Natural scrub typhus antibody suppresses HIV CXCR4(X4) viruses

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Abstract

Viral load generally rises in HIV-infected individuals with a concomitant infection, but falls markedly in some individuals with scrub typhus (ST), a common Asian rickettsial infection. ST infection appears to shift the viral population from CXCR4-using (X4) to CCR5-utilizing (R5) strains, and there is evidence of cross-reactivity between ST-specific antibodies and HIV-1. We examined the mechanism of ST suppression of HIV by measuring the effects of ST infection on X4 and R5 viruses *in vivo* and *in vitro*, and assessing the relative contributions of antibodies and chemokines to the inhibitory effect. *In vivo*, a single scrub typhus plasma infusion markedly reduced the subpopulation of HIV-1 viruses using the X4 co-receptor in all 8 recipients, and eliminated X4 viruses 6 patients. *In vitro*, the 14 ST sera tested all inhibited the replication of an X4 but not an R5 virus. This inhibitory effect was maintained if ST sera were depleted of chemokines but was lost upon removal of antibodies. Sera from ST-infected mice recognized a target that co-localized with X4 HIV gp120 in immunofluorescent experiments. These *in vivo* and *in vitro* data suggest that acute ST infection generates cross-reactive antibodies that produce potent suppression of CXCR4- but not CCR5-using HIV-1 viruses. ST suppression of HIV replication could reveal novel mechanisms that could be exploited for vaccination strategies, as well as aid in the development of fusion inhibitors and other new therapeutic regimens. This also appears to be the first instance where one pathogen is neutralized by antibody produced in response to infection by a completely unrelated organism.

Introduction

Coinfection generally raises HIV-1 viral load. One prospective study found that average plasma HIV-1 RNA loads increase approximately 8-fold following coinfection by an assortment of opportunistic pathogens,[1] and rises in viral load have been observed with a variety of concomitant bacterial, parasitic, and viral diseases.[2,3] Surprisingly, a few coinfections have the opposite effect. Transient declines in HIV-1 RNA load have been reported for acute dengue and measles infections, both of which induce HIV-suppressive chemokines that bind to the CCR5 HIV coreceptor.[4-6] The first infectious agent found to exhibit an HIV-1 suppressive effect was *Orientia tsutsugamushi*, the causative agent of scrub typhus.[7] A mite-borne rickettsial disease generally unfamiliar to Western scientists because its distribution is limited to Asia, scrub typhus (ST) affects an estimated one million people annually and one billion people are thought to be at risk of infection.[8] Earlier studies demonstrated that acute *O. tsutsugamushi* infection was associated with a substantial decrease in HIV-1 RNA levels in some ST-infected patients in northern Thailand and viral load sometimes fell below the limits of detection.[7] ST also appeared to shift the viral population from CXCR4-using (X4) to CCR5-utilizing (R5).[7] To explore the mechanism by which ST coinfection suppresses HIV, we tested *in vivo* and *in vitro* the hypothesis that the reduction in HIV-viral load associated with ST infection was attributable to effects on X4 viruses.

There is evidence of cross-reactivity between ST-specific antibodies and HIV-1. Immune sera from mice experimentally inoculated with *O. tsutsugamushi* have been shown to selectively stain with HIV-1 infected lymphocytes in an *in vitro* immunofluorescence assay.[7] Several studies have proposed a protective role for chemokines in HIV-1 infection, demonstrating an inverse relationship between chemokine production and plasma viral load.[9,10] We therefore assessed the relative contributions of antibodies and chemokines to ST-associated HIV inhibition.

Materials and Methods

HIV-1 coreceptor usage was determined in longitudinal plasma samples from antiretroviral *naïve* HIV-1 infected individuals being treated by passive transfer of ST plasma.[11] Individual units of plasma from donors of one unit of whole blood with mild, acute scrub typhus were safety-tested for HIV, HBV, and HCV viral markers, subjected to virucidal heat treatment, and administered to HIV-1-infected

recipients.[11] Plasma processing exceeded the safety requirements of both the Thai Red Cross and US FDA at the time of the study. Plasma recipients were all late-stage AIDS patients for whom antiretroviral drugs were not an option under Thai Ministry of Public Health HIV Treatment guidelines at the time. Informed consent was obtained under a protocol approved by both the Thai Ministry of Public Health and the Walter Reed Army Institute of Research. Samples collected from three individuals who received placebo infusions of saline were included as controls.

