Role of translocated bacterial flagellin in monocyte activation among individuals with chronic HIV-1 infection

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Conclusions
Our results indicate that translocated bacterial flagellin contributes to systemic monocyte activation in HIV-1 infected patients, through activation of Toll-like receptor 5 (TLR5):

- Circulating anti-flagellin IgG correlated to plasma markers of microbial translocation and monocyte activation
- Circulating anti-flagellin IgG correlated with TLR5 expression and cytokine production in monocytes; both basal and response to flagellin (“TLR5 responsiveness”)

Introduction
Microbial translocation is considered to be an important underlying factor of chronic immune activation characteristic of HIV-1 infection. Monocyte activation in particular has been associated to morbidity and mortality (Wilson et al., 2014).

We hypothesised that bacterial flagellin, a Toll-like receptor 5 (TLR5) agonist, co-translocates with other microbial products and contributes to systemic monocyte activation.

Aim
This study aimed to assess circulating flagellin and anti-flagellin levels in HIV-infected subjects, their correlation to other markers of microbial translocation and immune activation, the effect of antiretroviral therapy (ART), and their relationship to TLR5 responsiveness.

Study population
Blood samples were collected from four groups: 1) 23 ART-naïve HIV-positive subjects; 2) 24 HIV-positive subjects with samples prior to initiation of ART as well as after 5-12 months on therapy; 3) 21 HIV-negative subjects (“negative controls”); and 4) 27 individuals with infectious diarrhea (“positive controls”).

Methods
Serum levels of flagellin, anti-flagellin IgG, total IgG were measured by ELISA and compared to established plasma markers of microbial translocation (LPS, LBP) and monocyte activation (sCD14, sCD163). To assess TLR5 responsiveness, PBMCs from HIV-positive and HIV-negative individuals were exposed to flagellin in vitro, followed by flow cytometry analysis of cell surface activation markers (CD14, CD163, HLA-DR) and cytokine production (TNF, IL-1beta, IL-6, IL-8).

Results
- Flagellin and anti-flagellin IgG were not significantly higher in HIV+ vs. HIV-, but were correlated to each other. Anti-flagellin IgG was reduced significantly after ART (Figure 1).
- Anti-flagellin IgG was correlated to plasma LPS, LBP, sCD14, and sCD163 (Figure 2).
- Trend to correlation between circulating anti-flagellin IgG and basal monocyte cell surface TLR5 expression, and a significant correlation to reduction in TLR5 upon flagellin stimulation (Figure 3).
- TLR5 cell surface expression was highly correlated to basal monocyte cytokine production and response to flagellin stimulation, and anti-flagellin IgG displayed similar correlation patterns (Table 1).

Table 1. Cytokine production following flagellin stimulation

<table>
<thead>
<tr>
<th>% cytokine-producing monocytes</th>
<th>Correlation with basal surface TLR5 (HIV+ and HIV-)</th>
<th>Correlation with serum anti-flag/flagG (HIV+ only)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Spearman’s rho</td>
<td>P-value</td>
</tr>
<tr>
<td>IL-1β+ IL-8+</td>
<td>0.126</td>
<td>0.001</td>
</tr>
<tr>
<td>induction</td>
<td>0.401</td>
<td>0.001</td>
</tr>
<tr>
<td>IL-6+ IL-8+</td>
<td>0.19</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>induction</td>
<td>0.129</td>
<td>0.302</td>
</tr>
</tbody>
</table>

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