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Host *CCL3L1* Gene Copy Number in Relation to HIV-1–Specific CD4⁺ and CD8⁺ T-Cell Responses and Viral Load in South African Women

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Summary

HIV-specific T-cell responses play an important role in control of infection. Because *CCL3* has immune modulatory and antiviral activities, we hypothesized that host *CCL3* genotype (*CCL3L1* gene duplications) would influence the development of effective HIV-specific immune responses. Copy numbers of *CCL3L1* were determined for 71 HIV-infected women, and HIV-specific CD4⁺ and CD8⁺ T-cell responses to overlapping peptide pools spanning the HIV-1 subtype C genome were simultaneously measured by an interferon- γ and interleukin-2 whole-blood flow cytometric assay. Host *CCL3L1* copy number correlated negatively with viral load ($r = -0.239$, $P = 0.045$), as did magnitudes of Gag CD4⁺ ($r = -0.362$, $P = 0.002$) and CD8⁺ ($r = -0.261$, $P = 0.028$) T-cell responses. Patients with a Gag CD4⁺ response ($P = 0.002$) or dominant Gag CD8⁺ ($P = 0.006$) response had significantly lower viral loads than those whose dominant response targeted another region of the genome, whereas a dominant Nef-specific CD8⁺ T-cell response was associated with higher HIV viral load. *CCL3L1* copy number greater than or equal to the population median of 5 was significantly associated with increased magnitude of CD4⁺ Gag responses ($P = 0.017$), and women who had CD4⁺ and CD8⁺ Gag-specific responses had significantly lower viral loads ($P = 0.004$) and higher *CCL3L1* copy number ($P = 0.015$) than those women with only CD8⁺ Gag-specific responses.

Keywords

CCL3L1 copy number; CD4⁺ and CD8⁺ T cell responses; HIV-1 subtype C; viral load

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AUTHOR CONTRIBUTIONS C. Tiemessen and L. Kuhn conceived and designed the experiments. S. Shalekoff, S. Meddows-Taylor, D. Schramm, and S. Donninger performed the experiments. S. Shalekoff and C. Tiemessen analyzed the data. S. Shalekoff and C. Tiemessen wrote the paper. Patient recruitment and provision of clinical data were provided by G. Gray, G. Sherman, and A. Coovadia.

Innate immunity is central to immune responses to infectious organisms and is instrumental in driving the development and maintenance of adaptive immune responses. The host innate response to infection, acute or chronic, is accompanied by the orchestrated regulation of chemoattractant molecules (chemokines) produced by activated leukocytes, which govern the outcome of an infectious insult, resulting in clearance or persistence of the organism.

There is substantial support for a positive role of elevated levels of the CC chemokines CCL3 (macrophage inflammatory protein [MIP]-1 α), CCL4 (MIP-1 β), and CCL5 (RANTES) in attenuation of HIV-1 disease progression.¹⁻⁴ This association is usually inferred to be attributable to the role of CC chemokines as HIV-1 suppressor factors.⁵ Aside from their role in chemotaxis, however, CC chemokines also play an important role in T-cell activation⁶ and in directing and enhancing adaptive immune responses. For example, they are used as adjuvants for DNA vaccines,^{7,8} highlighting how manipulation of the chemokine environment at the site of vaccine exposure can tailor vaccines to achieve certain types of immune responses to antigen.

In humans, CCL3 protein is encoded by 2 functional genes (*CCL3/LD78 α* and *CCL3L1/LD78 β*), occurring as 2 copies and as variable copy numbers, respectively, in different individuals.⁹ We refer throughout to the proteins CCL3 and CCL3L1 collectively as “CCL3” unless otherwise stated. Variation in copy number of *CCL3L1* has been associated with HIV-1 disease progression in an extensive study conducted on numerous population groups.¹⁰ CCL3 may mediate its protective effects in several ways, which may include its ability to enhance adaptive immune responses, and underlying host CCL3 genotype may therefore have an impact on the development and maintenance of effective HIV-1-specific immune responses.¹¹ Recent data have shown that variations in the genes encoding *CCL3L1* and *CCR5* influence cell-mediated immunity to recall antigens (delayed-type hypersensitivity skin tests) in HIV-1-infected and healthy individuals,¹² but correlations with HIV-1-specific responses have not yet been reported.

