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Cellular Immune Responses and Susceptibility to HIV-1 Superinfection: A Case-Control Study

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Abstract

A case control study was performed to determine the effects of HIV-1-specific cellular immune responses on the odds of acquiring a second HIV-1 infection (superinfection). Changes in the frequency of cytokine-producing or cytolytic CD8+ or CD4+ T cells were not associated with significant alterations in the odds of superinfection, suggesting that HIV-1 specific cellular immune responses at the level induced by chronic infection do not appear to significantly contribute to protection from HIV-1 superinfection.

Keywords

HIV-1; cellular immunity; T cells; superinfection; re-infection

Despite 25 years of research, relatively little is known about the ability of HIV-1-specific immune responses to modulate the risk of infection in humans exposed to HIV-1. While many studies have shown that CTL contribute to the control of an established HIV-1 infection [1], their role in preventing new infections are challenging to ascertain. Aside from vaccine trials, studies of superinfection provide one of the only settings to evaluate the protective role of HIV-1-specific immune responses in humans exposed to diverse, circulating strains. HIV-1 superinfection occurs when an individual infected with one strain of HIV-1 becomes infected with a second strain from a different source partner [2]. The protective role of pre-existing HIV-1-specific immunity in the setting of superinfection remains unclear. In six superinfected individuals examined in prior cases studies, at least some of the targeted cytotoxic T lymphocyte (CTL) epitopes were altered in the superinfecting strain in comparison to the initial strain, potentially contributing to the ability of the superinfecting strains to establish infection despite the ongoing CTL response [3–7].

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However, no prior studies of superinfection have compared the breadth, magnitude, or polyfunctionality of the T cell responses between superinfected individuals and individuals who were not superinfected. To better define correlates of protection from a second HIV-1 infection, we undertook the first case-control study to assess HIV-1-specific cellular immune responses in HIV-1-infected women with continued exposure to HIV-1, some of whom went on to become superinfected.

The superinfected cases (n=12) and non-superinfected controls (n=36) were previously identified among 56 women enrolled in a prospective cohort study of high-risk women [8–10]. Informed consent was obtained from all participants, and the ethical review committees of the University of Nairobi, the University of Washington, and the Fred Hutchinson Cancer Research Center approved this study. None of the individuals were treated with antiretroviral medications or were in an advanced state of immunosuppression at the time of superinfection. Controls (3 per case) were matched to cases according to the HIV-1 subtype, the timing of samples in relation to initial infection, and the viral load, as described previously [11]. Cases and controls had similar levels of ongoing reported risk for exposure to HIV-1 during the follow-up period, with an average of 1.46 sex partners per week for the cases and 1.42 sex partners per week for the controls. The sample dates ranged from 21 to 1150 days (median 285 days) prior to documented superinfection.

We determined the frequency of antigen-specific CD8+ and CD4+ T cell responses by intracellular cytokine staining (ICS) following stimulation with HIV-1 and CMV peptide pools, as described [12, 13]. The CMV pp65 peptides, HIV-1 potential T cell epitope (PTE) gag peptides, and HIV-1 PTE env peptides were obtained from the AIDS Research and Reference Reagent Program, NIH; PMA/ionomycin (Sigma) was used as a positive control. The following antibodies were used: CD107a-PE-Cy5, CD4-Alexa700, CD8-PerCP-Cy5.5, GranzymeB-Alexa700, IFN- γ -PE-Cy7, IL-2-APC, MIP-1 β -PE, TNF- α -FITC (BD Biosciences); CD3-Qdot605 (Molecular Probes). The percentage of CD8+ and CD4+ T cells producing the cytokines IL-2, TNF- α , IFN- γ , and MIP-1 β was determined. Cytolytic activity was also assessed based on expression of CD107a, using Granzyme B expression to confirm that degranulating CD107a+ CD8+ T cells had cytolytic potential [14]. Data were analyzed using FlowJo (version 9.1; Tree Star, Inc.), and background from wells stimulated with DMSO alone was subtracted. Samples were rejected for 10 of the 48 subjects for low viability (<50% after resting and stimulation) and/or for low cell numbers (<1000 live CD4+ or CD8+ T cells), leaving 10 superinfection cases and 28 controls. Of the superinfection cases evaluated, three were superinfected with a virus of the same subtype (intrasubtype superinfection), while seven were intersubtype superinfections. Cytokine production and cytolytic activity were similar to that observed in other cohorts of chronically HIV-1 infected individuals [15–20].

