Altered Properties of Mucosal NK Cell Subsets during Acute HIV-1 Infection

Poster 272

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ABSTRACT

The role of mucosal innate immunity during acute HIV infection (AHI) is poorly understood, however increasing evidence supports a beneficial role of natural killer (NK) cells. Here, we investigate the distribution and activation of mucosal NK cell subsets during early AHI.

27 subjects in Fiebig (F) I (n=11) and FIII (n=16) underwent sigmoid biopsy at the time of AHI diagnosis. Ten gender- and age-matched HIV-uninfected (HIV-) and 5 ART-naïve chronically HIV-infected (CHI) subjects served as controls. Mucosal mononuclear cells were isolated, and NK cell subsets were defined by the expression of $CD56^{(bright,\ dim,\ neg)}$ and NKp44 using multi-parameter flow cytometry.

At the time of diagnosis, FI subjects had similar frequencies of total NK cells compared to HIV- and CHI, while FIII subjects showed a significant expansion (Table 1). In contrast surface expression of the activating natural cytotoxicity receptor, NKp44, was significantly increased on total NK cells at FI compared to FIII, HIV- and CHI. $CD56^{dim}$ cytolytic NK cells were the dominant subset of NK cells and were significantly decreased in FI and FIII patients compared to HIV- and CHI. The expression of NKp44 on $CD56^{dim}$ NK cells correlated inversely with plasma HIV RNA (r=-0.48, p=0.01). However, FI patients showed a significantly higher expression of NKp44 compared to FIII, HIV- and CHI. The frequency of immunoregulatory NK cells ($CD56^{bright}$) also decreased in FI and FIII compared to HIV-, and did not recover during CHI. In contrast, a significant expansion of the $CD56^{neg}$ NK cell subset, associated with chronic viral infection, was observed in FI and FIII compared to HIV- and CHI.

Distribution of mucosal NK cells subsets is altered during early AHI characterized by the loss of cytolytic $CD56^{dim}$ and immunoregulatory $CD56^{bright}$ NK cells and the expansion of $CD56^{neg}$ NK cells, a dysfunctional subset associated with impaired cytotoxicity. The reason for the early loss, without recovery, during CHI of the $CD56^{bright}$ subset warrants further investigation in the pathogenesis of HIV infection.

Table 1: Frequencies (%) of NK cell populations.

NK Cell Population	Fiebig I	Fiebig III	HIV-	CHI
total NK	20.3	28.5*	20.9	22.0
total NK NKp44+	9.9	4.4*	5.3*	6.3*
$CD56^{dim}$ NK	65.9	64.5	82.5***	87.1***
$CD56^{dim}$ NK NKp44+	12.1	4.9*	4.4***	2.1**
$CD56^{bright}$ NK	3.4	3.2	6.3*	2.7
$CD56^{neg}$ NK	24.6	26.7	8.9**	8.2***

All data are median (interquartile rang); All comparisons were made to Fiebig I: *p≤0.05, **p≤0.01 and ***p≤0.001

BACKGROUND

- NK cells are critical for defense against many viral infections and have been linked to control of HIV infection *(Alter et al., J Intern Med, 2009).*
- Acute HIV infection is associated with expansion of peripheral NK cells suggesting a virus-driven increase in cytolytic activity *(Alter et al., Blood, 2005).*
- In the NHP model $NKp44^+$ NK cells are restricted to the mucosa, noncytotoxic and likely to mediate mucosal homeostasis by the expression of IL-17 and IL-22 *(Liyanage et al., Virology, 2013; Reeves et al., Blood, 2011).*
- In the NHP model activated $NKp44^+$ mucosal NK cells are depleted during infection and show altered function *(Reeves et al., Blood, 2011).*
- Mucosal NK cells seem to play a role in defenses in the female reproductive tract against vaginal SIV challenge *(Shang et al., J Immunol, 2014).*

MATERIALS AND METHODS

- 27 acutely HIV-infected subjects at Fiebig I (n=11) and Fiebig III (n=16) from RV254/SEARCH 010.
- 10 gender- and aged-matched HIV-uninfected and 5 ART-naïve chronically HIV-infected subjects (6 to 12 months from infection) from RV304/SEARCH 013.
- All subjects underwent sigmoid biopsy. The distribution of mucosal NK cell subsets defined by the expression of CD56 and CD16 and the expression of NKp44 was assessed by multi-parameter flowcytometry.
- Cross-sectional analysis (Mann-Whitney Test); Correlation (Spearman Correlation).