To date, CC chemokines have not been studied in the context of their ability to influence or instruct adaptive anti-HIV T-cell immune responses in HIV-1-infected individuals. Given the role of CCL3 in HIV-1 protective immunity and attenuation of disease progression and the described roles of CD4⁺¹³⁻¹⁸ and CD8⁺¹⁹⁻³⁹ T cells in control of HIV-1 infection, we questioned whether gene duplications of *CCL3L1*, selected as a measure of host CCL3 chemokine production capacity,^{10,40,41} influenced the integrity of HIV-1-specific CD4⁺ and CD8⁺ T-cell responses and viral load in HIV-1-infected women.

MATERIALS AND METHODS

Study Participants

As part of a study to investigate determinants of maternal-infant HIV-1 transmission, 71 HIV-1-infected women were recruited soon after delivery (50 from Chris Hani Baragwanath Hospital in Soweto and 21 from Coronation Hospital in Johannesburg, South Africa). Of the 71 women (median age = 28 years, range: 18 to 39 years), only 3 had begun antiretroviral therapy a few months before sample collection (their exclusion did not alter any of the result outcomes; therefore, data are presented from the total group). The median viral load at enrollment was 3.9 log (range: 2.6 to 5.69 log), and the median CD4⁺ T-cell count was 436 cells/ μ L (range: 40 to 1655 cells/ μ L; <200 cells/ μ L in 8 [14.8%] of 54 women, 200 to 500 cells/ μ L in 29 [53.7%] of 54 women, and >500 cells/ μ L in 17 [31.5%] of 54 women) for the cohort. This study was approved by the University of the Witwatersrand Committee for Research on Human Subjects, and written informed consent was obtained from the women.

HIV-1 Quantitation

HIV-1 RNA levels (expressed as log₁₀ units) were quantitated using the Roche Amplicor RNA Monitor assay version 1.5 (Roche Diagnostic Systems, Inc., Branchburg, NJ), with a lower detection limit of 400 HIV-1 RNA copies/mL.

Quantitation of CD4⁺ T-Cell Counts

CD4 T-cell counts were determined using the commercially available FACSCount System from Becton Dickinson (San Jose, CA).

CCL3L1 Copy Number Determination

Real-time polymerase chain reaction (PCR) was performed using an ABI PRISM 7500 (Applied Biosystems, Foster City, CA), and the following primers and probes were synthesized (DNA Synthesis Laboratory, Department of Molecular and Cellular Biology, University of Cape Town, Cape Town, South Africa) for quantitation of CCL3-L1 copy number: β -globin gene upstream 5' -ggcaaccctaaggtgaaggc-3', β -globin gene downstream 5' -ggtagccaggccatcacta-3', β -globin gene probe 5' -catggcaagaaagtgcgtgcct-3', CCL3L1 gene upstream 5' -tctccacagcttctaaccaaga-3', CCL3 and CCL3L1 genes downstream 5' -ctggaccactcctcactgg-3', and CCL3L1 gene probe 5' -aggccggcaggtctgtgctga-3'.⁴¹ In addition, CCL3 gene upstream 5' -tctccacagcttctaaccaagc-3' and CCL3 gene probe 5' -aagccggcaggtctgtgctga-3' were designed and synthesized. All probes were labeled with 5' 6-carboxyfluorescein (FAM) and a 3' 6-carboxytetramethylrhodamine (TAMRA) quencher.

For each sample, the β -globin, CCL3, and CCL3L1 genes were amplified in duplicate, using approximately 20 ng of genomic DNA per sample. CCL3 gene copy number was confirmed at 2 copies per diploid genome (pdg) for each sample, calculated using the Relative Quantification method (as per the protocol supplied) and using β -globin (present at 2 copies pdg) as the endogenous control. CCL3 was then used as the endogenous control to calculate CCL3L1 copy number, again using the Relative Quantification method against a known copy control. Samples giving a result of a single CCL3L1 gene copy pdg were confirmed by sequencing to ensure homozygosity.

