

SHORT REPORT: HIGHER PERCENTAGES OF CIRCULATING MAST CELL PRECURSORS CORRELATE WITH SUSCEPTIBILITY TO REINFECTION WITH *SCHISTOSOMA MANSONI*

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Abstract. A high level of serum IgE is generally associated with human resistance to schistosomes, though the protective mechanisms of IgE remain undefined. We recently reported that whereas some individuals who are occupationally hyperexposed to *Schistosoma mansoni* display resistance to reinfection, others remain highly susceptible, in some cases due to HIV-1 co-infection. As IgE functions, in part, through FcεRI on mast cells, we characterized circulating CD117⁺ FcεRI⁺ mast cell precursors in this population. Surprisingly, a higher percentage of CD117⁺ cells correlated with a susceptible phenotype in HIV-1 seronegative participants with schistosomiasis. There was no association between percentages of peripheral CD117⁺ cells and susceptibility to reinfection in persons with HIV-1. Serum levels of polyclonal IgE were inversely correlated with percentages of CD117⁺ cells regardless of HIV-1 status. Thus, immature mast cells may affect IgE availability, or IgE may affect immature mast cells, altering the balance of host susceptibility and resistance to schistosomes.

Parasite-specific IgE has been associated with resistance to schistosomes in several studies.^{1–5} Although the mechanism by which IgE affords protection against parasites has not been elucidated, it likely acts through high affinity IgE receptors on granulocytes, such as mast cells, basophils, and eosinophils.^{6–10} As mature, functional mast cells reside in anatomic sites most likely to be invaded by schistosomes, such as dermal tissues, they may be among the first cells encountered by this pathogen.¹¹ Furthermore, the majority of somatic IgE is bound to tissue mast cells by surface FcεRI α chain.¹² Effector mechanisms, such as degranulation, result when multivalent antigen crosslinks cell-bound IgE.^{6,13} Dermal mast cells release immunoregulatory molecules such as IL-8, TNF-α, and histamine, which are important in stimulating early host immune responses.¹⁴

While there is evidence that IgE and granulocytes play an important role in human resistance to reinfection with schistosomes, information is only beginning to emerge regarding the role of the high-affinity Fc epsilon receptor (FcεRI) in the context of human immunity to infection with schistosomes and other helminths.¹⁵ Recently, we reported that increased percentages of FcεRI β⁺ eosinophils correlated with resistance to schistosomiasis, although the mechanism by which eosinophils contribute to immunity is undefined.¹⁰ In contrast, basophilia does not develop during helminth infections, including schistosomiasis, and schistosome antigens may play a role in the desensitization of basophils.^{8,16,17} With respect to human mast cells, there are few reports defining their behavior during schistosomiasis.

Our group is currently investigating a population of adult male car washers who are occupationally exposed to infective *Schistosoma mansoni* cercariae. Individuals who develop increased resistance after cycles of reinfection and praziquantel (PZQ) treatment as well as those who remain highly suscep-

tible have been identified.¹⁸ Because mature mast cells do not circulate in blood, we evaluated levels of peripheral immature mast cells, to determine whether an increased circulation of these cells might be associated with resistance or susceptibility to infection with schistosomes. As approximately one third of the study population is seropositive for HIV-1, we also evaluated the effect of HIV-1 co-infection on mast cell precursor levels.

This study was approved by the Institutional Review Boards of the Centers for Disease Control and Prevention and the University of Georgia, the Scientific Steering Committee of the Kenya Medical Research Institute, and the National Ethics Review Board of Kenya. It was performed in western Kenya along the shores of Lake Victoria as previously described.¹⁸ Study participants, aged 18–55 (mean age ± standard deviation: 27.4 ± 9.4), included occupationally exposed car washers or fishermen, who had been observed for up to 9 years (Table 1). HIV-1 infection in this population can lead to lower levels of eggs per gram of feces in those with equal worm burdens as HIV-1 seronegative individuals.¹⁹ HIV-1 co-infections also increase susceptibility to reinfection by schistosomes in relationship to levels of circulating CD4⁺ T cells¹⁸ and eosinophils.¹⁰

Upon informed consent, blood was drawn into heparinized vacutainers by venipuncture. Absolute CD4⁺ and CD8⁺ T cell counts were determined by FACsCount. Whole blood cells were stained and analyzed by flow cytometry¹⁰ (Figure 1A–1D) to determine the percentage of CD117⁺ (c-kit), FcεRI⁺, CD3[–], CD19[–], CD14[–], CD11b[–] cells (staining reagents obtained from BD Pharmingen and Upstate Biotechnology). These CD117⁺ cells are considered to be mast cell precursors.^{20–23} Data were collected on a FACSCalibur (BD Biosciences) using CellQuest software and analyzed with WinMDI software (Joseph Trotter, The Scripps Research Institute, CA). HIV-1 screening was performed on study participants' plasma samples by Sero Strip HIV (Saliva Diagnostic Systems, Vancouver, WA) and Uni-Gold HIV Recombinant (Trinity Biotech, Wicklow, Ireland) tests according to the manufacturers' specifications.

