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Association between Cellular Immune Activation, Target Cell Frequency, and Risk of Human Immunodeficiency Virus Type 1 Superinfection

Catherine A. Blish,[a,b*] Ozge C. Dogan,[a] Walter Jaoko,[c] R. Scott McClelland,[b,c,d,e] Kishorchandra Mandaliya,[f] Katherine Odem-Davis,[a] Barbra A. Richardson,[e,g,h] Julie Overbaugh[a]

Division of Human Biology[a] and Vaccine and Infectious Disease Division,[h] Fred Hutchinson Cancer Research Center, Seattle, Washington, USA; Departments of Medicine,[b] Epidemiology,[d] Global Health,[e] and Biostatistics,[g] University of Washington, Seattle, Washington, USA; Department of Medical Microbiology, University of Nairobi, Nairobi, Kenya[c]; Coast General Hospital, Mombasa, Kenya[f]

We performed a case-control study of women at risk of HIV-1 superinfection to understand the relationship between immune activation and HIV-1 acquisition. An increase in the frequency of HIV-1 target cells, but not in other markers of T cell activation, was associated with a 1.7-fold increase in the odds of superinfection. This suggests that HIV-1 acquisition risk is influenced more by the frequency of target cells than by the generalized level of immune activation.

Inflammation and immune activation promote HIV-1 disease progression, presumably because activated target cells support high levels of viral replication (1, 2); however, their role in driving HIV-1 transmission is less clear. A number of studies have examined immune activation in HIV-exposed but uninfected individuals. The majority of these studies support the idea that low levels of immune activation at the time of exposure reduce the risk of HIV-1 infection (3–16). However, one recent study indicated that condom use was associated with lower levels of immune activation, potentially confounding some of the prior results (10). Further, in other studies, exposure to HIV-1 is associated with increased levels of immune activation (17–20) and with the development of HIV-1-specific immune responses (18, 21–26) that could play a role in protection. Differences in study design, control groups, and the type of immune response that was assessed and classified as immune activation may be partly responsible for these somewhat conflicting results from exposed, uninfected cohorts.

HIV-1 vaccine trials have done little to clarify the role of immune activation in driving HIV-1 acquisition. In the Step Study, the enhanced risk of infection among the members of a subset of vaccinees was attributed to their circumcision status and prior elevated antibody titers with respect to the Ad5 vector rather than to increased immune activation of T cells (27, 28). In the RV144 vaccine trial, generalized immune activation was not directly linked to either protection or susceptibility (29). The factors that contribute to HIV-1 acquisition in the setting of natural exposure to diverse circulating strains remain incompletely understood.

Here, we examined the association between immune activation and HIV-1 acquisition in a cohort of HIV-1-infected women at ongoing risk of HIV-1 superinfection through sex work. A subset of these women went on to become superinfected with a second HIV-1 strain (30–32). Superinfection provides a unique opportunity to evaluate correlates of HIV-1 acquisition, since both immune activation and HIV-1-specific immune responses can be evaluated. Our previous studies of this cohort found no significant differences in preexisting HIV-1-specific antibody or T cell responses in women who went on to be superinfected versus those who did not (33–35). However, individuals in other cohort studies of mostly male subjects who acquired a second HIV-1 infection within ~1 year of initial infection were found to have weak neutralizing antibody responses to their initial infection, suggesting the possibility that neutralizing antibodies can play some role in mediating susceptibility (36–38). Superinfection has also been noted to occur despite broad $CD8^+$ T cell responses (39). None of these studies explored the role of generalized immune activation in HIV-1 superinfection, though such activation could mitigate the beneficial effects of HIV-1-specific immunity.