HIV-1 Peptides

A total of 396 synthetic overlapping peptides spanning 9 HIV-1 subtype C gene regions (Gag, Pol, Nef, Env, Tat, Rev, Vif, Vpu, and Vpr) were supplied by the South African Vaccine Initiative (SAAVI) Repository (Immunology Laboratory, AIDS Virus Research Unit, National Institute for Communicable Diseases) for use in this study. Gag, Vif, Vpu, and Vpr amino acid sequences were based on the HIV-1 subtype C consensus sequence, whereas Pol, Nef, Tat, Rev, and gp160 were designed to match gene regions that had been chosen for inclusion in HIV-1 subtype C candidate vaccines (Du151 and Du179). Peptides varied from 15- to 18-mers in length and overlapped by at least 10 amino acids. Peptides were resuspended in 100% dimethyl sulfoxide (DMSO) at a concentration of 10 μ g/mL and were then pooled at 40 μ g/mL per peptide stock in phosphate-buffered saline (PBS) at a final DMSO concentration of <0.5%. Peptide pools were composed of the following numbers of peptides: Gag, 66; Pol, 92 (excluding integrase); Env, 114; Nef, 50; and the regulatory regions combined (Tat, Rev, Vif, Vpu, and Vpr), 70.

Intracellular Cytokine Staining

Blood was collected in sodium heparin tubes. Stimulation was performed within 6 hours of collection by incubating 200 μ L of whole blood with 1 μ g of the costimulatory antibodies CD28 and CD49d (BD Biosciences, San Jose, CA) and a final concentration of 10 μ g/mL of each peptide, together with the secretory inhibitor Brefeldin A (10 μ g/mL; Sigma-Aldrich Corp.,

St. Louis, MO) for 6 hours at 37°C. Thereafter, the samples were cooled to 18°C. To control for nonspecific cytokine release, a negative control tube incubated with costimulatory antibodies and the equivalent amount of DMSO as the peptide tube was prepared for each patient. *Staphylococcus enterotoxin B* (SEB) was included as a positive control. Twenty microliters of ethylenediaminetetraacetic acid (EDTA) was added to all samples for 15 minutes, after which the samples were transferred to fluorescent activated cell sorting (FACS) tubes, and red blood cells were lysed with 2 mL of FACS lysing solution (BD Biosciences) for 10 minutes at room temperature. After centrifugation, the samples were permeabilized with 500 µL of FACS perm 2 (BD Biosciences) for 10 minutes at room temperature. Samples were then washed and stained with specific fluorescent antibodies (CD3 allophycocyanin [APC], CD8 peridinin chlorophyll [PerCP], and interferon [IFN]-γ phycoerythrin [PE] and interleukin [IL]-2 PE) for 60 minutes in the dark at room temperature. Samples were washed and resuspended in 150 µL of 1% paraformaldehyde (1:10 ratio in PBS) and stored at 4°C until acquisition using a FACSCalibur flow cytometer (Becton Dickinson Immunocytometry Systems, San Jose, CA) within 24 hours. After acquisition, data were analyzed using FlowJo version 6.3.2 software (Tree Star, San Carlos, CA). The lymphocyte gate was identified based on the forward and side scatter characteristics for each sample. CD4⁺ T cells were defined as the CD3⁺CD8⁻ cells within the lymphocyte gate, and CD8⁺ T cells as the CD3⁺CD8⁺ cells within the lymphocyte gate. Initial work included the activation marker CD69 fluorescein isothiocyanate (FITC), and CD4⁺ and CD8⁺ T cells that secreted cytokine were defined as those that were positive for CD69 and IL-2 plus IFN_γ. Because its inclusion did not enhance the analysis of results, however, it was omitted from future staining panels. Significant IL-2 plus IFN_γ production was defined as responses of ≥0.1% after subtracting the background staining from cells stimulated with anti-CD28 and anti-CD49d antibodies in the absence of antigen.

Statistical Analysis

Statistical analysis was performed using SPSS version 14.0.2 software (SPSS Inc., Chicago, IL). Comparison between study groups was done using the Mann-Whitney *U* test, and the Spearman correlation coefficient was calculated to determine correlations of immune responses with markers of disease progression.

RESULTS

Quantitation of CD4⁺ and CD8⁺ T-Cell Responses

CD8⁺ T-cell responses to overlapping peptide pools spanning the HIV-1 genome were higher in frequency (Table 1) and in magnitude than CD4⁺ T-cell responses to these peptide pools measured by an intracellular cytokine (ICC) assay (Figs. 1A, B). A representative example of the CD4⁺ and CD8⁺ T-cell responses to the different peptide pools is shown (see Figs. 1C, D). Sixty-eight (96%) of 71 women had at least 1 detectable HIV-1-specific CD8⁺ T-cell response, with Gag (76%), Pol (76%), and Nef (83%) being the peptides most frequently targeted. The magnitude of the responses ranged between 0.1% and 5.5%, with responses of the highest magnitude to Gag. Forty-nine (69%) of 71 women had HIV-1-specific CD4⁺ T-cell responses, with responses to Gag (39%) and Env (37%) being the most frequently detected. These responses ranged between 0.1% and 3.8%, with the highest magnitude of responses to Env. Moreover, 66% of women had CD8⁺ T-cell responses to 4 or 5 peptide pools, whereas approximately half of the women had CD4⁺ T-cell responses to only 1 or 2 pools (Table 2).