To determine whether individuals with deficits in T cell functions were more likely to be superinfected, the odds of superinfection were evaluated in relation to T cell function by exact conditional logistic regression (Table 1). For cases, analyses were performed using samples collected at the visit prior to documented superinfection in order to assess immunity at the time point most relevant in terms of exposure to, and lack of protection from, the second virus. The samples for the controls were taken from approximately the same time following initial infection. Statistical analyses were performed using the Open Source statistical package R www.r-project.org; ISBN 3-900051-07-0) and LogXact (Cytel Incorporated, Cambridge, MA). Overall, changes in the frequency of cytokine-producing or cytolytic CD8+ or CD4+ T cells were not associated with significant alterations in the odds of superinfection (Table 1). For instance, the odds of being superinfected were a negligible 1.02 times greater (95% confidence intervals (CI) 0.95, 1.09) for every increase of 0.1 in the percentage of CD8+ T cells expressing CD107a following stimulation with gag peptides,

with a *P*-value of 0.47 (Table 1). Similar results were observed for every other function of CD8+ and CD4+ T cells, including production of IFN- γ , IL-2, MIP-1 β , or TNF- α , and for every possible combination of functions as determined by Boolean gating (Table 1 and data not shown). Thus, CD8 or CD4 T cells responses to global HIV-1 peptide pools were not significantly associated with the odds of superinfection.

These findings are admittedly disappointing: there is little evidence that HIV-1 specific cellular immune responses, at least at the level observed during chronic infection, play a significant role in protection from HIV-1 acquisition in the setting of superinfection. Similar results were recently attained in SIV infected macaques, in which CTL contributed to control of chronic infection but not to protection from superinfection [21]. While these data do not necessarily mean that HIV-specific T cells are incapable of contributing to protection from HIV-1, they do suggest that protection may require T cells at higher frequency or with different specificity than those found in chronically HIV-1 infected individuals. Further studies of humans exposed to diverse HIV-1 strains are therefore necessary to determine which immune responses, if any, contribute to protection.

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Table 1

Odds of superinfection based upon an increase of 0.1 in the percentage of CD8+ and CD4+ T cells performing a specific function following *in vitro* stimulation with HIV-1 gag PTE, HIV-1 env PTE, or CMV pp65 peptide pools

Cell type	Stimulation condition	Function ¹	Odds ratio ²	95% Confidence intervals	Nominal P-value
CD8	HIV-1 gag	CD107a	1.02	0.95 – 1.09	0.47
		IFN- γ	1.13	0.97 – 1.42	0.14
		IL-2	1.14	0.62 – 2.14	0.66
		MIP-1 β	1.05	0.95 – 1.17	0.32
		TNF- α	1.47	0.55 – 4.09	0.42
	HIV-1 env	CD107a	1.00	0.99 – 1.01	0.96
		IFN- γ	0.22	0.02 – 1.05	0.062
		IL-2	1.16	0.81 – 1.71	0.41
		MIP-1 β	0.62	0.25 – 1.19	0.17
		TNF- α	0.27	0.00 – 1.44	0.33
	CMV pp65	CD107a	0.93	0.84 – 1.01	0.11
		IFN- γ	0.94	0.84 – 1.03	0.18
		IL-2	1.27	0.59 – 3.20	0.56
		MIP-1 β	0.95	0.88 – 1.02	0.19
		TNF- α	0.93	0.83 – 1.04	0.20
CD4	HIV-1 gag	IFN- γ	1.23	0.51 – 2.95	0.63
		IL-2	0.77	0.10 – 4.23	0.82
		MIP-1 β	0.45	0.07 – 2.12	0.54
		TNF- α	0.90	0.49 – 1.42	0.89
	HIV-1 env	IFN- γ	1.15	0.69 – 1.92	0.53
		IL-2	1.01	0.73 – 1.38	0.96
		MIP-1 β	0.48	0.10 – 1.11	0.16
		TNF- α	1.02	0.62 – 1.80	0.79
	CMV pp65	IFN- γ	1.05	0.85 – 1.30	0.63
		IL-2	0.39	0.05 – 1.50	0.24

Cell type	Stimulation condition	Function ¹	Odds ratio ²	95% Confidence intervals	Nominal <i>P</i> -value
		MIP-1β	1.10	0.84 – 1.46	0.49
		TNF-α	0.99	0.80 – 1.21	0.99

¹Function was assessed as the percentage of cells expressing either CD107a (to indicate cytotoxicity) or a given cytokine following stimulation.

²Odds ratios were calculated based on exact conditional logistic regression