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Research paper

A new antigen scanning strategy for monitoring HIV-1 specific T-cell immune responses

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ABSTRACT

Delineation of the immune correlates of protection in natural infection or after vaccination is a mandatory step for vaccine development. Although the most recent techniques allow a sensitive and specific detection of the cellular immune response, a consensus on the best strategy to assess their magnitude and breadth is yet to be reached. Within the AIDS Vaccine Integrated Project (AVIP http://www.avip-eu.org) we developed an antigen scanning strategy combining the empirical-based approach of overlapping peptides with a vast array of database information. This new system, termed Variable Overlapping Peptide Scanning Design (VOPSD), was used for preparing two peptide sets encompassing the candidate HIV-1 vaccine antigens Tat and Nef. Validation of the VOPSD strategy was obtained by direct comparison with 15mer or 20mer peptide sets in a trial involving six laboratories of the AVIP consortium. Cross-reactive background responses were measured in 80 HIV seronegative donors (HIV-), while sensitivity and magnitude of Tat and Nef-specific T-cell responses were assessed on 90 HIV+ individuals. In HIV−, VOPSD peptides generated background responses comparable with those of the standard sets. In HIV-1+ individuals the VOPSD pools showed a higher sensitivity in detecting individual responses (Tat VOPSD vs. Tat 15mers or 20mers: $p \leq 0.01$) as well as in generating stronger responses (Nef VOPSD vs. Nef 20mers: $p < 0.001$) than standard sets, enhancing

Abbreviations: VOPSD, Variable Overlapping Peptide Scanning Design;ELISpot, enzyme-linked immunospot assays;SFU, spot forming units;ICS, intracellular cytokine staining;CTL, CD8 cytotoxic T-cell.

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both CD4 and CD8 T-cell responses. Moreover, this peptide design allowed a marked reduction of the peptides number, representing a powerful tool for investigating novel HIV-1 candidate vaccine antigens in cohorts of HIV-seronegative and seropositive individuals.

1. Introduction

The growing knowledge of how the human immune system deals with different types of pathogens has allowed us to better define the immune correlates that lead to the protective effects exerted by efficacious vaccines. Therefore, delineation of the immune correlates of protection occurring during natural infection, or following vaccination, has become a fundamental step in the development of new vaccines. Indeed, induction of strong and durable T-lymphocyte responses against HIV-1-infected cells has become an important immunogenicity outcome in vaccine trials, given the increasing evidence that HIV-1-specific cell-mediated immune responses partially protect macaques experimentally infected with SIV or SHIV (Schmitz et al., 1999; Barouch et al., 2000) and contribute substantially to the control of HIV-1 disease in humans (Koup et al., 1994; Mazzoli et al., 1997; Musey et al., 1997; Ogg et al., 1998). Therefore, methods for detecting both CD4 and CD8 T cell responses have been significantly improved by the introduction of cytokine enzyme-linked immunospot assays (particularly IFN-γ ELISpot) and intracellular cytokine staining (ICS) assays (Schmittel et al., 1997; Betts et al., 2001b). This has led to more sensitive detection and quantification of the cellular immune response but, despite greater ease and rapidity of assessment there is not yet a consensus for assaying the magnitude and the breadth of T-cell dependent adaptative responses. In this regard, two strategies are currently used to define T-cell epitopes: bioinformatic approaches utilizing algorithms to predict epitopes containing peptide-binding 'motifs' and 'supermotifs' for the major histocompatibility complex (MHC) classes I and II molecules (De Groot et al., 2003) or via overlapping peptides that span the viral proteins of interest (Betts et al., 2001b; Addo et al., 2003; Draenert et al., 2003). In this latter case the length and degree of overlap of peptides are the subject of scientific debate (Betts et al., 2001b; Draenert et al., 2003; Draenert et al., 2004). The use of computer-assisted bioinformatics approaches, based on HLA peptide binding motifs and available viral sequences, has been shown to be a useful tool for identifying CD8 cytotoxic T-cell (CTL) epitopes derived from HIV-1(De Groot et al., 2003). However, since peptide binding is not the sole determinant of epitope dominance, not all CTL epitopes will be identified by HLA motif-screening algorithms (Draenert et al., 2003; Beattie et al., 2004). On the other hand, the methods based on a comprehensive screening of HIV-1 encoded antigens via overlapping peptide pools are attractive because they make no assumptions about peptides that do not conform exactly to the predicted peptide-binding motif, although they still do not detect all of the T cell responses present in HIV-1 infected individuals (Beattie et al., 2004). The main limitation to this type of screening assay is that a consensus on the best design with regards to peptide length and overlap has not been reached yet. Indeed, the length and degree of peptide overlap not only influences the detection of T-cell responses but also has a major impact on cost, labor intensiveness and amount of blood needed to perform such studies. Therefore, the identification of an optimal peptide design strategy represents a first priority for feasible study design in the context of vaccine clinical trials. Optimal CD8 CTL epitopes are 8–11 amino acid (aa) residues in length, and, therefore, an overlap of at least 10 aa between adjacent overlapping peptides is required to ensure that no 11-mer is missed in the screening process. Increasing the number of overlapped aa (11 vs 10 aa) has not produced a clear advantage in detecting CTL responses (Draenert et al., 2003), although it does result in substantial increase in the number of peptides necessary to perform a complete HIV-1 peptide-scan (746 vs 600). A documented disadvantage of overlapping peptides is that epitopes placed in the middle of longer peptides may not be always detectable because of suboptimal epitope presentation. This implies that longer peptide sets (e.g. 20-mers) may be less sensitive in detecting CD8 T-cell responses (Betts et al., 2001b; Maecker et al., 2001; Draenert et al., 2003), whereas they seem to be more efficient in resolving class II restricted CD4 T-cell responses (Draenert et al., 2003). Furthermore, even overlapping 15-mers are less efficient than optimized epitopes in detecting low frequency CD8 responses (200 spot forming units [SFU]/million PBMC), when the epitope is located centrally in the peptide (Beattie et al., 2004).

A novel strategy that combines database information with a systematic peptide scanning has been described (Draenert et al., 2003) which takes advantage of the well established notion that only a minority of the 20 possible aa residues at the peptide C-terminus, a primary anchor position that contributes substantially to binding of the peptide to the MHC class I molecule will be present in CD8 CTL epitopes (only 9 of them served as the C-terminal anchor position in 96% of described optimal epitopes and in 95% of peptide-binding motifs described for over 60 HLA class I alleles). The direct comparison between "gold standard" 15/11 and 15/10 overlapping residue peptide sets has demonstrated the efficacy of this approach that allowing the use of longer peptides (18mers) without any loss in magnitude and/or breadth of the immune response, permits a substantial reduction in the number of peptides needed (410 vs 746 for a complete HIV-1 genome scan). Although an optimal overlapping set has still to be obtained (13% of the CD8 CTL responses were not shared between different peptide sets), this result suggests that more rational approaches to overlapping peptide design are feasible and they might represent an interesting alternative to the completely empirical strategy. In this study we present a novel strategy for T-cell epitope screening that, without compromising the detection of the CD4 T-helper responses, significantly enhances the investigation of CD8 CTL responses while at the same time reducing both the amount of labor required and assay costs.

Therefore, we propose this peptide design strategy as feasible for the screening of HIV-1 candidate vaccine antigens in large cohorts of HIV-seronegative and seropositive individuals.