Immune activation was assessed by examining differentiation and activation markers on T cell subsets by flow cytometry analysis of peripheral blood mononuclear cells (PBMCs) from 10 superinfected women and 29 nonsuperinfected controls as previously described (34). Cases and controls were matched based on the timing of sample collection with respect to initial infection and their HIV-1 plasma viral loads (Table 1). All 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. Cases and controls did not differ significantly in their sex frequencies (mean of 1.4 versus 1.5 self-reported sex acts in the preceding week averaged over follow-up prior to sample collection; Table 1). CD4 counts were not routinely available but were >200 cells/mm^3 either immediately prior to or within 2 years of superinfection in all cases (30–32). As expected based on prior results (1, 40–43), HIV-infected individuals showed higher levels of $CD4^+$ and $CD8^+$ T cell activation markers (Ki-67, CD38, HLA-DR, CCR5) and had perturbations in the expression of memory

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Address correspondence to Catherine A. Blish, cblish@stanford.edu.
* Present address: Catherine A. Blish, Division of Infectious Diseases and Geographic Medicine, Department of Medicine, Stanford University School of Medicine, Stanford, California, USA.

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TABLE 1 Sample timing and demographics for superinfection cases and controls[a]

Case no.	SI case ID	SI testing ypi[b]	SI VL (log10 copies/ml)	SI sex frequency[c]	Cont case ID	Cont testing ypi	Cont VL (log10 copies/ml)	Cont sex frequency
1	QA013	0.73	5.12	1.4	QA465	0.62	5.05	1.8
					QC370	0.83	5.05	1.7
					QD435	0.82	4.29	1.1
2	QB008	0.83	4.52	1.1	QB099	0.77	4.93	4.3
					QC406	1.0	4.77	0.8
					QD370	0.76	4.22	1.7
3	QA413	2.0	4.95	0.8	QA509	2.1	4.61	1.0
					QC036	2.1	5.30	0.6
					QC890	1.8	4.84	0.2
4	QB045	4.6	4.50	0.3	QA281	4.7	4.98	0.8
					QA692	4.8	5.15	0.9
					QB247	4.7	4.70	0.9
5	QB726	2.8	3.77	1.0	QA874	2.9	4.68	1.6
					QB670	2.8	4.18	1.3
					QC413	2.7	3.38	1.9
6	QB850	0.14	5.22	3.0	QB424	0.086	5.52	2.5
					QB543	0.14	5.52	0.5
					QC805	0.12	5.16	2.0
7	QC885	0.16	5.56	1.4	QB461	0.16	5.58	1.2
					QB765	0.22	5.50	1.0
8	QB609	0.28	2.39	1.3	QB857	0.30	4.17	2.3
					QC048	0.20	5.05	4.7
					QF481	0.26	3.97	0
9	QA252	2.7	4.02	0.8	QC100	2.7	1.69	1.7
					QD470	3.0	4.31	0
					QD834	2.9	4.91	1.6
10	QC858	0.93	4.29	2.9	QA560	0.96	4.07	0.7
					QB936	0.59	5.12	2.4
					QC888	1.07	4.50	2.4

[a] Abbreviations: Cont, control; ID, identification number; SI, superinfection; ypi, years postinfection; VL, viral load.
[b] Samples were tested at the time point immediately prior to detection of superinfection in order to best assess the status of the immune response at the time point most relevant in terms of exposure to, and lack of protection from, the second virus. Controls were matched to this time postinfection.
[c] Data represent averages of self-reported frequencies of sexual intercourse in the preceding week from all follow-up visits up to the time of sample collection. This measure includes both protected and unprotected visits. The median number of visits contributing to this summary was 37 (interquartile range, 20 to 64 visits) over a median of 2 years (interquartile range, 1 to 5 years).

and differentiation markers in comparison to HIV-1-uninfected controls (Fig. 1).