Relation Between Magnitudes of CD4⁺ and CD8⁺ T-Cell Responses

Previous studies have detected positive correlations between CD8⁺ Gag-specific precursor frequency and CD4⁺ T-cell proliferative p24-specific responses⁴² and between the magnitude of CD4⁺ and CD8⁺ T-cell responses,³⁰ whereas another study⁴³ found no correlation between

the frequency of the total or Gag-specific CD4⁺ and CD8⁺ T-cell responses. We therefore examined whether a relation between CD4⁺ and CD8⁺ responses could be found in our study. There was no correlation between the magnitude of CD4⁺ and CD8⁺ T-cell responses to the Gag, Pol, Nef, and Reg peptide pools; however, there was a positive relation between the magnitude of CD4⁺ and CD8⁺ T-cell responses to Env ($r = 0.4$, $P < 0.001$).

Markers of Disease Progression and HIV-1-Specific CD4⁺ and CD8⁺ T-Cell Responses

The relation between plasma viral load and HIV-1-specific T-cell responses is controversial, with some studies reporting a positive correlation,⁴³ which suggests that the immune response may be driven by the level of antigenic exposure. Other studies have reported a negative correlation,^{24,27,29,30,42,44,45} suggesting that high levels of viral replication may result in the depletion of HIV-1-specific T cells, whereas still others have found no correlation between the magnitude of CD4⁺ and CD8⁺ T-cell responses measured by IFN γ and viral load, which suggests that using IFN γ production as the sole indicator of antiviral T-cell function may not be adequate.⁴⁶⁻⁴⁹

Therefore, we assessed whether there was any relation between the magnitude of CD4⁺ and CD8⁺ HIV-1-specific T-cell responses and CD4⁺ T-cell counts. There was no correlation between the magnitude of any of the HIV-specific CD4⁺ T-cell responses and CD4⁺ T-cell counts. Likewise, there was no correlation between HIV-specific CD8⁺ T-cell responses and CD4⁺ T-cell counts, with the exception of a positive correlation between the magnitude of Gag-specific CD8⁺ T-cell responses and CD4⁺ T-cell counts ($r = 0.288$, $P = 0.037$).

With regard to viral load, we observed a significant negative correlation between the magnitude of CD4⁺ T-cell responses to Gag and viral load ($r = -0.362$, $P = 0.002$; Fig. 2A). There was no association between CD4⁺ T-cell responses to Pol, Nef, Reg, or Env and viral load (see Figs. 2B–E). The patients were stratified according to whether they had a CD4⁺ T-cell Gag response and those who had no Gag response (no response or response to other regions). Those women who had a CD4⁺ T-cell response to Gag had a significantly lower viral load compared with those with no Gag response ($P = 0.002$; see Fig. 2F).

The CD8⁺ Gag-specific response correlated inversely with virus load (Fig. 3A; $r = -0.261$, $P = 0.028$). Conversely, the CD8⁺ Nef-specific response correlated positively with viral load (see Fig. 3C; $r = 0.256$, $P = 0.031$), with a positive trend for CD8⁺ Pol- and Env-specific T cells (see Figs. 3B, E). On stratification of patients into groups based on dominant Gag and Nef responses, individuals with a dominant CD8⁺ Gag-specific T-cell response had lower viral loads than those whose dominant response was to another region ($P = 0.006$; see Fig. 3F), and individuals with a dominant Nef response had higher viral loads than those whose dominant response was to another region ($P = 0.035$; see Fig. 3G).