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TABLE 1
Comparison of parasitologic and immunologic measures by HIV-1 status

	HIV negative	HIV positive
N	32	23
EPG (95% CI)	233 (13–454)	86 (20–149)
CD4 (95% CI)	839 (690–988)*	490 (393–587)
CD8 (95% CI)	583 (499–666)*	1007 (788–1226)
CD117 (95% CI)	3.71 (3–4.4)	3.58 (2–4.6)

* $P < 0.01$ compared with HIV-1 positive cohort as determined by Mann-Whitney test.

Subjects' feces (3 stools per person, 2 slides per stool) were screened for *S. mansoni* eggs using the modified Kato Katz technique (Helm Tec R Kato/Katz kit; Pesquisas E Desenvolvimento Limitada, Brazil). Infected individuals were treated with PZQ (40 mg/kg). A person was considered to be reinfected with *S. mansoni* if found to have a positive egg count after successful treatment (drug treatment followed 6 weeks later by three egg-negative stools).

A mathematical formula was created to generate a numerical value representing relative susceptibility to reinfection, which took into account the number of cars washed (infested water exposure), length of time in study, and the number of times reinfected while in study.¹⁰

$$\frac{\text{Number of times reinfected} \times 100}{\text{Amount of time followed (weeks)} \times \text{mean number of cars washed per week}} = \text{IoS/R}$$

In relation to this formula, the lower the magnitude of the

IoS/R, the more resistant the individual is to reinfection by schistosomes. Statistical analyses were performed using GraphPad InStat version 3.05 (GraphPad Software, San Diego, CA). Nonparametric comparisons of groups were made with the Mann-Whitney U test. Spearman nonparametric rank correlation test was used to evaluate associations between experimental measures.

The overall mean percentages of CD117⁺ cells were similar in HIV-1 positive and negative study participants (Table 1) and most CD117⁺ cells expressed low levels of FcεRI (Figure 1C). Percentages of circulating CD117⁺ cells were plotted against the IoS/R values. Surprisingly, a positive correlation between a susceptible phenotype and a higher percentage of circulating CD117⁺ cells was observed in the HIV-1 seronegative cohort (Figure 2A, $P = 0.040$). There are several possible explanations for this observation. For example, higher levels of immature mast cells in circulation might be a result of aberrant cellular trafficking in persons who become reinfected or a dysregulation of mast cell development due to altered levels of specific cytokines involved in mast cell maturation. We recently reported that higher percentages of peripheral blood eosinophils in HIV-1 seronegative individuals correlate with resistance (low IoS/R values) in this car washer study group.¹⁰ In the current study, we predicted that preferential generation of immature mast cells by the bone marrow would explain the elevation of CD117⁺ cells in the peripheral blood of those who are more susceptible to reinfection. However, there was no correlation (either direct or inverse) between percentages of circulating eosinophils and mast cells (Figure 2D), suggesting that there are likely separate mecha-