We used a method developed previously to compute the change in proportion of HIV virus using each coreceptor and to make numerical comparisons of coreceptor use over time and in different individuals.[12,13] Briefly, HIV-1 virions were isolated from plasma samples and

subjected to RT-PCR amplification, and 920bp amplicons spanning the V3 region of the *env* gene were sequenced. Envelope sequences were used to predict coreceptor usage on the basis of the overall charge of the V3 loop and the presence of basic or acidic residues at positions 275 and 287 of the *env* gene.[12,13] In this model, λ is a variable that represents the fraction of virus in a specimen using the R5 coreceptor. If $\lambda=1$, almost all of the viruses in a population use R5; if $\lambda=0$, almost all use X4. If $\lambda=0.50$, half of the HIV-1 viruses in a blood specimen use the R5 and half use the X4 co-receptor. We calculated the proportion of X4-specific virus for each plasma specimen according to the formula: X4 viral load $=1-\lambda$/total viral load. We analyzed total, R5-specific, and X4-specific HIV-1 RNA levels in these patients immediately prior to plasma infusion and 3, 14 and 28 days following plasma transfer. Virus production from triplicate cultures of infected peripheral blood mononuclear cells (PBMCs) was assayed at day 14 by measuring p24 antigen production. Virus was cultured with admission sera from 14 HIV-uninfected ST patients and with fetal bovine serum controls. Experiments were conducted on an exclusively X4-coreceptor-using virus (LAV) and on a solely R5-coreceptor-using virus (SF162).

We assessed possible mechanisms by which ST could inhibit HIV by depleting selected chemokines or antibodies from the serum of an HIV-uninfected scrub typhus patient. The chemokine ligands of the HIV-1 coreceptors CCR5 and CXCR4 (MIP-1α, MIP-1β, RANTES, and SDF-1α) were removed by adsorption of the sera using monoclonal antibodies immobilized on a plastic microtiter plate. Following overnight incubation, the concentration of these four chemokines was below the level of detection, as measured by using commercial ELISA kits (Invitrogen, Carlsbad, California, USA). Serum antibodies were depleted by using a protein A column.

Immune sera from mice experimentally inoculated with *O. tsutsugamushi* have been shown previously to selectively stain with HIV-1 infected lymphocytes in an immunofluorescence assay.[7] We therefore attempted to identify the HIV-1 target of potentially cross-reactive ST-induced antibodies by performing co-localization experiments to see if ST-sera targeted with HIV-1 gp120 envelope protein in LAV-infected GHOST-CXCR4 cells. We used i) DAPI nuclear stain; ii) convalescent sera from ST-infected mice, conjugated with a FITC-labeled secondary antibody; iii) red fluorescence of Texas Red-labeled secondary antibodies marking gp120; iv) co-localization mask of blue (i), green (ii), and red (iii) fluorescence. GHOST-CXCR4 cells were infected with the X4 LAV virus and GHOST-CCR5 cells were infected with an X4-using virus (SF162).

Results

Previous work showed that after a single infusion of scrub typhus plasma, the total HIV-1 RNA copy number (R5+X4 viruses) fell modestly in 7 of 10 plasma recipients.[11] The mean age of the 5 male and 5 female plasma recipients was 29 years (SD±5 years). The median HIV-1 RNA copy number prior to plasma infusion was 114,438 (range 43,150-193,597 copies/µL). Median CD4^{+} cell count prior to plasma transfer was 63 cells/µL (range 15-130) and rose to 72 cells/µL (range, 10-147) one month after plasma infusion (P<0.05, Wilcoxon signed rank test). We made viral isolations at least twice from every patient and all viruses were subtype E.

Mixed-effects analysis showed that this reduction in HIV RNA level was accompanied by a significant shift in the viral population from X4 to R5 strains (P=0.0008).

Here, we computed the proportion of HIV virus using each coreceptor before and after scrub typhus plasma infusion and made numerical comparisons of coreceptor use over time and in different individuals. We found that 2 patients who showed no drop in total HIV viral load after receiving ST plasma had only R5 virus circulating preceeding plasma infusion. Viral load and coreceptor usage did not change in 3 patients who received placebo infusions of saline. However, ST infusion to the to the eight HIV-1-infected individuals in whom X4-viruses were present at the time ST plasma was infused markedly reduced the proportion of X4-using viruses (Figure 1). The X4-specific viral load decreased to zero in 6 of 8 patients (Figure 1; patients *A,B,C,D,F,H*). The inhibitory effect was prolonged, and X4-using viruses remained undetectable one month after a single infusion of ST plasma in 5 of 8 patients (Figure 1; patients *B, C, D, F, H*).