CCL3L1 Copy Number, Markers of Disease Progression, and HIV-1-Specific T-Cell Responses

CCL3L1 gene copy number has been shown to be directly correlated with CCL3 production^{10,40,41} and to influence disease progression in HIV-1 infection.¹⁰ *CCL3L1* gene copy numbers were determined for all the HIV-1-infected women (median = 5, interquartile range [IQR]: 4 to 5, mean = 4.8, SD = 1.68; Fig. 4A) and correlated to their viral loads and CD4 T-cell counts (see Figs. 4B, C). There was a decrease in viral load with an increase in host *CCL3L1* copy number ($r = -0.239$, $P = 0.045$), with CD4⁺ T-cell counts showing a positive relation that was not significant. Next, we determined if there was any relation between *CCL3L1* copy number and the magnitude or breadth of CD4⁺ and CD8⁺ T-cell responses. The only significant finding was that a *CCL3L1* copy number greater than or equal to the population

median of 5 was significantly associated with an increased magnitude of Gag-specific CD4⁺ T-cell responses ($P = 0.017$; Fig. 5).

This association, taken together with our prior results (see Figs. 2A, 3A), led us to hypothesize that the presence of detectable Gag-specific CD4⁺ T-cell responses would “mark” more effective Gag-specific CD8⁺ T-cell responses. To test this, we examined the women who had CD4⁺ and CD8⁺ T-cell responses to Gag. Of the 71 women, 76% had Gag-specific CD8⁺ T-cell responses and 39% had detectable Gag-specific CD4⁺ T-cell responses. Thirty-two women (45%) had only Gag-specific CD8⁺ T-cell responses, whereas 22 (31%) had Gag-specific CD4⁺ and CD8⁺ T-cell responses (Fig. 6). When these 2 groups were compared, viral load was significantly reduced ($P = 0.004$) only when CD8⁺ Gag responses were combined with CD4⁺ Gag responses. Likewise, *CCL3L1* copy number was significantly increased ($P = 0.015$) in those women who had both CD4⁺ and CD8⁺ T-cell responses to Gag.

DISCUSSION

In light of recent data highlighting the important role of gene duplications of *CCL3L1* in HIV protective immunity^{10,40,50} and disease progression,¹⁰ we sought to establish the relation between the integrity of HIV-1 protein-specific T-cell responses, some of which associate with viral control in chronic HIV-1 infection, and host duplications of the gene *CCL3L1*. Evaluating these relations therefore first warranted a detailed evaluation of CD4⁺ and CD8⁺ T-cell responses to pools of peptides representing the various protein regions of HIV-1, which were measured by a whole-blood IL-2 plus IFN γ flow cytometric assay in our case.

Our findings with respect to the magnitude and the breadth of HIV-specific T-cell responses were in agreement with a number of studies that have simultaneously evaluated CD4⁺ and CD8⁺ T-cell responses.^{28,30,43,46,51-53} Data are limited, however, on responses among subtype C-infected populations, and only 3 of these studies have evaluated HIV-1 subtype C responses: 2 in South Africa^{28,30} and 1 in India.⁵³ One difference noted in our study was that the Env peptide pool elicited CD4⁺ T-cell responses of the highest magnitude, contrasting with results from another study,³⁰ which found that the Env peptide pool elicited responses of the smallest range and magnitude. This inconsistency between results may, however, reflect differences in assay design, because these investigators used only IFN γ for detection of responses, which may have resulted in some loss in sensitivity of detection and quantitation of CD4⁺ T-cell responses.

Our data support other studies that have shown preferred targeting of Gag was associated with enhanced immune control,²⁷⁻³² suggesting an important role for Gag-specific CD8⁺ T-cell response immune responses. A reason for this may be that Gag is a highly conserved protein that tolerates fewer mutations. A large population-based study has shown that the extent of conservation alone cannot explain the protein-specific differences in CD8⁺ T-cell responses, however.³² The targeting of a conserved protein such as Pol is not associated with effective immune control.³² It was interesting that in our study, Gag-specific CD4⁺ T-cell responses were associated with virus control but that Env responses, which occurred in as many individuals and were higher in magnitude than Gag responses, did not. Therefore, it may be that CD4⁺ T-cell responses that are of greater breadth and lower magnitude than Gag are more effective at controlling virus than more focused higher magnitude responses in a more variable region like Env. Differential timing of intracellular processing of proteins such as Gag versus Env or other proteins requiring de novo synthesis on cell infection has been suggested as a possible explanation for protein-specific differences in CD8⁺ T-cell responses.³² This would, however, be an unlikely explanation in the context of class II-restricted CD4⁺ T-cell responses to these proteins.

