Alterations in the Course of Experimental Syphilis Associated with Concurrent Simian Immunodeficiency Virus Infection

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Case reports suggest that the course of syphilis is altered in patients infected with human immunodeficiency virus (HIV). To investigate this issue, a model of syphilis in rhesus macaques with and without simian immunodeficiency virus (SIV) was developed. After intradermal inoculation with Treponema pallidum, 2 SIV-infected monkeys had persistent ulcerative primary lesions and 1 developed secondary syphilis. Two SIV-uninfected controls developed transient nonulcerative primary lesions. Only the controls showed consistent VDRL antibody responses. In contrast, reciprocal antibody titers to T. pallidum detected by microhemagglutination were higher in SIV-infected animals (>20,480) than controls (>1280). All 4 animals developed a full range of T. pallidum antigen–specific antibodies shown by immunoblot and had similar peak lymphocyte proliferative responses to T. pallidum antigens. These results support the contention that retrovirus-induced immunodeficiency delays clearance of T. pallidum from sites of infection and may impair the humoral immune response to syphilis.

In the past 10 years, a number of case reports have suggested that the clinical course of syphilis and the response to antibiotic therapy may be altered in patients who are also infected with human immunodeficiency virus (HIV). Most of these reports describe development of syphilitic meningitis or cerebral vasculitis and ocular syphilis, previously uncommon manifestations of syphilis, in patients coinfected with HIV and syphilis [1, 2]. Several of these patients had prior treatment with benzathine penicillin G for early syphilis. Less commonly, unusual or more severe cutaneous manifestations of secondary syphilis and delayed healing of skin lesions after therapy have been described [3-5]. A few HIV-infected persons with biopsy-proven secondary syphilis and nonreactive serum nontreponemal serologic tests have been reported [3, 6, 7].

Because these observations are based on case reports, however, and because similar atypical cases occurred before the advent of HIV, the true frequency and relative importance of these unusual manifestations of syphilis and their relationship to HIV infection are unknown. Prospective studies in humans will help clarify these issues but will suffer from inability to define the precise time of Treponema pallidum infection, noncompliance, incidental antibiotic therapy, inability to study untreated controls, and other difficulties. An animal model of syphilis in conjunction with retrovirus-induced immunodeficiency might help elucidate the natural course of syphilis in HIV-infected patients. The immunodeficiency and illness caused by simian immunodeficiency virus (SIV) in macaques is similar to those produced by HIV in humans [8]. Therefore, we conducted a pilot study to investigate the course of experimental primary syphilis in rhesus macaques with and without SIV infection and here report the results of the first 6 months of observation.

Materials and Methods

Animals. Juvenile (2-3 years old), male, colony-born Macaca mulatta with nonreactive VDRL tests and microhemagglutination assays for T. pallidum (MHA-TP) were housed individually at 25°C and were fed antibiotic-free food and water. Adult male New Zealand White rabbits without evidence of Treponema paraluiscuniculi infection (nonreactive VDRL and fluorescent treponemal antibody absorption [FTA-ABS] tests) were housed individually at 19-20°C and were fed antibiotic-free food and water.

Source of organisms. T. pallidum subspecies pallidum, Nichols strain, was propagated in rabbits as previously described [9]. After sacrifice, infected testes were sliced longitudinally and extracted by gentle rotation in 50% normal rabbit serum in 0.9% saline. The extraction medium was centrifuged at 280 g for 10 min, and motile organisms in the supernatant were counted by darkfield microscopy and adjusted to 5.0 × 10^7 organisms/ml.

Experimental design and procedures. Four macaques were inoculated intradermally with 10^7 live T. pallidum (in 0.2 ml) at each of four sites on the back, and the back was kept clipped free of hair. Two of the 4 animals had been intravenously inoculated...
with $10^3$ TCID of wild-type SIVmne [10] 3 months before \textit{T. pallidum} infection. This timing was chosen to model syphilis infection in an immunocompromised individual without AIDS; experience at the University of Washington Regional Primate Center suggests that rhesus macaques develop opportunistic infection 6–18 months after inoculation with this strain.

Animals were sedated with ketamine (10 mg/kg) and examined for clinical manifestations of syphilis three times weekly for the first 12 weeks, twice weekly for weeks 13–16, and weekly for weeks 17–24. Touch preparations were made from skin lesion exudate, and biopsies of skin lesions were obtained on days 14 and 95. Blood samples were collected by venipuncture before (baseline) and every 2 weeks after syphilis infection for VDRL test, MHA-TP, immunoblot, and lymphocyte culture. Proportions of peripheral blood T lymphocyte subsets were determined at 0, 4, 6, 8, 12, 16, and 20 weeks.