RESULTS

Acutely infected subjects were separated in two groups, subjects in Fiebig I (n=11) and subjects in Fiebig III (n=16). In Fiebig I subjects the frequency of total NK cells was comparable to HIV-uninfected subjects, while in Fiebig III the frequency of total NK cells significantly increased compared to Fiebig I subjects (p≤0.05; **Figure 1A**). The observed increase in frequency of total NK cells with progression of Fiebig stage correlated with the colonic HIV RNA suggesting a virus-driven mechanism (r=0.40, p=0.04; **Figure 1B**).

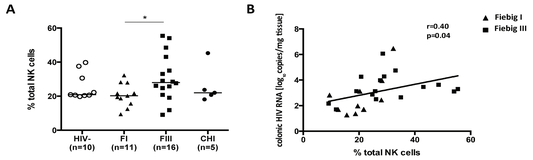

Figure 1: The frequency of total NK cells increases significantly with progression of Fiebig stage, possibly virus driven. (A) Frequency of total NK cells in Fiebig I subjects is comparable to HIV-uninfected subjects, however there is a significant increase in the frequency of total NK cells observed in Fiebig III subjects compared to Fiebig I subjects (A). This increase in frequency of total NK cells with progression of Fiebig stage correlates with the colonic HIV RNA. *p≤0.05

We then further looked at the distribution of the different NK cell subsets, defined by the expression of CD56 ($CD56^{bright}$, $CD45^{dim}$, $CD56^{neg}$). For all three subsets changes in the frequency as well as in the number of cells per gram of tissue were observed already at Fiebig I compared to HIV-uninfected subjects (**Figure 2**). The frequency of $CD56^{bright}$ and $CD45^{dim}$ NK cells decreased significantly in Fiebig I and Fiebig III compared to HIV-uninfected individuals ($CD56^{bright}$: p≤0.05 and p≤0.01, respectively; $CD45^{dim}$: p≤0.001; **Figure 2A and B**). In contrast the frequency of $CD56^{neg}$ NK cells increased significantly in Fiebig I and Fiebig III compared to HIV-uninfected subjects (p<0.001; **Figure 2C**). These changes are also reflected in the absolute number of NK cell subsets per gram of tissue, indicating changes in the total number of those cells subsets (**Figure 2 D, E and F**).

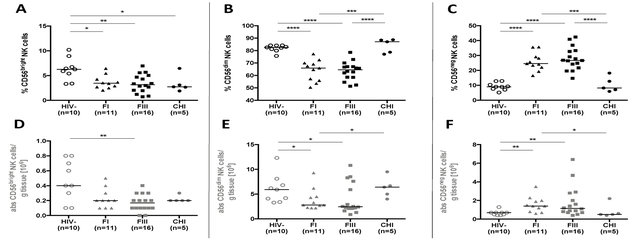

Figure 2: During acute HIV infection (Fiebig I and Fiebig III) a significant decrease in the frequency and number of cells is observed for $CD56^{bright}$ and $CD56^{dim}$ NK cells, while the frequency and number of $CD56^{neg}$ NK cells was increased. The frequency and number of cells per gram of tissue decreased significantly in the subset of $CD56^{bright}$ (A and D) and $CD56^{dim}$ (B and E) NK cell subsets in Fiebig I and Fiebig III compared to HIV-uninfected subjects. In contrast the frequency and number of $CD56^{neg}$ NK cells increased in Fiebig I and Fiebig III compared to HIV-uninfected subjects (C and F). *p≤0.05, **p≤0.01, ***p≤0.001