Aside from the aspect of protein specificity of T-cell responses in control of HIV-1 infection, another important consideration is defining T-cell functionality. Assessing quality of T-cell responses has included measures of induced production of a number of intracellular cytokines and the degranulation marker Cd107a.^{33,34} Interestingly, recent studies have described a role for the inhibitory receptor programmed death-1 (PD-1), upregulated in the presence of high antigen load, in the induction of T-cell dysfunction or “exhaustion” that contributes to poor control in chronic infections.³⁵⁻³⁹ It has also been shown that in chronic infections, cytokine production in CD8⁺ T cells may be impaired, whereas functions such as degranulation or cytotoxicity are not compromised, indicating the involvement of distinct pathways for the different processes.⁵⁴ Therefore, it should be kept in mind that using cytokines as markers of integrity of immune responsiveness does not necessarily reflect the full functionality of those T cells and should be seen as reflecting cytokine production potential of HIV-specific T cells that, as shown, can be related to markers of disease progression.

This said, it was clear from our data that the inclusion of IL-2 in the ICC assay represented an important marker, particularly for detecting relations seen between Gag CD4⁺ T-cell responses and viral load, consistent with findings from several studies.¹³⁻¹⁵ Importantly, it has been shown that only Gag-specific IL-2⁺IFN γ ⁺ and not IL-2⁻IFN γ ⁺ cells were inversely related to viral load¹⁴ and that individuals who could control viral replication had higher levels of Gag-specific CD4⁺ T cells secreting IL-2 and IFN γ .¹⁶ Studies finding no correlation between Gag-specific CD4⁺ T-cell responses and viral load^{30,43,55} and no association with disease progression^{43,56-59} all used IFN γ alone for detection purposes.

Our approach in this study was to use host genotype (defined as numbers of copies of *CCL3L1*) as a measure of the host's ability to produce CCL3. Increased *CCL3L1* gene copy number has been correlated with increased CCL3 production in stimulated monocytes⁴¹ and in phytohemagglutinin (PHA)-stimulated mononuclear cells from HIV-1-infected mothers and cord blood mononuclear cells of HIV-exposed uninfected infants.⁴⁰ The relations that we found between *CCL3L1* copy number and the magnitude of Gag-specific CD4⁺ T-cell responses and viral control suggest one of the potential pathways through which *CCL3L1* may attenuate disease progression in chronically HIV-infected individuals. This is further supported by the fact that women who had both CD4⁺ and CD8⁺ Gag-specific responses had significantly lower viral loads and higher *CCL3L1* copy numbers than those women with only CD8⁺ Gag-specific responses. Demonstrable Gag-specific CD4⁺ T-cell responses are indicative of better immune integrity and function and can serve as markers of more effective Gag-specific CD8⁺ T-cell responses. Our data are consistent with findings from studies of long-term non-progressors, which have observed vigorous HIV-specific CD4⁺ T-cell responses associated with virus control that are linked to enhanced production of CC chemokines.¹ It remains to be determined whether CCL3 is necessary to support the development of effective CD4⁺ T-cell responses or whether it is the maintenance of these responses in a milieu of higher CCL3 production that is advantageous to the host to control viremia.

CC chemokines have received most attention in the context of HIV-1 infection through their abilities to block CCR5-utilizing HIV-1 strains from entering permissive cells, earmarking this antiviral role as the underlying mechanism most likely to counter virus replication. Their role as innate factors in an adaptive immune response to HIV-1, however, has received somewhat less attention. Of the many possible functions of CCL3,¹¹ the role of these molecules in supporting the development and maintenance of adaptive immunity (HIV-1-specific T-cell responses) may be important in virus control in chronically HIV-infected individuals. We propose that CCL3L1 production capacity, inferred here by *CCL3L1* copy numbers, influences T-cell responsiveness by having an “adjuvant effect” or by preserving CD4⁺ T-cell function through contributing to control of viral replication through their antiviral activities mediated through binding to CCR5.

Our data do not distinguish the underlying mechanism but emphasize the importance of host innate immune capability (*CCL3L1* gene duplications) in influencing the integrity of HIV-1-specific T-cell responses associated with virus control in HIV-1-infected individuals. These findings are consistent with the data showing that variations in genes encoding CCL3L1 (and CCR5) influence cell-mediated immunity to recall antigens in HIV-1-infected and healthy individuals.¹² Our data extend these observations to demonstrate further the influence of *CCL3L1* duplications on HIV-1-specific CD4⁺ and CD8⁺ T-cell responses. The link between innate immunity and subsequent development and maintenance of adaptive immunity has emerged as an essential area of immunologic research necessary for the development of effective approaches for vaccines and therapeutics.