Cerebrospinal fluid (CSF) was collected by lumbar puncture with a 22-gauge 3.8-cm spinal needle before (baseline) and every 2 weeks (except for week 6) after syphilis infection. CSF samples containing $\geq 2000$ red blood cells/$\mu$l were discarded because this amount of CSF blood contamination has produced false-positive CSF FTA-ABS tests (but not CSF VDRL tests) in humans with secondary syphilis [11]. CSF white blood cell counts were determined at each time point. CSF VDRL test, CSF MHA-TP, and CSF and serum determinations of total IgG and albumin were done 4, 8, 12, 16, 20, and 24 weeks after infection. MHA-TP index (CSF MHA-TP titer $\times$ serum IgG/CSF IgG) and albumin ratios were calculated to determine intrathecal antitreponemal antibody synthesis and blood-brain barrier integrity, respectively [12].

\textbf{Antibody responses.} The serum and CSF VDRL slide flocculation tests and serum MHA-TP were done by standard methods [13]. CSF MHA-TP was done as for serum except that CSF was not heated and was diluted 1:2.5 in absorbing diluent [14]. Serum VDRL and FTA-ABS tests for recipient rabbits were done as previously described [15]; known reactive and nonreactive rabbit sera were used as controls. VDRL reagents were obtained from Difco Laboratories (Detroit). FTA-ABS antigen and sorbent were obtained from SciMedx (Denville, NJ). Fluorescein-labeled goat anti-rabbit IgG (Zymed, South San Francisco) for the FTA-ABS test was used at a final dilution of 1:2000. Reagents for MHA-TP were obtained from Miles (Elkhart, IN). Primate sera were examined for IgG antibody reactivity to individual antigens of \textit{T. pallidum} by immunoblot, as previously described [16].

\textbf{Infectivity of CSF.} At weeks 2, 4, 8, 12, 16, 20, and 24, CSF was analyzed by rabbit inoculation for viable \textit{T. pallidum}. CSF samples (0.5 ml) were mixed with an equal volume of normal rabbit serum and inoculated intrathecally into separate rabbits within 1 h of collection. Serologic conversion of VDRL and FTA-ABS tests within 3 months after inoculation was considered proof of infectivity of the original sample.

\textbf{Immunofluorescence staining for \textit{T. pallidum}.} Lesion exudate was air-dried on glass slides, fixed in acetone, and stained for \textit{T. pallidum} with pathogen-specific, fluorescein-conjugated monoclonal antibody H9-1 as previously described [17].

\textbf{Lymphocyte proliferation assays.} Peripheral blood lymphocytes for culture were separated from whole heparinized blood on 95% Histopaque (Sigma, St. Louis) in RPMI 1640, washed three times in balanced salt solution, and resuspended at a final concentration of $1.25 \times 10^6$ lymphocytes/ml in complete medium containing RPMI 1640, penicillin, streptomycin, and 10% fetal bovine serum (FBS). Quadruplicate cultures containing 200 $\mu$l of cell suspension/well ($2.5 \times 10^5$ cells) were stimulated with 10 $\mu$l (10$^6$ organisms) of sonicated \textit{T. pallidum} antigen [9]. Quadruplicate cultures incubated with a mixture of monoclonal antibodies 9.3 (anti-CD28, 1.0 $\mu$g/ml) and FN18 (anti-CD3, 1.0 $\mu$g/ml) and 12-$\alpha$-tetradecanoylphorbol 13-acetate (0.2 ng/ml; provided by E. A. Clark, University of Washington Regional Primate Center), previously shown to produce optimal stimulation of macaque T lymphocytes [18], served as positive controls, and samples without antigen were included as unstimulated negative controls. The geometric mean 24-h incorporation of [$^3$H]-thymidine (0.5 $\mu$Ci in 10 $\mu$l/well) of replicate cultures for individual animals was determined after 3, 4, and 5 days of culture, and the corresponding unstimulated background values were subtracted. The peak mean response $\pm$ SD was calculated for each group.

