

C-Reactive Protein Levels Increase During HIV-1 Disease Progression in Rakai, Uganda, Despite the Absence of Microbial Translocation

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on behalf of the Rakai Health Sciences Program

Introduction: Microbial translocation has been implicated as a contributing factor to the heightened immune activation observed during HIV-1 disease progression. When examined in a longitudinal study of HIV-1 seroconverters in Rakai, Uganda, microbial translocation was not associated with HIV-1 disease progression. However, the role of general immune activation in HIV disease progression in this population was not fully examined.

Methods: Longitudinal serum samples of HIV-1 seroconverters in three HIV-1 disease progression groups [long-term nonprogressors (LTNP), standard progressors (SP), and rapid progressors (RP)] from Rakai, Uganda, were tested for levels of C-reactive protein (CRP), a marker for immune activation.

Results: CRP levels significantly increased in the SP group ($P < 0.0001$) but not in the RP group or the LTNP group. CRP levels during the first year post-HIV seroconversion in the RP group were significantly higher than those observed in the LTNP group ($P < 0.05$). For the entire population, CRP levels negatively correlated with lipopolysaccharide levels ($P < 0.05$) and were not associated with endotoxin antibody levels.

Conclusions: This study suggests that in this population, increased immune activation is significantly associated with HIV-1 disease progression but not microbial translocation.

Key Words: Africa, HIV disease progression, immune activation, microbial translocation

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INTRODUCTION

Immune activation has been demonstrated in multiple studies to be a significant contributing factor to HIV-1 disease progression.^{1–4} In a cross-sectional analysis of subjects in the United States, it was observed that this immune activation was associated with increased levels of the bacterial components in the blood, which was hypothesized to be due to increased microbial translocation from the lumen of the gastrointestinal tract.⁵ This increase in microbial translocation was hypothesized to contribute to HIV-1 disease progression.^{5,6} When this theory was examined in a longitudinal study of HIV-1 seroconverters with known progression outcomes from Rakai, Uganda, it was found that microbial translocation or its subsequent innate cytokine response had no association with HIV-1 disease progression.⁷ However, this study did not measure general immune activation specifically. CRP is an indicator of immune activation in response to inflammatory damage or infection and has been shown to increase in HIV-1–infected individuals.^{8–11} Therefore, we examined C-reactive protein (CRP) levels in these subjects to determine if immune activation was associated with disease progression in this population.

METHODS

The study population has been described in detail previously.⁷ Briefly, serum samples of HIV-1 seroconverters from Rakai, Uganda, were tested for levels of CRP by enzyme-linked immunosorbent assay using manufacturer's recommendations (Invitrogen, Carlsbad CA; or Biovender Laboratory Medicine, Czech Republic for samples that were out of range on the Invitrogen assay). Longitudinal samples from pre-infection throughout disease progression were tested, and the subjects were divided into three progression groups [long-term nonprogressors (LTNP, CD4 count >600 cells/ μ L at 7+ years after seroconversion; $n = 27$), standard progressors (SP, death