In vitro, we tested sera collected from 14 HIV-negative patients with acute ST for its ability to inhibit HIV replication. All sera markedly inhibited the replication of an X4 virus (Figure 2). However, R5 viral replication was virtually unaffected by these same 14 ST sera (Figure 2).

Compared with control serum, whole sera from a patient with acute scrub typhus
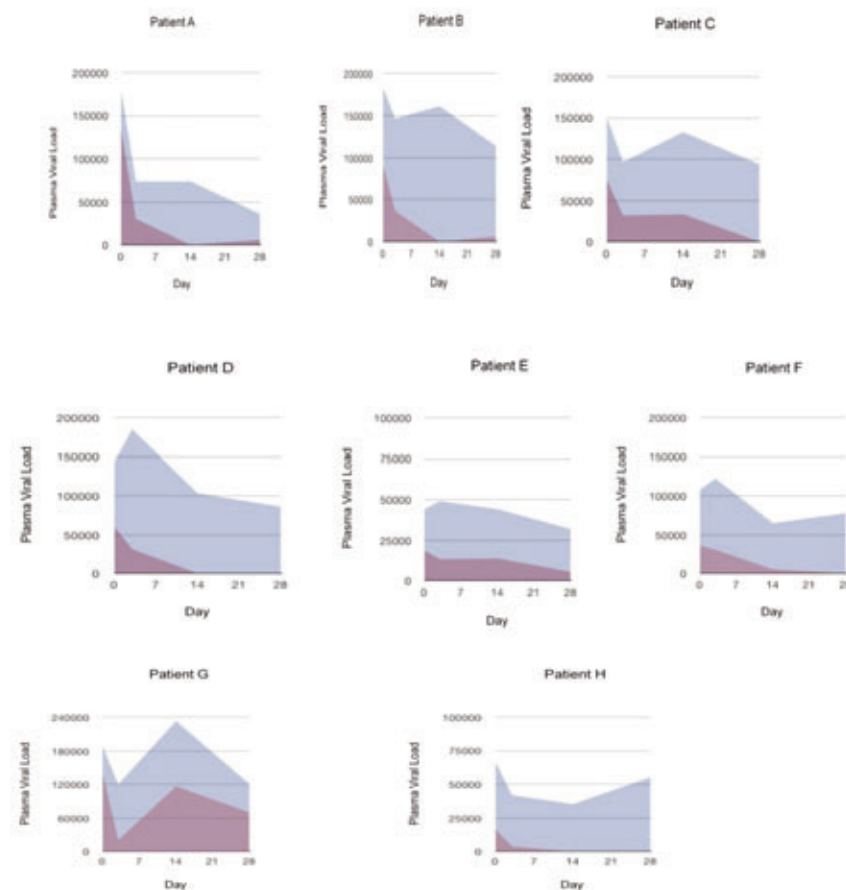

Figure 1. The effects of a single infusion of scrub typhus plasma administered on Day 0 to 8 HIV-infected recipients are shown as the area under the curve (AUC) for the total HIV-1 RNA copy number (shaded in blue) and for the X4-specific viral load (shaded in purple) immediately prior to and then 3, 14 and 28 days after infusion.

markedly inhibited the replication of an X4-using virus in culture (Figure 3, blue column). When this serum was depleted of the chemokine ligands of the HIV-1 coreceptors CCR5 and CXCR4 (MIP-1α, MIP-1β, RANTES, and SDF-1α), its inhibitory effect on HIV replication persisted (Figure 3, red column). However, the inhibitory effect was lost when antibodies were removed (Figure 3, green column).

Sera from ST-infected mice recognized a target that co-localized with gp120 in GHOST-CXCR4 cells infected with the X4 LAV virus (Figure 4). This staining pattern was not seen with pre-immune sera or with uninfected target cells. No co-localization was seen after GHOST-CCR5 cells were infected with the R5 virus SF162.

Discussion

The infusion of plasma from HIV-1 seronegative patients with acute ST completely eliminated circulating X4-using viruses in 6 of 8 HIV-infected recipients, and markedly reduced the proportion of X4 viruses in the remaining two individuals (Figure 1). *In vitro*, all 14 ST sera tested markedly inhibited the replication of an X4-using HIV virus but had no inhibitory effect on an R5 virus (Figure 2). This inhibitory effect was maintained if ST sera were depleted of chemokines, but was lost upon removal of antibodies (Figure 3). These data suggest that the inhibitory effect of ST coinfection on HIV is attributable to the induction of cross-reactive antibodies that neutralize X4 strains of the HIV-1 virus. Infectious agents sometimes share antigenic sites with each other or with host cell components, a phenomenon known as molecular mimicry. Several studies have demonstrated mimicry between HIV-1 and other infectious organisms. Rabies vaccination can induce the production of anti-HIV-1 gp120 antibodies,[14] and active measles virus infection often results in the production of antibodies that are cross-reactive with HIV-1 proteins.[15] Similar results have been reported using sera obtained from patients with malaria, leishmaniasis, and leprosy.[16-19] This is, however, the first time that suppression of HIV-1 by cross-reactive antibodies has been seen. Indeed, to date this appears to be the first case where natural antibodies made in response to infection with one organism – a rickettsial intracellular bacteria, both cross-react with and suppress a completely unrelated infectious agent – HIV X4-using viruses.