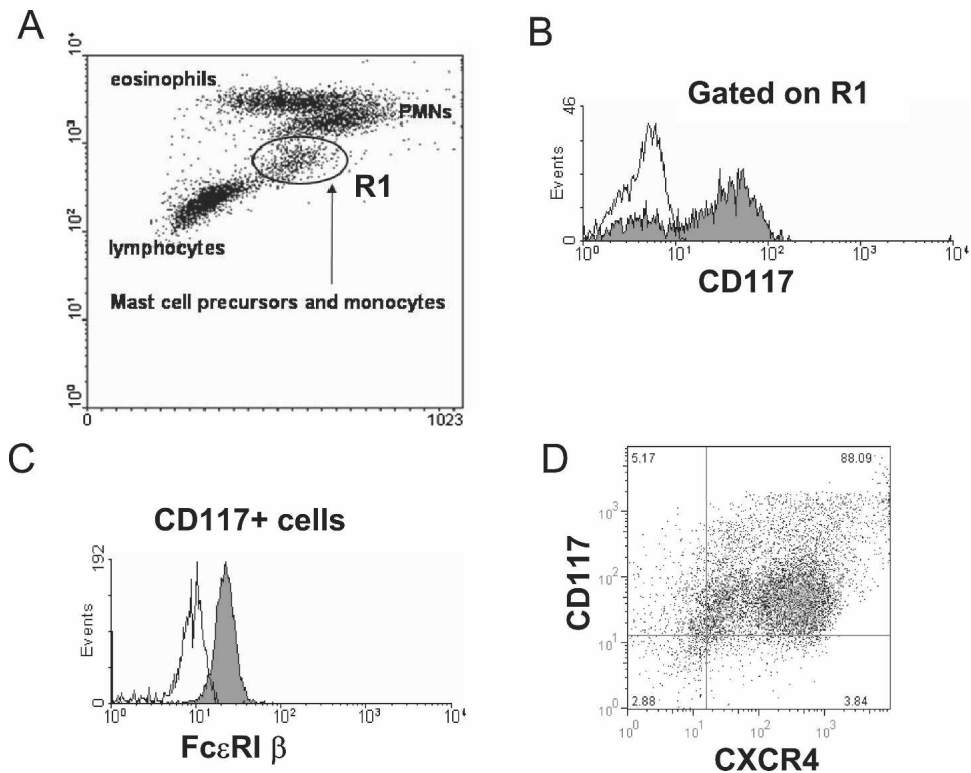


FIGURE 1. Circulating CD117⁺ cells in schistosomiasis. A, Whole blood was electronically separated by linear/log, forward/side scatter plot. Four distinct leukocytic groups were apparent and include lymphocytes, monocytes, and mast cell precursors (R1), polymorphonuclear cells (PMN), and eosinophils. B, Cells in R1 contain CD117⁺ and CD117⁻ cells (gray fill: anti-CD117; black line: isotype control). C, CD117⁺ cells express low levels of FcεRI β chain (gray fill; black line: isotype control). D, CD117⁺ cells co-express CXCR4.