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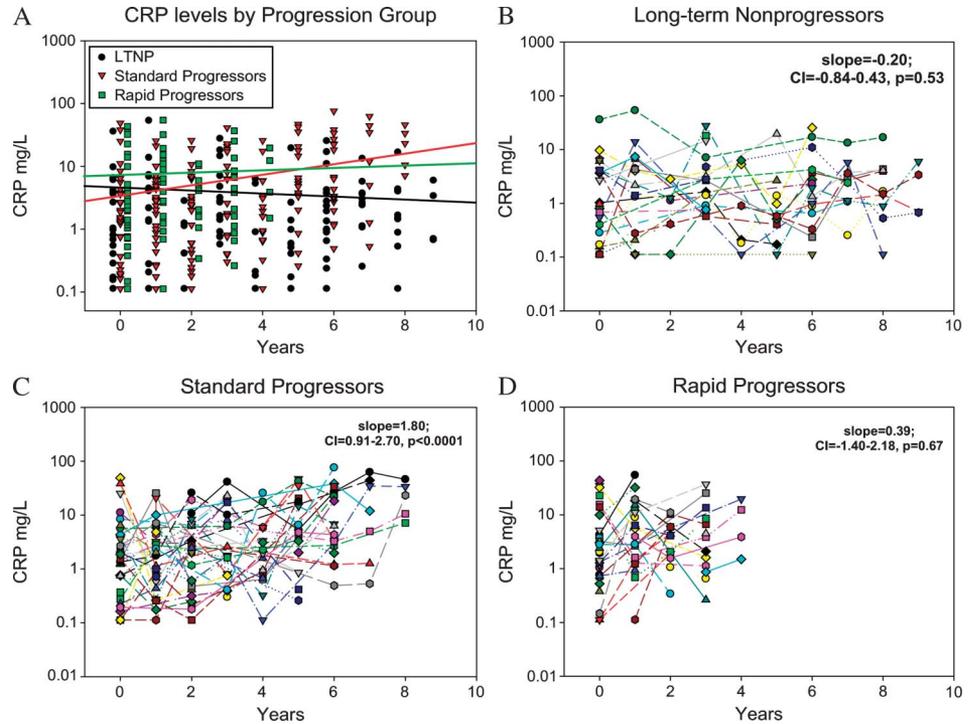


FIGURE 1. Longitudinal changes of CRP levels according to disease progression groups. The last HIV-negative time point for each patient (year 0) and all subsequent years postseroconversion are shown together (A) and for each progression group [LTNP (B) (circles, n = 27), SP (C) (inverted triangles, n = 41), and RP (D) (squares, n = 39)]. Longitudinal variation in patient levels was determined using linear multilevel modeling fit with slopes and 95% confidence intervals shown for each group. The resulting regression line for each group is shown in the respective color (A).

>5 years but <9 years after seroconversion; n = 41), and rapid progressors (RP, death <4 years after seroconversion; n = 39)]. The change in CRP level by progression group was then examined using a linear mixed-effects model, and the slopes (mg/year, ie, the change of CRP along time) were tested and

compared between progression groups at significance level of $\alpha = 0.05$.^{7,8} CRP levels at the first year postseroconversion were compared using an Analysis of Variance on ranks test, and Dunn method for pair-wise comparisons ($P < 0.05$). Due to the variability observed in the longitudinal CRP levels, the

