Dysfunctional Neutrophil Response to SIV Infection

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ABSTRACT

Recent studies indicate that individuals with low neutrophils (PMN) are at increased risk of HIV infection. Also, the RV144 vaccine trial implicated non-neutralizing antibodies and associated Fc-mediated functions in vaccine-induced protection. These studies suggest that early innate antiviral functions and Fc-mediated functions of PMN may play an important role in mediating protection or early viral control, yet the role of innate cellular responses in preventing infection or the establishment of the viral reservoir remains unknown. The potential to contribute to the early control of viral spread may depend on the kinetics of PMN mobilization, activation, and recruitment during acute HIV/SIV infection. We assessed kinetic changes in PMNs in peripheral and mucosal tissues during acute SIV infection in six rhesus macaques challenged i.r. with 100,000 TCID50 of SIVmac239X. Flow cytometry, CBC, and luminex were used to assess PMN and cytokine levels and related PMN functional markers. Samples were collected pre-SIV and days 3, 7, 14, 21, 28, 42, and 63 post-SIV. We observed trending decreases in systemic IL-17 (p=0.0793) and G-CSF (p=.0625) early after SIV. Surprisingly, blood PMN concentrations steadily decreased after infection, and no significant increase of PMN was detected in gut or lymphoid tissues, as would be expected after viral infection. In addition, blood PMN numbers and rectal PMN percentages significantly correlated (p=0.0032). Lastly, HLA-DR (p=0.0185), CD86 (p=0.0335), and FcγRI (p=<0.001) were significantly upregulated on PMN during acute SIV infection, suggesting PMN activation without concurrent tissue recruitment, indicating a dysfunctional innate response. In contrast to other acute viral infection models, PMN-supporting cytokines are decreased or not induced during acute SIV, potentially contributing to lack of PMN mobilization and recruitment to the tissues. Further, blood and rectum PMN levels correlate post-SIV, suggesting that blood PMN concentration may directly impact recruitment to gut tissues. Lastly, upregulation of markers involved in antigen presentation and Fc-mediated functions highlights the potential diverse functional roles of neutrophils during acute SIV infection and the mechanisms by which neutrophils, if induced, could contribute to more effective viral control. However, lack of neutrophil induction during acute SIV may contribute to productive infection and the establishment of the viral reservoir.

BACKGROUND AND RATIONALE

Neutrophils in HIV/SIV

Neutrophil dysfunction in infected individuals:

- Neutropenia
- Increased apoptosis
- Reduced function
- Impaired tissue recruitment

→ **Increased Secondary infections**

Neutrophils in mucosal barrier repair:

- Mouse model evidence for a critical role of neutrophils in epithelial repair

→ **Microbial translocation and non-AIDS morbidity/ mortality**

Potential role for neutrophils in protection/pathogenesis:

- **Anti-HIV functions shown *in vitro* (reactive oxygen species, defensin activity, NETs, ADCC-like killing of infected T cells)**
- **Antigen presentation**
- **Recent reports indicate individuals with low neutrophil counts have increased risk of HIV infection**

Raffatellu M., et. Al, Nature Medicine, 2008
Ramsuran V., et. Al, CID, 2011
Reviewed by Kuritzkes, CID 2000
Reviewd by Drescher B., et. al, Virus Research, 2012

Neutrophil Response to non-HIV/SIV Pathogens

(as shown in influenza, human rhinovirus, and many bacterial infections)

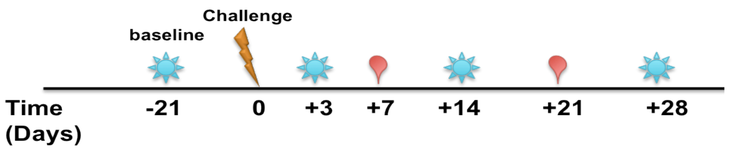
Increase in neutrophil supporting cytokines and chemokines

IL-17 → G-CSF, GM-CSF, IL-8 → **Neutrophil Mobilization (blood concentration)** **AND** **Recruitment to infection site**

QUESTION

What are the kinetics of the neutrophil response to acute SIV infection?