Sera from ST-infected mice recognize a target that co-localizes with X4 HIV gp120 in immunofluorescent co-localization experiments (Figure 4). Short amino acid matches between *O. tsutsugamushi* surface antigens

and HIV-1 have been found by searching the NCBI database,[11] although the relevance of these sequence similarities remains to be determined. For example, protein sequence comparisons have revealed a striking similarity between an immunodominant 47kDa ST antigen (positions 433 to 442) and HIV gp120 (codons 214 to 223):

ST 47kDa sAg: T L R E I V T N I K
HIVgp120:   T L R Q I V T N L K

This potential mimiotope is intriguing in that it is a relatively conserved portion of the HIV envelope protein that is just C-terminal of the V3 loop, and is both a principal neutralizing determinant and a key determinant of viral coreceptor specificity. Crystallographic data suggests that this region should be *visible* on the native envelope.[20] The scrub typhus 47kDa is also a surface antigen. The interaction between surface ST and HIV antigens could explain both the suppression and its X4 specificity.

The ST suppressive effect is potent. In two HIV-infected patients not receiving antiretroviral treatment, viral load fell below the limits of detection during naturally acquired scrub typhus infection.[7] Just one infusion of ST plasma eliminated circulating X4 viruses from 6 of

8 plasma recipients (Figure 1). ST suppressor factors appear to be produced commonly during scrub typhus infection. All ST plasma tested inhibited X4 viruses *in vivo* (Figure 1) and all ST sera tested inhibited X4 viruses *in vitro* (Figure 2).

Unfortunately, we did not have any opportunity to evaluate the effect of ST co-infection in primary HIV-1 infection. However, the R5 coreceptor is used by almost all primary HIV-1 isolates regardless of viral genetic subtype. Since ST neutralizes X4 but not R5 viruses, it is unlikely that ST co-infection would have a significant impact on either HIV-1 transmission or on the course of early infection, which are mediated almost entirely by R5-using viruses. However, by elucidating the mechanism of viral suppression by ST and characterizing potential cross reactive epitopes, our understanding of the natural correlates of protection and control of HIV-1 infection may be expanded. The search for antibodies that potently neutralize different HIV strains is a key element of rational vaccine design.[21,22]

Understanding how ST antibodies achieve such potent inhibition of X4-viruses could be of help in this search and identify additional targets for drug development. Preferential suppression of X4 viruses, associated with disease progression and pathogenicity, suggests the
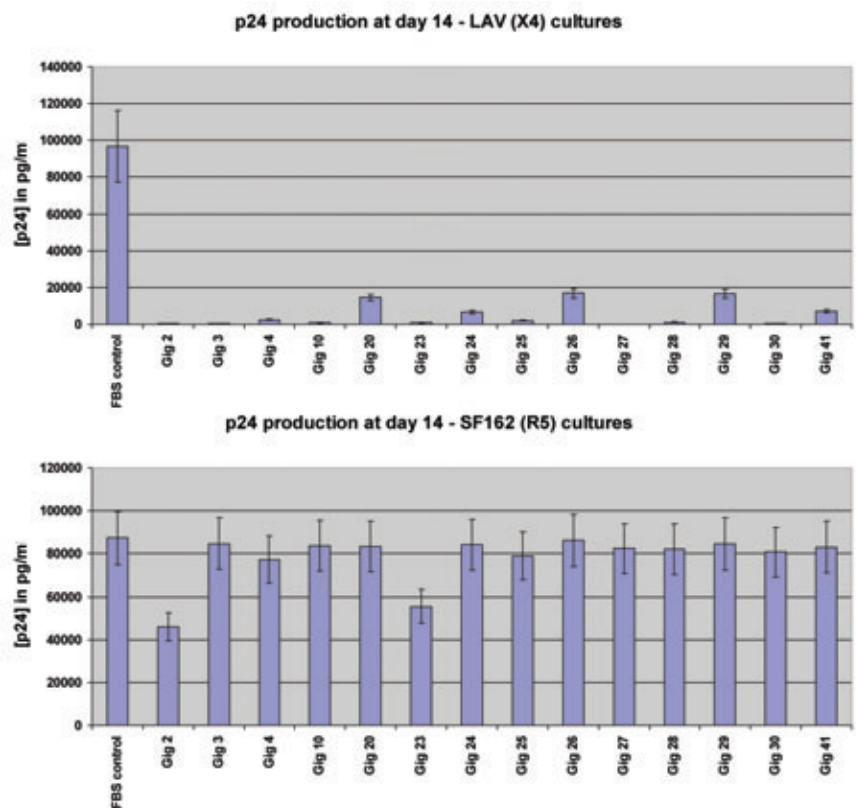