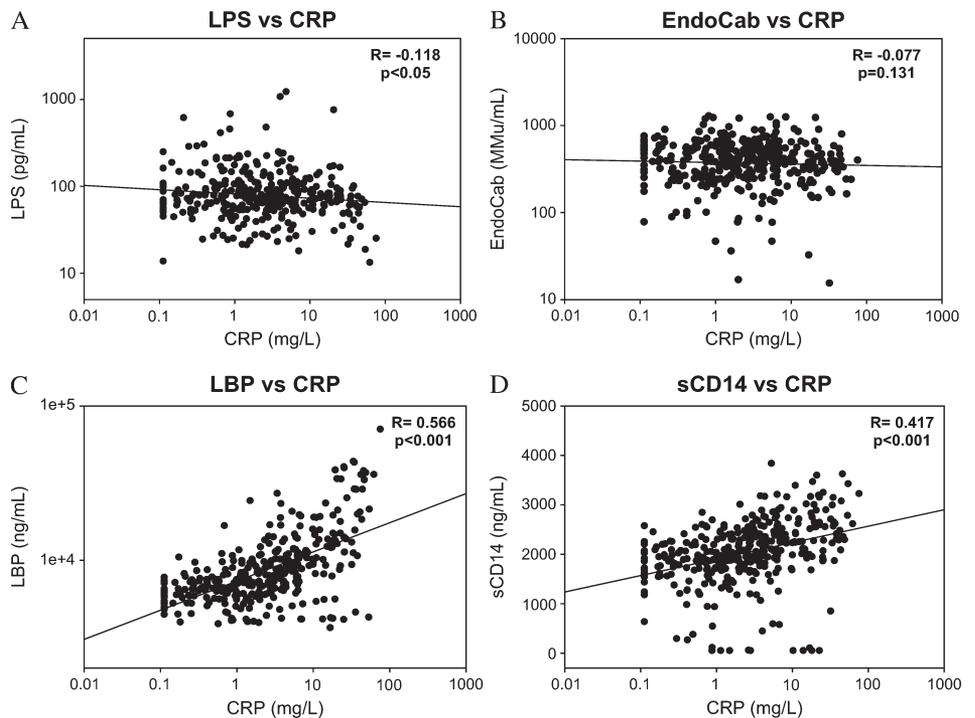


FIGURE 2. Comparisons of CRP and LPS (A), EndoCab (B), LBP (C), and sCD14 (D) levels for the total population were calculated using Spearman rank order analysis. Correlation coefficients (R) for all cytokines and microbial translocation markers are shown with corresponding P values (significance was determined to be $P < 0.05$). EndoCab, endotoxin core antibody.

TABLE 1. Spearman Correlations of CRP and Microbial Translocation Markers According to Disease Progression Groups

| Progression Group | LPS | EndoCab | LBP | sCD14 |
|-------------------|--|-----------------------|--|--|
| LTNP | -0.050 ($P = 0.73$) | -0.033 ($P = 0.73$) | 0.255 ($P < 0.01$) | 0.265 ($P < 0.01$) |
| SP | -0.228 ($P < 0.01$) | -0.069 ($P = 0.38$) | 0.737 ($P < 0.001$) | 0.585 ($P < 0.001$) |
| RP | -0.008 ($P = 0.94$) | -0.039 ($P = 0.73$) | 0.601 ($P < 0.001$) | 0.287 ($P < 0.01$) |

Correlation coefficients and corresponding P values are shown with significant correlations shown in bold. EndoCab, endotoxin core antibody.

population was divided into those that showed signs of consistent immune activation (serially increasing CRP levels in 80% of subsequent time points, $n = 39$) and inconsistent activators (all other subjects, $n = 68$). CRP levels were compared with indicators of microbial translocation tested previously [lipopolysaccharide (LPS), endotoxin core antibody, LPS-binding protein (LBP), soluble CD14 (sCD14)] with a Spearman correlation.⁷ The cohort was approved by Institutional Review Boards in Uganda (the Uganda Virus Research Institute's Science and Ethics Committee and the Uganda National Council for Science and Technology) and from the Institution Review Boards of collaborating US institutions (Walter Reed Army Institute of Research, Columbia University and Johns Hopkins University).

RESULTS

It was observed that CRP levels significantly increased in the SP group (slope = 1.80 mg/year, $P < 0.0001$) but not in the RP group (slope = 0.39, $P = 0.67$) or the LTNP group (slope = -0.20, $P = 0.53$) (Fig. 1). The increase in CRP levels observed in the SP group was significantly higher than in the LTNP group ($P = 0.0005$) (Fig. 1A). The slope of the increase in CRP levels in the SP group is similar to levels observed by Lau et al⁸ in US subjects who progress to AIDS (slope = 1.39). CRP levels during the first-year postseroconversion in the RP group [median = 4.76 mg/L, interquartile range (IQR) = 1.37–13.8] were significantly higher than those observed in the LTNP group (median = 0.57; IQR = 0.21–4.21) ($P < 0.05$) but not the SP group (median = 2.55, IQR = 0.65–5.08).

CRP levels were slightly negatively correlated with LPS ($r = -0.12$, $P < 0.05$) but were significantly correlated to LBP ($r = 0.57$, $P < 0.001$), and the monocyte activation marker sCD14 ($r = 0.42$, $P < 0.001$) (Figs. 2A, C, D). There was no correlation between CRP and endotoxin core antibody levels (Fig. 2B). When these parameters were examined according to progression group, consistent patterns were seen for LBP and sCD14, but LPS was negatively correlated to CRP in the SP group only (Table 1). CRP did not correlate with LPS in those subjects with consistent immune activation ($r = -0.08$, $P = 0.325$) but was negatively correlated in those individuals with inconsistent activation ($r = -0.204$, $P < 0.01$).