STUDY DESIGN AND METHODS



Legend:

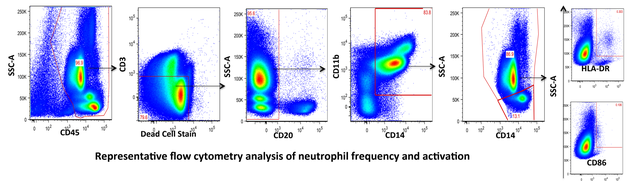
IR Challenge with 100,000 TCID50 $SIV_{MAC239X}$

Comprehensive sampling: Blood and biopsies (colon, jejunum, rectum)

Minimal (non-invasive) sampling: blood draw

n= 6 male rhesus macaques

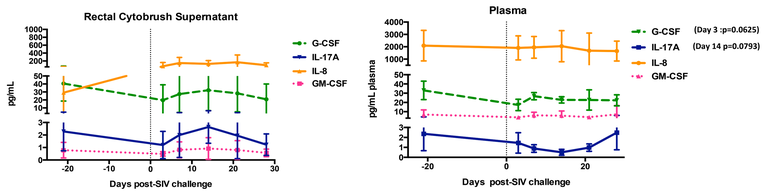
- **Complete blood count and flow cytometry were used to assess neutrophil numbers and activation in blood and mucosal tissues.**
- **Luminex was used to assess cytokine/chemokine levels in plasma and rectal cytobrush supernatant.**



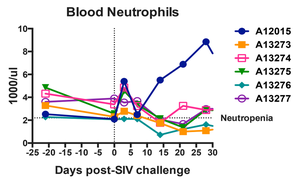
Representative flow cytometry analysis of neutrophil frequency and activation

RESULTS

Cytokines necessary for neutrophil mobilization and recruitment are not induced locally or systemically during acute SIV infection.

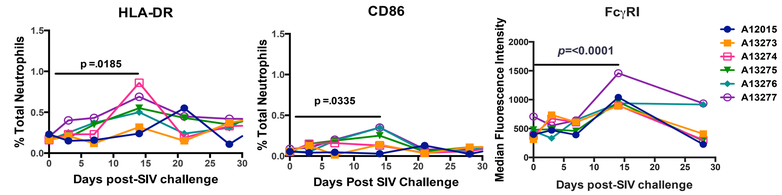


Blood neutrophils progressively decrease during acute SIV infection.

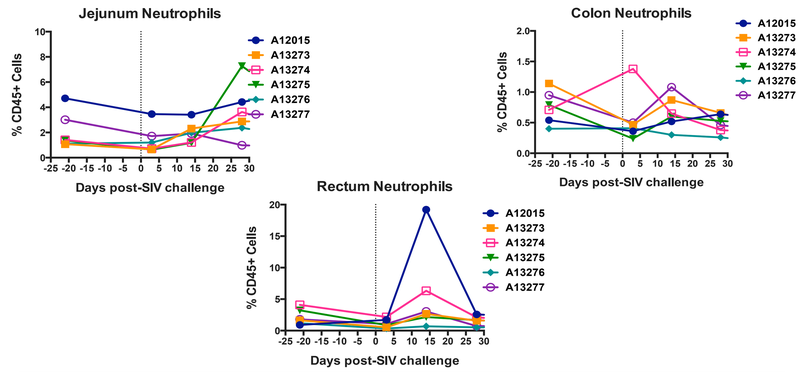


RESULTS (CONT.)

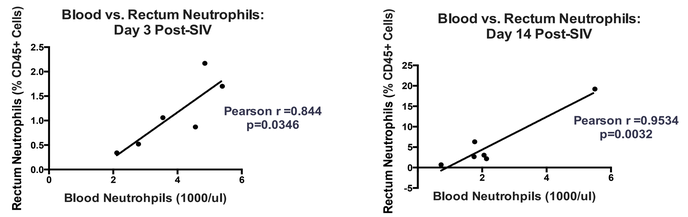
Blood neutrophils upregulate markers involved in antigen presentation and Fc-mediated functions during acute SIV infection.



Neutrophils do not significantly increase in intestinal tissues during acute SIV infection.



Blood neutrophil counts correlate with rectal neutrophil counts during acute SIV infection.



CONCLUSIONS

- In contrast to other infection models, neutrophil-supporting cytokines are not induced during acute SIV, potentially contributing to the observed decrease in blood neutrophils and lack of recruitment to the tissues.
- During acute SIV, neutrophils upregulate markers involved in antigen presentation and Fc-mediated functions highlighting the potential for diverse functionality of neutrophils during acute SIV infection.
- Blood and rectum neutrophil levels correlate post-SIV, suggesting that blood neutrophil concentration may directly impact recruitment to gut tissues.

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