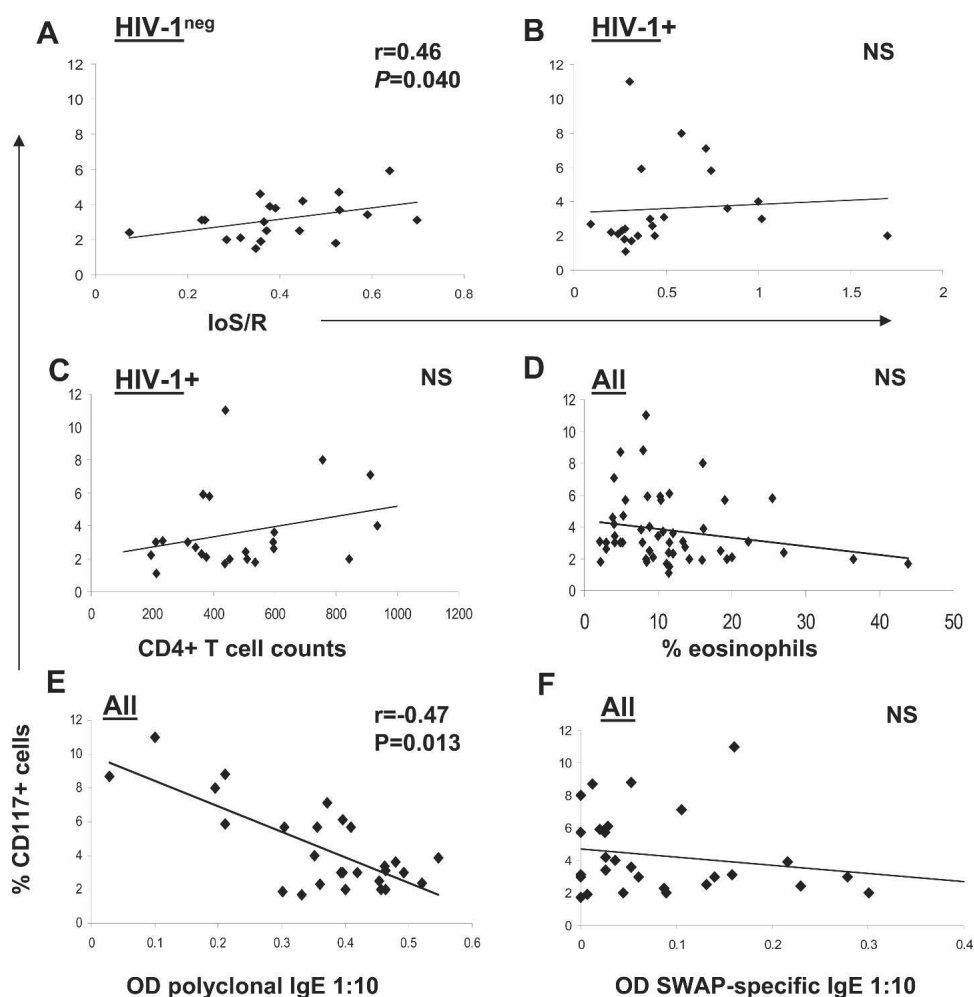


FIGURE 2. Higher percentages of CD117⁺ cells correlate with a history of susceptibility in HIV-1 seronegative individuals and low concentrations of serum polyclonal IgE. A, Percentages of circulating CD117⁺ cells were plotted against IoS/R of HIV-1 seronegative study participants. Higher percentages of CD117⁺ cells are associated with a history of susceptibility ($r = 0.46$; $N = 20$; $P = 0.04$, Spearman rank correlation). B, Percentages of circulating CD117⁺ cells were plotted against IoS/R of HIV-1 seropositive study participants and demonstrate no relationship. C, Percentages of CD117⁺ cells do not correlate with CD4⁺ T cells in HIV-1 seropositive individuals. D, Percentages of CD117⁺ cells are not related to the percentages of eosinophils in either cohort. Shown are data from both HIV-1 positive and negative individuals. E, Concentrations of polyclonal IgE were measured by standard isotype-specific ELISA and optical densities (OD) were plotted against percentages of CD117⁺ cells using data from both cohorts. Higher percentages of CD117⁺ cells are associated with a low level of IgE ($r = -0.47$; $N = 27$; $P = 0.013$). F, Concentrations of SWAP (soluble worm antigen preparation)-specific IgE were measured by standard antigen-specific isotype-specific ELISA using 5 $\mu\text{g/ml}$ of SWAP and 1:10 dilution of sera. ODs representing relative concentrations of SWAP-specific IgE were plotted against percentages of CD117⁺ cells using data from both cohorts. No relationship was observed. Samples sizes differ for different tests due to the unavailability of certain samples from some study participants.

nisms in the generation or distribution of eosinophils and mast cell precursors in persons with schistosomiasis.

The association of susceptibility to reinfection with schistosomes with an increased level of CD117⁺ cells was not observed in the HIV-1 seropositive cohort (Figure 2B). Similarly, there was no correlation in either HIV-1 seropositive or seronegative study participants between their CD4⁺ T cell counts and their levels of CD117⁺ cells (Figure 2C; the results obtained from the HIV-1⁺ group are shown). This is in contrast to what we have observed in regard to eosinophil percentages, which did correlate with CD4⁺ T cell counts in the HIV-1⁺ study participants.¹⁰ Therefore, although T cells appear to augment eosinophilia, they may not be as critical for the generation or maintenance of mast cell precursors in schistosomiasis.

CD4^{low} mast cells and basophils may express receptors for HIV-1 including CCR5 and CXCR4 and have been shown to

be susceptible to infection with the virus despite the low expression of CD4.^{22,24–26} CD117⁺ cells in the car washer population were positive for CXCR4 (Figure 1D) and CCR5, but were CD4^{neg/low} (data not shown). It is therefore possible that CD117⁺ cells are infected with the virus in the HIV-1 seropositive group but we did not determine infection status of these cells in this study. Furthermore, HIV-1 synthesizes two proteins that have direct effects on mast cell and basophil function.²⁷ First, HIV-1 Tat has the unique ability to induce chemotaxis of basophils and mast cells that may affect systemic trafficking of mast cell precursors.²⁸ Second, gp120 is a member of the Ig superantigen family and has been shown to crosslink surface-bound IgE on Fc ϵ RI-bearing cells thereby inducing IL-4 and IL-13 secretion.^{29,30} Thus, HIV-1 co-infection in this study population may impede our ability to accurately evaluate the role of circulating mast cells in schistosomiasis.

In addition to a putative role for host protection against infection with schistosomes, there is emerging evidence that IgE may promote mast cell survival in the absence of antigen.^{31,32} Therefore, we plotted the levels of serum IgE, as measured by standard ELISA,¹⁰ against percentages of CD117⁺ cells. There was an inverse correlation between the percentages of CD117⁺ cells with concentrations of total IgE (Figure 2E) but no relationship with levels of adult worm-specific IgE, regardless of HIV-1 status (Figure 2F). These results suggest a possible cross-regulatory interplay in the biology of IgE and immature mast cells, which express FcεRI at lower levels than that described for mature cells.

Mast cells are normally strategically positioned as sentinels in tissues such as the skin and mucosa that are also most likely to first encounter infection by schistosomes. Because IgE is associated with resistance, strategically located mast cells could have an important role in host resistance or susceptibility. For example, because mast cell degranulation increases vascular permeability, anti-schistosome IgE-coated mast cells could either assist in cercarial penetration (increased susceptibility) or enhance the access of immune effector cells to the areas of tissue penetration (increased resistance) when they encounter schistosome antigens. While it is difficult to conjecture what may be occurring in regard to tissue mast cells, our data show a correlation between increased percentages of circulating mast cell precursors and susceptibility to reinfection and could indicate a dysregulation of mast cell maturation that contributes to susceptibility. Because our cohort size was relatively small and the range of mast cell precursors was narrow, continued studies are warranted to further our understanding of the role of different types of granulocytes and their IgE receptors in the mechanisms of host protection.

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