To determine whether higher levels of activation were associated with altered odds of superinfection, exact conditional logistic regression analysis was performed (Table 2). Increased frequencies of both $CD3^+$ T cells and, more specifically, $CD3^+$ $CD4^+$ $CCR5^+$ cells, primary targets of HIV-1 infection, were associated with increased odds of superinfection (Table 2). For every 1% increase in the frequency of $CD3^+$ $CD4^+$ $CCR5^+$ HIV-1 target cells among total lymphocytes, the odds of superinfection were 1.69-fold higher (95% confidence interval [CI], 1.02 to 3.36), with a *P* value of 0.04. This elevation in risk was reflected only in the frequency of these cells as a percentage of total lymphocytes, as the percentage of CCR5-expressing $CD4^+$ T cells was not associated with an elevated risk of superinfection (odds ratio [OR], 0.99; 95% CI, 0.94 to 1.05). There was no association between expression of other immune activation markers such as Ki-67, CD38, or HLA-DR among T cells and the odds of superinfection (Table 2). Furthermore, the levels of activation among either $CD4^+$ or $CD8^+$ cells, as measured by expression of any combination of Ki-67, CD38, and HLA-DR, was not associated with the odds of superinfection (not shown). Finally, differences in the frequencies of regulatory T cells, naive cells, and various memory and effector T cell subsets were not associated with significant alterations in the odds of superinfection (Table 2). Thus, only increases in the frequency of $CD3^+$ and $CD3^+$ $CD4^+$ $CCR5^+$ target cells, but not increases in the frequencies of any other activated subsets, were associated with increased odds of superinfection.

These data leverage long-term, regular follow-up of a high-risk population and the setting of superinfection to evaluate,