DISCUSSION

The similarity between these results and those seen in US-based studies would suggest that at least in the case of longitudinal CRP levels, the two populations are similar.⁸ This

is surprising because the previous findings found significant differences in a variety of markers for immune activation in HIV-uninfected individuals.⁷ Additionally, it is interesting that CRP levels did not significantly increase in the RP group, but this may be due to the higher initial levels of CRP seen in this group. The short lifespan of the RP group also may not provide enough time for the CRP levels to noticeably increase. It is also possible that the higher initial level of immune activation seen in the RP group contributes to rapid disease progression. The absence of change in the LTNP group was expected because lower rates of CRP increase have been seen in individuals who do not progress to AIDS.⁸ These results are also somewhat divergent from a study done in Zambia that found that increased CRP levels only associated with HIV-1 disease progression in patients who had diarrhea, although that was a cross sectional study, and therefore is not strictly comparable to the current longitudinal study.¹⁰ It has previously been speculated that CRP levels may be used as a marker for disease progression in resource-poor settings, but the variability observed in individual CRP trajectories overtime most likely limits its clinical utility.¹²

The negative correlation of CRP with LPS levels in the total population and the SP group, and the lack of any significant correlation in those individuals that show consistent immune activation, suggests that the activation observed in this population is not caused by an increase in microbial translocation, even though CRP correlated with other markers for immune activation (LBP and sCD14) that have been linked to microbial translocation.⁵ In conclusion, these data suggest that in this Ugandan population, immune activation is associated with HIV-1 disease progression in the absence of microbial translocation.

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REFERENCES

- Bentwich Z, Kalinkovich A, Weisman Z. Immune activation is a dominant factor in the pathogenesis of African AIDS. *Immunol Today*. 1995;16:187–191.
- Rizzardini G, Piconi S, Ruzzante S, et al. Immunological activation markers in the serum of African and European HIV-seropositive and seronegative individuals. *AIDS*. 1996;10:1535–1542.
- McCune JM. The dynamics of CD4+ T-cell depletion in HIV disease. *Nature*. 2001;410:974–979.
- Douek DC. Disrupting T-cell homeostasis: how HIV-1 infection causes disease. *AIDS Rev*. 2003;5:172–177.

5. Brechley JM, Price DA, Schacker TW, et al. Microbial translocation is a cause of systemic immune activation in chronic HIV infection. *Nat Med*. 2006;12:1365–1371.
6. Brechley JM, Price DA, Douek DC. HIV disease: fallout from a mucosal catastrophe? *Nat Immunol*. 2006;7:235–239.
7. Redd AD, Dabitao D, Bream JH, et al. Microbial translocation, the innate cytokine response, and HIV-1 disease progression in Africa. *Proc Natl Acad Sci U S A*. 2009;106:6718–6723.
8. Lau B, Sharrett AR, Kingsley LA, et al. C-reactive protein is a marker for human immunodeficiency virus disease progression. *Arch Intern Med*. 2006;166:64–70.
9. Pepys MB, Hirschfield GM. C-reactive protein: a critical update. *J Clin Invest*. 2003;111:1805–1812.
10. Zulu I, Hassan G, Njobvu RNL, et al. Cytokine activation is predictive of mortality in Zambian patients with AIDS-related diarrhoea. *BMC Infect Dis*. 2008;8:156.
11. Melchior JC, Niyongabo T, Henzel D, et al. Malnutrition and wasting, immunodepression, and chronic inflammation as independent predictors of survival in HIV-infected patients. *Nutrition*. 1999;15:865–869.
12. Drain PK, Kupka R, Msamanga GI, et al. C-reactive protein independently predicts HIV-related outcomes among women and children in a resource-poor setting. *AIDS*. 2007;21:2067–2075.