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FIGURE 1.

Relative magnitudes of CD4⁺ (A) and CD8⁺ (B) T-cell responses measured by ICC assay for each of the peptide pools are shown for 71 HIV-1-infected women. Flow cytometric plots of a representative example of the CD4⁺ (C) and CD8⁺ (D) T-cell responses to the different peptide pools of 1 individual are shown. The percentage of IL-2- plus IFN- γ -positive cells was calculated after subtraction of the background staining. Responses $\geq 0.1\%$ are considered positive.

FIGURE 2.

Scatter plots show the magnitude of HIV-1-specific CD4⁺ T-cell responses (x axis) against HIV viral load (y axis). The magnitude of the association is described using the Spearman correlation coefficient (r) and is shown if the P value was <0.05 . A–E, Linear regression lines are shown. The relation between viral load and presence or absence of CD4⁺ Gag-specific T-cell responses is shown. Data are presented as medians (horizontal bar), 25th and 75th percentiles (boxes), and 10th and 90th percentiles (bars) of viral load among those with and without Gag-specific CD4⁺ T-cell responses. F, Significant differences between groups and sample numbers per group are indicated.

FIGURE 3.

Scatter plots show the magnitude of HIV-1-specific CD8⁺ T-cell responses (x axis) against HIV viral load (y axis). A–E, Spearman correlation coefficient (r) is shown if the P value was <0.05 . Distribution of viral load between those with (F) and without (G) dominant CD8⁺ Gag- and Nef-specific T-cell responses is shown. Data are presented as medians (horizontal bar), 25th and 75th percentiles (boxes), and 10th and 90th percentiles (bars) of viral load within groups. Significant differences between groups and sample numbers per group are indicated.

FIGURE 4.

A, Distribution of *CCL3L1* gene copies pdg among 71 HIV-1–infected women. Correlation between CCL3L1 copy number and viral load (B) and CD4 T-cell count (C) is demonstrated.

FIGURE 5.

Relation between magnitude of Gag-specific CD4⁺ T-cell responses and *CCL3L1* copy number stratified as <5 or ≥5 (population median = 5). Data are presented as medians (horizontal bar), 25th and 75th percentiles (boxes), and 10th and 90th percentiles (bars) of the magnitude of Gag-specific CD4 T cells among those with < or ≥5 *CCL3L1* gene copies pdg. Significant differences between groups and sample numbers per group are indicated.

FIGURE 6.

Comparison of viral loads and *CCL3L1* copy numbers among women with CD8⁺ Gag-specific T-cell responses with and without concurrent CD4⁺ Gag-specific T-cell responses. Proportions of total individuals with and without CD4⁺ and CD8⁺ T-cell responses to Gag (and the overlap between the 2) are indicated at the top of the figure. Sample numbers and proportions of CD8⁺ Gag (no CD4 response to Gag) and CD8⁺ Gag (with a CD4 response to Gag) responders are indicated underneath the graphs. Data are presented as medians (horizontal bar), 25th and 75th percentiles (boxes), and 10th and 90th percentiles (bars) of viral load and *CCL3L1* copy number, respectively. Significant differences between groups and sample numbers per group are indicated.

TABLE 1
Frequency of CD4⁺ and CD8⁺ T-Cell Responses to Gag, Pol, Nef, Reg, and Env Among 71 HIV-1–Infected Women in South Africa

T-Cell Response	Gag No. (%)	Pol No. (%)	Nef No. (%)	Reg No. (%)	Env No. (%)
CD4 ⁺	28 (39.4)	16 (22.5)	13 (18.3)	18 (25.4)	26 (36.6)
CD8 ⁺	54 (76.1)	54 (76.1)	59 (83.1)	43 (60.6)	47 (66.2)

TABLE 2Peptide Pools Eliciting CD4⁺ and CD8⁺ T-Cell Responses Among 71 HIV-1–Infected Women in South Africa

No. Peptide Pools Positive	No. (%) Patients With CD4 ⁺ T-Cell Response	No. (%) Patients With CD8 ⁺ T-Cell Response
5	0	20 (28.2)
4	6 (8.4)	27 (38.0)
3	9 (12.7)	11 (15.5)
2	16 (22.5)	3 (4.2)
1	18 (25.4)	7 (9.9)
0	22 (31.0)	3 (4.2)