Impaired *T. pallidum* Opsonic Antibody Function in HIV-infected Patients with Neurosyphilis

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Background

Compared to HIV-uninfected patients, HIV-infected patients may be at increased risk of neurosyphilis. *Treponema pallidum*, the bacterium that causes syphilis, is cleared from sites of infection by activated macrophages that ingest and kill opsonized organisms. We hypothesized that HIV-infected individuals might have a defect in opsonic antibody production or function that could impair bacterial clearance and predispose them to neurosyphilis.

Methods

Patients were enrolled in a study of cerebrospinal fluid (CSF) abnormalities in syphilis. Eligibility criteria included clinical or serological evidence of syphilis, and assessment by the referring provider that the patient was at risk for neurosyphilis. Reasons for referral to the study included 1) neurological findings, particularly vision or hearing loss; 2) serum RPR titer $\geq$ 1:32, and 3) in HIV-infected individuals, peripheral blood CD4+ T cell count $\leq$ 350/ul. Patients underwent a structured history and neurological examination, lumbar puncture (LP), and venipuncture. A convenience sample of HIV-uninfected and HIV-infected patients with normal and abnormal CSF measures were chosen for study.

Monocyte derived macrophages (MDMs) were derived from peripheral blood mononuclear cells from normal donors by culture with 1 ng/ml macrophage colony stimulating factor. MDMs were incubated with Nichols strain *T. pallidum* at a bacterium to cell ratio of 25:1 with 17% patient sera in quadruplicate for 4 hrs. at 37C in 5% CO_2, fixed and stained by immunofluorescence for *T. pallidum*. Opsonic capacity of sera was determined as % MDMs ingesting opsonized *T. pallidum* and was normalized to the result of a pool of sera from 55 patients with syphilis (RPR titer 1:64). Normal human AB sera served as the negative control. Mann-Whitney U test was used to compare continuous variables between groups and linear regression was used for multivariable analysis; Spearman's rank order correlation was used to compare continuous variables.

Financial Support

This work was supported by NIH/NINDS NS34235 and NS082120.

Results

Table 1. Participant Characteristics

	HIV-uninfected n=37	HIV-infected n=83
Male	36 (97%)	82 (99%)
Age	37 (26-45)	41 (33-46)*
Early stage	23 (66%)	64 (78%)
Treated for syphilis before entry	25 (68%)	35 (42%)*
Days before entry	7 (4-12)	7 (3-13)
Previous syphilis	5 (14%)	26 (31%)
Serum RPR titer	64 (32-128)	128 (64-256)**
Opsonic capacity of sera	0.62 (0.40-1.20)	0.47 (0.29-0.73)*
Reactive CSF-VDRL	10 (27%)	22 (27%)

Results are expressed as percent or median (IQR)
*P<0.05, **P<0.01

- Opsonic capacity of sera did not differ by gender, age, syphilis stage or previous syphilis
- Opsonic capacity of sera was significantly higher in patients who had been treated for uncomplicated syphilis before study entry (P=0.002).
- While serum RPR titer did not differ between those who had or had not been previously treated (P=0.30), higher opsonic capacity of sera was seen with higher serum RPR titers, especially in untreated patients (**Figure 1**).

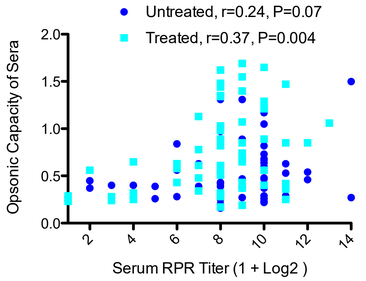


Figure 1. Scatterplot of opsonic capacity of sera vs. serum RPR titer.

Results

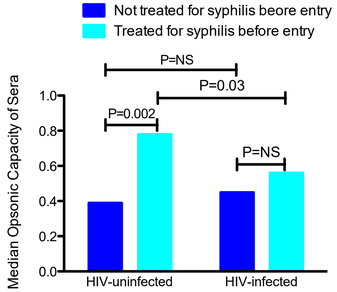


Figure 2. Opsonic capacity of sera from HIV-uninfected and HIV-infected patients as a function of syphilis treatment before study entry.

- Among the HIV-uninfected patients, but not HIV-infected patients, opsonic capacity of sera was higher in treated compared to untreated patients (**Figure 2**).
- Among treated patients, opsonic capacity of sera was significantly higher in HIV-uninfected compared to HIV-infected patients. This difference remained significant after taking into account serum RPR titer (P=0.01).

To test whether serum opsonic capacity contributed to development of neurosyphilis, we divided patients into four groups to account for the impact of HIV and treatment for uncomplicated syphilis before entry (and before LP) on opsonic capacity of sera (**Figure 3**).

- Opsonic capacity of sera in HIV-uninfected patients differed significantly between the groups, even after taking into account serum RPR titer (P=0.002).
- Opsonic capacity of sera in HIV-infected patients did not differ between the groups.
- Among treated patients with neurosyphilis, serum opsonic capacity was significantly lower in the HIV-infected patients (P=0.04). This finding was less significant after taking into account serum RPR titer (P=0.07), likely due to small numbers.

Results

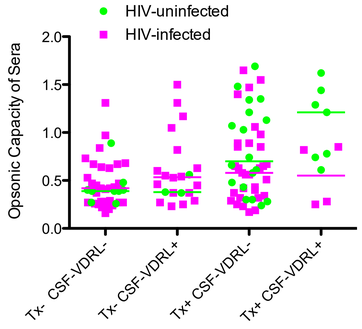


Figure 3. Opsonic capacity of sera from HIV-uninfected and HIV-infected patients as a function of treatment for uncomplicated syphilis before LP and CSF-VDRL reactivity. Tx-, no treatment for syphilis before study entry; Tx+, treated for syphilis before study entry.

Conclusions

We measured the opsonic capacity of sera from patients with syphilis. This is the ability of sera to facilitate ingestion of *Treponema pallidum*, the bacterium that causes syphilis. *T. pallidum* is cleared from sites of infection by opsonophagocytosis by activated macrophages.

Opsonic capacity of sera is higher when serum RPR titer is higher

There is an interaction between HIV, recent treatment for uncomplicated syphilis and opsonic capacity of sera

- **In HIV-uninfected patients, but not HIV-infected patients, opsonic capacity is higher after recent treatment for uncomplicated syphilis**
  - **Higher serum opsonic capacity after treatment may be due to greater antigenic stimulation**
- **Among recently treated patients, serum opsonic capacity is higher in HIV-uninfected than HIV-infected patients with neurosyphilis**

HIV-infected patients with syphilis may have a defect in production or function of opsonic antibody

- **This defect could contribute to increased risk of neurosyphilis**