower viral loads [3–5]. By contrast, most untreated persons with recent seroconversion or with chronic HIV-1 infection have low to undetectable levels of HIV-1-specific Th cells, particularly when measured in vitro by antigen-induced lymphoproliferation [6–8].

However, less is known of the characteristics of CD4 epitopes in HIV. This reflects, in part, the difficulty in identifying CD4 T helper responses in HIV-1-infected subjects, due to either dysfunction or deletion of specific CD4 T cells, and also due to the lower frequencies of CD4 T cell responses compared to CD8 T cell responses [9,10]. CD4 T cell responses found in LTNPs and treated patients

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are predominantly directed against Gag epitopes [11,12]. However, little is known about the role of Th responses to epitopes expressed by other HIV-1 genes and how these responses evolve in early infection and following treatment. Such findings could support efforts to improve immunity against HIV-1 infection by augmenting the Th responses. Peptide-based immunotherapeutic strategies offer considerable advantages over conventional approaches, particularly regarding safety. Peptide design itself is becoming increasingly sophisticated, with the rapid evolution of bioinformatics tools that can analyse not only T cell epitopes, but also their potential for successful presentation on diverse human leucocyte antigen (HLA) class I or II following intracellular processing by antigen-presenting cells (APCs) [13]. Moreover, by targeting conserved viral domains, peptide acquired improved reactivity to diverse viral strains. Our first search for Nef-derived epitopes identified the peptide 56–68 as the first promiscuous HLA-DQ HIV-derived peptide capable to induce HIV-specific memory $CD4^+$ T cells producing IFN-γ in all the healthy donors tested [14]. In this paper, we selected three Nef-derived epitopes as HLA-DR-restricted helper epitopes with the use of TEPITOPE software. We tested the corresponding peptides in cocktail with the HLA-DQ-restricted Nef peptide for their capacities to stimulate *ex vivo* CD4 response in treatment-naïve asymptomatic patients and in LTNPs which were followed over a 24-month period. By analyzing specific cytokine and proliferative responses we correlated strong cellular responses to these Nef-derived peptides with non-progression in infection. So, a good T CD4 response to peptides of the Nef protein could thus be regarded as a factor of good prognosis in HIV infection and a tool of importance in the decision to put or not a patient under treatment.

2. Material and methods

2.1. Synthetic peptides

Nef 56–68 (AWLEAQEEEEVGF) (peptide Nef-4), Nef 66–97 (VGFPVTPQVPLRPMTYKAAVDLSHFLKEKGGL) (peptide Nef-2), Nef 133–159 (VRYPLTFGWCYKLVPVEPDKVEEANKG) (peptide Nef-3), Nef 180–202 (VLEWRFDSRLAFHHVARELHPEY) (peptide Nef-1), Gag 253–284 (NPPIPVGEIYKRWIILGLNKIVRMYSPTSILD) were synthesized on an Advanced Chemtech model 357 MPS Synthesizer (Advanced Chemtech Europ, Brussels, Belgium). Homogeneity was confirmed by analytical HPLC.

2.2. Study subjects

PBMCs from HIV-infected individuals recruited from the Centre d'Information et de Soins de l'Immunodéficience Humaine (CISIH, Tourcoing, France) were obtained for this study with written informed consent. The clinical status, the $CD4^+$ cell counts and the viral RNA loads from patients are summarized Table 1. Group A represented 28 asymptomatic patients, HIV-seropositive for less 6 years and that have never received any antiretroviral therapy. Group B includes 6 patients seropositive for more than 8 years and defined as treatment naïve Long-term Non-Progressors (LTNPs).

2.3. Lymphoproliferative assay

PBMC were isolated from fresh whole blood by Ficoll-Hypaque (Sigma–Aldrich, St. Louis, MO) density gradient centrifugation within 16 h of venipuncture. PBMC were then plated at 10^5 cells/well into six replicate wells of a 96-well plate containing PHA (1 μg/ml), Nef (Nef 1, 2, 3, 4) or Gag peptides, cocktail of Nef peptides (Nef-cocktail) (5–25 μg/ml) or medium alone (RPMI-1640 (Sigma–Aldrich) supplemented with penicillin-streptomycin, HEPES buffer, L-glutamine, sodium pyruvate and 10% human AB serum (ML-10). After 5 days, each well was pulsed with 1 μCi [^{3}H] thymidine for 6 additional hours before harvesting of cells onto glass fiber filters using the Packard Filtermate Harvester. ^{3}H incorporation was measured by TopCount scintillation counter (Packard Instruments, Meriden, CT). A stimulation index (SI) was calculated for each Ag as the cpm of stimulated wells divided by the cpm of control wells. For categorical analysis, an SI of ≥ 5 was considered significant.

2.4. Ex vivo immunization

30×10^6 PBMCs of patients defined as non-responders in our study (S2.15, S2.17, S2.21, S2.28, S2.29) were *ex vivo* immunized for 8 days at 37 °C with 50 μg/ml of Nef-cocktail in 15 ml of ML-10 and then tested for proliferative activity as described above.

2.5. Cytokine detection

IL-2, IL-4, IL-5, IL-10 and IFN-γ in culture supernatants of PBMCs stimulated 24 h with PHA (5 μg/ml) or 5 days with Nef-cocktail or Gag peptide (50 μg/ml) were detected using sandwich ELISA. The antibody pairs used for the detection were provided by BD PharMingen (San Diego, CA, USA). Absorbance at 492 nm was measured using a multi-channel spectrophotometer (Titertek Multiskan MCC 1340). Results were expressed as the mean of duplicate wells after subtraction of the background.

3. Results

3.1. Selection of HLA class II restricted epitopes in Nef protein

TEPITOPE is a prediction model used to predict promiscuous and allele-specific DR-restricted T cell epitopes in silico [15]. We applied TEPITOPE to analyze the sequence of