It is known from the NHP model that NKp44 expressing NK cells are mainly noncytolytic and potentially involved in the maintenance of the mucosal homeostasis by the expression of IL-17. In addition, we showed earlier that in acute HIV infection that $CD4^+$ T cells expressing IL-17 were depleted during Fiebig III, leading to subsequent local and systemic immune activation (*Schuetz et al., PLoS Pathogens, 2014*). Therefore we determined the expression of NKp44 on total NK cells and on the different NK cell subsets (**Figure 3**). We observed a significant increase in the frequency of $NKp44^+$ total NK cells in Fiebig I compared to HIV-uninfected subjects (p≤0.05), which decreased again in Fiebig III subjects (p≤0.05; **Figure 3A**). This increase observed in Fiebig I subjects was mainly due to an increase in the frequency of $NKp44^+$ $CD56^{dim}$ NK cells (p≤0.01) that decreased again in Fiebig III subjects (p≤0.05; **Figure 3C**). The frequency of $NKp44^+$ $CD56^{bright}$ cells decreased significantly in Fiebig III compared to HIV-uninfected subjects with no difference between the frequency of Fiebig I and HIV-uninfected subjects (p≤0.05; **Figure 3B**). There was no change in the frequency of $NKp44^+$ $CD56^{neg}$ NK cells observed during acute infection (**Figure 3D**). In addition the decrease in the frequency of $NKp44^+$ $CD56^{dim}$ NK cells correlated inversely with the plasma HIV RNA (r=-0.48, p=0.01; **Figure 4A**) and $CD8^+$ T cell activation in the periphery measured by the expression of Ki67 (r=-0.39, p=0.04; **Figure 4B**). This possibly indicates a role of $NKp44^+$ $CD56^{dim}$ NK cells in the maintenance of the mucosal homeostasis and that a depletion of this cell subset could contributes to a subsequent immune activation.

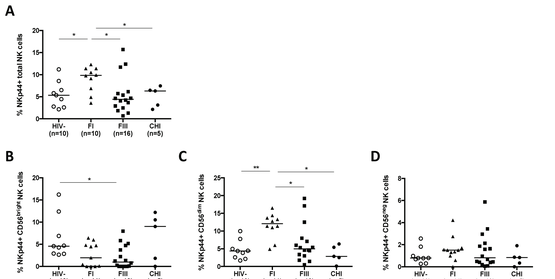

Figure 3: The frequency of $NKp44^+$ NK cells, possibly involved in the maintenance of the mucosal homeostasis, increased in Fiebig I but decreased again once subjects had progressed to Fiebig III. The frequency of total NK cells expressing NKp44 increased in Fiebig I compared to HIV-uninfected individuals but decreased again in Fiebig III (A). This change in frequency is mainly due to the expression of NKp44 on $CD56^{dim}$ NK cells (C). In contrast the frequency of $NKp44^+$ $CD56^{bright}$ NK cells decreased significantly in Fiebig III compared to HIV-uninfected subjects with no difference in the frequency between Fiebig I and HIV-uninfected subjects (B). There was no change in the frequency of $NKp44^+$ $CD56^{neg}$ NK cells observed (D). *p≤0.05, **p≤0.01

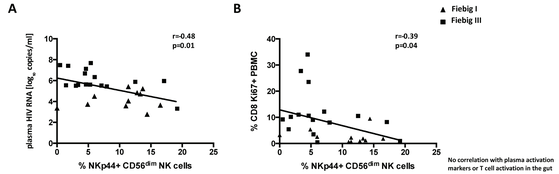

Figure 4: The frequency of $NKp44^+$ $CD56^{dim}$ NK cells inverselycorrelates with the plasma HIV RNA (A) and the immune activation of peripheral $CD8^+$ T cells (B).

SUMMARY

- Frequency of total NK cells increases with progression of Fiebig stage potentially driven by the virus
- The increase in total NK cells was mainly attributed to the increase in the subset of $CD56^{neg}$ NK cells
- At the same time a decrease of immunoregulatory $CD56^{bright}$ and cytolytic $CD56^{dim}$ NK cells was observed in acute HIV-infection compared to HIV-uninfected individuals
- Mucosal $NKp44^+$ NK cells associated with IL-17 expression and mucosal homeostasis, increased significantly in Fiebig I, but decreased by Fiebig III and chronic HIV infection
- The decrease of $CD56^{dim}$ mucosal NKp44+ NK cells by Fiebig III correlates inversely with plasma VL and $CD8^+$ T cell activation in the periphery

CONCLUSION

$NKp44^+$ NK cells play a potential role in the maintenance of the mucosal homeostasis. The increase in the frequency of $NKp44^+$ NK cells in Fiebig I, observed to our knowledge for the first time in acute HIV infection, could potentially pose a window of opportunity for intervention. However, further studies evaluating the functional profile of those cells in humans are warranted with regard to HIV pathogenesis.

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