Figure 2. Effects of sera from 14 scrub typhus-infected patients on HIV replication within X4-using lymphocytes (above) and R5-using lymphocytes (below).
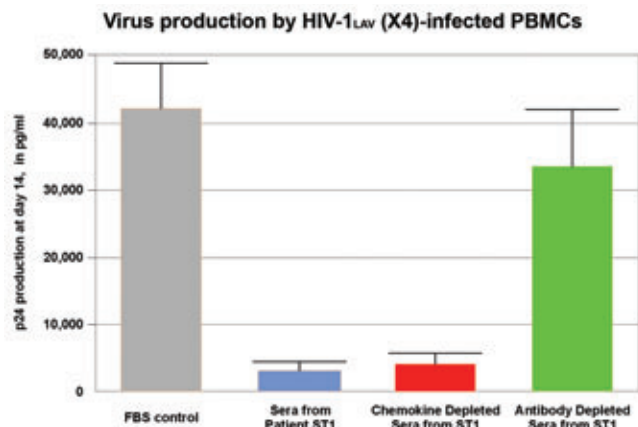
Figure 3. The effects of chemokine and antibody depletion on the inhibitory effects of scrub typhus serum on X4 virus replication. Selected chemokines or antibodies were depleted from the serum of an HIV-uninfected scrub typhus patient. Using p24 antigen as a marker, we measured HIV-1 viral replication in peripheral blood mononuclear cells.
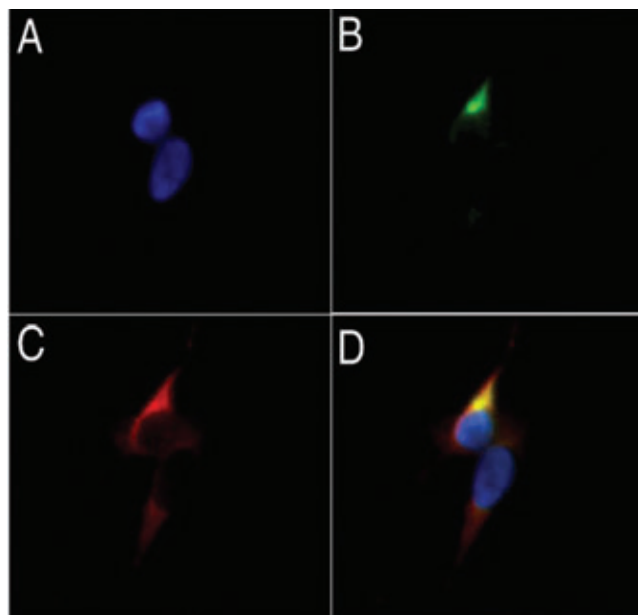

Figure 4. Co-localization of scrub typhus sera targets with HIV-1 gp120 envelope protein in LAV-infected GHOST-CXCR4 cells. a) DAPI nuclear stain; b) convalescent sera from ST-infected mice, conjugated with a FITC-labeled secondary antibody; c) red fluorescence of TexasRed-labeled secondary antibodies marking gp120; d) co-localization mask of blue (a), green (b), and red (c) fluorescence. Co-localization is revealed by yellow.

potential for a therapeutic vaccine aimed at slowing or halting HIV-1 disease progression. New weapons against HIV remain urgently needed. An effective HIV-1 vaccine is not on the horizon and there are approximately three times as many people who are estimated to need antiretroviral therapy than are estimated to be receiving it.[23]

Conclusions

In vivo and *in vitro* data suggest that acute ST infection generates cross-reactive antibodies that produce potent and long lasting suppression of CXCR4- HIV-1 viruses. Further study of the mechanism of suppression of HIV replication could reveal new mechanisms applicable to HIV-1 vaccine development and to therapies such as fusion inhibitors.

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