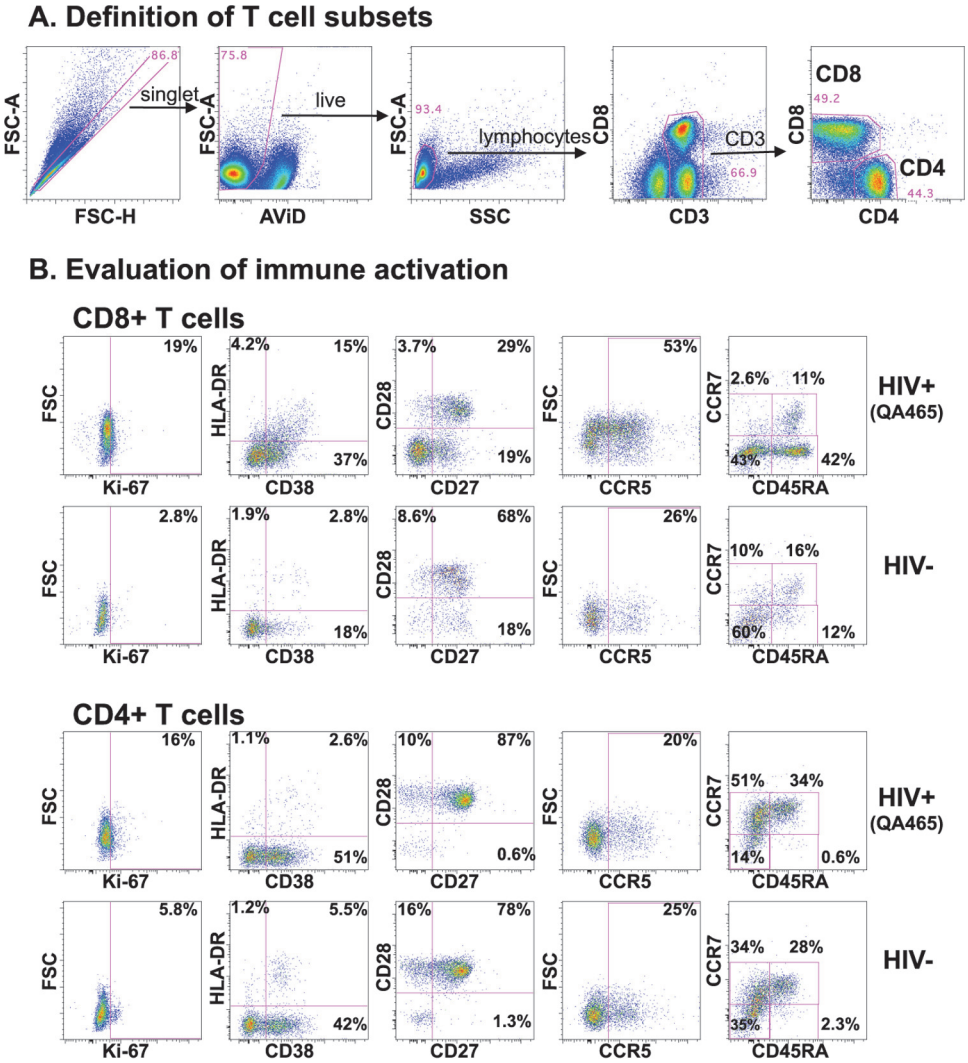

FIG 1 Representative flow cytometry data to assess T cell subsets and immune activation levels from superinfection cases and controls. (A) An example of the sequential gating tree used to identify T cell subsets. FSC, forward scatter; AViD, Live/Dead fixable aqua dead cell stain; SSC, side scatter. (B) Phenotypes of $CD8^+$ (top two rows) and $CD4^+$ (bottom two rows) T cells based on expression of the activation and memory markers Ki-67, HLA-DR, CD38, CD28, CD27, CCR5, CCR7, and CD45RA are shown. The top panels show the flow cytometry plots from HIV-infected subject QA465, and the bottom panels show the plots from cells from an HIV-uninfected individual that were cryopreserved and evaluated in each experiment in order to set consistent gating. The following antibodies were used: CD195 (CCR5)-phycoerythrin (PE)-Cy7, CD127-Alexa 647, CD25-PE-Cy7, CD28-PE-Cy5, CD38-PE, CD4-fluorescein isothiocyanate (FITC), CD8-peridinin chlorophyll protein (PerCP)-Cy5.5, GranzymeB-Alexa 700, and Ki-67-FITC (BD Biosciences); CD197 (CCR7)-allophycocyanin (APC) (R&D Systems); CD27-APC-efluor780 and Fox-p3-PE (eBioscience); CD3-Qdot605 (Molecular Probes); CD3-ECD and CD45RA–electron-coupled dye (ECD) (Beckman Coulter); and HLA-DR-Pacific blue (BioLegend).

for the first time, whether the activation and/or differentiation of T cells was associated with HIV acquisition. This is an important consideration, as the contributions of HIV-1-specific immune responses to protection remain uncertain. Some immune responses may even enhance infection risk, particularly if they increase recruitment of HIV-1 target cells to sites of viral entry without containing early replication. Our finding that an increased frequency of HIV-1 primary target cells is associated with increased odds of a second infection may partially explain the observation that the risk of superinfection appears to be highest in the first 6 months following initial infection (44, 45), before target cell depletion.

A strength of this study is the fact that superinfection cases were prospectively identified, allowing us to evaluate immunity in matched individuals with similar risk behaviors for exposure to HIV-1 but different outcomes. While this remains the largest superinfection cohort available with adequate sampling for such immunologic studies, we still had relatively modest power to detect significant differences between groups and therefore did not adjust for multiple comparisons. With the number of statistical tests, a single significant association may have been observed by chance, and our finding that increased numbers of HIV-1 target cells increase the odds of superinfection warrants follow-up in additional studies. Although the lack of association between other markers of immune activation and the risk of HIV-1 superinfection could be attributed to a lack of statistical power, this seems somewhat unlikely because the majority of observed associations were modest in magnitude. The median absolute estimated odds ratio for one

TABLE 2 Odds of superinfection based on frequency and expression of activation markers and effector and memory $CD8^+$ and $CD4^+$ subsets

Frequency (%) for indicated member of subset	Odds ratio[a]	95% CI[b]	*P* value
Total lymphocytes			
$CD3^+$	1.15	1.01–1.35	**0.03**
$CD3^+$ $CD8^+$	1.03	0.95–1.14	0.48
$CD3^+$ $CD4^+$	1.11	0.98–1.27	0.10
$CD3^+$ $CD4^+$ $CCR5^+$	1.67	1.02–3.29	**0.04**
$CD3^+$ T cells			
$CD8^+$	0.99	0.91–1.06	0.72
$CD4^+$	1.04	0.96–1.14	0.33
$CD4^+$ $CCR5^+$	1.13	0.89–1.45	0.33
Ki-67^+	0.97	0.92–1.01	0.20
$CD38^+$	0.96	0.89–1.03	0.33
HLA-DR^+	1.02	0.90–1.15	0.82
Ki-67^+ $CD38^+$ HLA-DR^+	0.97	0.75–1.24	0.83
$CD8^+$ T cells			
$CCR5^+$	0.99	0.94–1.05	0.85
Ki-67^+	0.98	0.92–1.02	0.34
$CD38^+$	0.96	0.89–1.02	0.24
HLA-DR^+	1.00	0.92–1.09	0.98
$CD38^+$ HLA-DR^+	0.97	0.87–1.08	0.63
Ki-67^+ $CD38^+$ HLA-DR^+	0.97	0.86–1.06	0.53
$GranzymeB^+$	0.98	0.93–1.03	0.48
Naive ($CD45RA^+$ $CCR7^+$)	1.03	0.87–1.22	0.7
Central memory ($CD45RA^-$ $CCR7^+$)	1.06	0.88–1.29	0.51
Effector memory ($CD45RA^-$ $CCR7^-$)	1.02	0.96–1.08	0.56
Terminally differentiated ($CD45RA^+$ $CCR7^-$)	0.96	0.89–1.03	0.32
$CD4^+$ T cells			
$CCR5^+$	0.99	0.93–1.04	0.83
Ki-67^+	0.97	0.91–1.01	0.18
$CD38^+$	0.97	0.9–1.04	0.41
HLA-DR^+	1.02	0.91–1.13	0.67
$CD38^+$ HLA-DR^+	1.00	0.8–1.22	0.91
Ki-67^+ $CD38^+$ HLA-DR^+	0.99	0.71–1.27	0.96
Treg (foxp3^+ $CD25^+$ $CD127^-$)	1.04	0.63–1.67	0.79
Naive ($CD45RA^+$ $CCR7^+$ $CD27^+$)	0.99	0.91–1.08	0.91
Transitional memory ($CD45RA^-$ $CCR7^-$ $CD27^+$)	1.02	0.92–1.14	0.64
Central memory ($CD45RA^-$ $CCR7^+$ $CD27^+$)	1.04	0.96–1.13	0.36
Effector memory ($CD45RA^-$ $CCR7^-$ $CD27^-$)	1.00	0.94–1.06	1.00
Terminally differentiated ($CD45RA^+$ $CCR7^-$ $CD27^-$)	0.95	0.80–1.09	0.48

[a] Data represent odds of superinfection for every 1% increase in the frequency of the indicated cell subset.
[b] CI, confidence interval.

unit increase in frequency was 1.03, equivalent in magnitude to the observed association (OR = 0.97) for percent $CD38^+$ HLA-DR^+ of CD8 T cells. Using cluster-level bootstrap sampling of the observed data for percent $CD38^+$ HLA-DR^+ of CD8 T cells, we would need approximately 300 case-control clusters (1,200 subjects with 1:3 case:control matching) to have 80% power to detect this observed association. Another potential limitation is that we were able to measure only reported risk behavior, and background variations in actual exposure to HIV-1 could have attenuated observed relationships between immune responses and HIV-1 acquisition.

This report highlights the complex interplay of different factors that influence the risk of HIV-1 acquisition in an HIV-1-infected individual. It is likely that both HIV-1-specific immunity and the availability of $CD3^+$ $CD4^+$ $CCR5^+$ HIV-1 primary target cells contribute to the risk of superinfection. Larger studies will be needed to confirm and fully define this dynamic. Here, we show that target cell availability appears to be a more significant contributor to superinfection risk than the generalized immune activation state.

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We declare that we have no conflicts of interest.

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