\textbf{Lymphocyte subset analysis.} A panel of mouse anti-human monoclonal antibodies that react with macaque lymphocytes was used to stain peripheral blood lymphocytes for two-color analysis using a FACScan flow cytometer (Becton Dickinson, Mountain View, CA). The antibodies were conjugated with fluorescein isothiocyanate (FITC) or with R-phycocerythrin (PE) as previously described [18]. The following pairs of antibodies were used: IF5-PE (anti-CD20) and $\delta$TA-4-FITC (anti-IgD), Leu-3A-PE (anti-CD4) and 3AC5-FITC (anti-CD45RA), Leu-3A-PE (anti-CD4) and Hutch-1-FITC (anti-CD44), and G10.1-PE (anti-CD8) and 60.3-FITC (anti-CD18).

\textbf{Isolation of SIV.} Peripheral blood mononuclear cells were separated from heparinized whole blood using 95% lymphocyte separation medium (Organon Teknika-Cappel, Durham, NC) in PBS according to the manufacturer’s instructions. About 2.0 $\times$ 10$^6$ cells were cocultured with an equal number of C8166-45 cells (obtained from R. Gallo, AIDS Research and Reference Reagent Program, National Institutes of Health, Bethesda, MD) and incubated at 37°C in 4 ml of RPMI 1640–10% FBS containing 1% phytohemagglutinin and 50 units/ml interleukin-2 (Amgen, Thousand Oaks, CA); after 24–48 h, RPMI–10% FBS was added to a final volume of 10 ml and the cultures were maintained for 4 weeks. Culture supernatant was tested for SIV at 2 and 4 weeks using a commercial HIV-1 antigen-capture test kit (Abbott Laboratories, Abbott Park, IL) according to the manufacturer’s instructions.

\textbf{Histology.} Skin biopsy samples were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned, and stained with hematoxylin-eosin for routine histologic examination.

\textbf{Results}

\textbf{Clinical course.} Both SIV-infected macaques had generalized lymphadenopathy, and SIV was isolated from their peripheral blood mononuclear cells at the time of \textit{T. pallidum} inoculation. Immunodeficiency was documented in both animals by reduced peripheral blood absolute CD4$^+$ lymphocyte counts and CD4$^+$ percentages compared with controls (table 1).
with endothelial proliferation and extensive infiltrates con-
vine syphilis infection, peaking at 12 and 18 weeks in the 2 ani-
mals showed no significant deviation from normal values
throughout the 6-month observation period. CSF MHA-TP reactiv-
ity was not evident at any time in the SIV-uninfected animals
but was evident at low titer (1:10–1:20) in both SIV-infected
animals from 12 to 24 weeks after syphilis infection. The
CSF-to-serum albumin ratio and the MHA-TP index re-
mained within normal limits in all 4 animals.

Immunologic responses. All 4 animals developed sero-
logic evidence of syphilis. All became serum MHA-TP-react-
tive by 8 weeks after syphilis infection and titers plateaued at
12 weeks. The MHA-TP titers were consistently four- to
eightfold higher in the SIV-infected animals than in controls
(figure 3).

Serum VDRL reactivity was evident in both SIV-unin-
fected animals by 8 weeks after infection, peaked at titers of
1:8–1:16 at 10–12 weeks, and then declined (figure 4). In
contrast, the serum VDRL test was reactive in only 1 SIV-infec-
ted animal at 4 weeks (at a titer of 1:2) and was only
weakly reactive or nonreactive thereafter (figure 4). This lack
of reactivity was not explained by a prozone phenomenon.

Specific antibody directed against the 47-kDa antigen of
T. pallidum was demonstrable by immunoblot by 4 weeks
after syphilis inoculation in 3 of 4 animals. All 4 developed
the full profile of specific antitreponemal antibodies by 8
weeks with no detectable differences between the control
and SIV-infected animals (figure 5).

Lymphocyte proliferative responses to T. pallidum anti-
gens were evident in all 4 animals by 4–20 weeks after infec-
tion and then declined (figure 6) with no obvious quantita-
tive difference between the control and SIV-infected animals.

Discussion

We describe the course of experimental syphilis in 2 con-
trol and 2 SIV-infected rhesus macaques over the first 6
months of infection. SIV exhibits ~50% sequence homol-
ogy with HIV-1 and 75% homology with HIV-2, and all of these
viruses have similar genomic organization of both structural
and functional genes, have similar protein composition, and
infect cells that express the CD4 receptor [8]. The immuno-
deficiency and illness caused by SIV in macaques is similar to
that produced in humans by HIV and includes CD4+ cell
depletion, anemia, fever, opportunistic infections, and death
after several months or years [8].

Few data on the course of experimental syphilis in maca-
ques are available. In 1906 Metchnikoff and Roux [19] de-
scribed development of primary lesions in 4 of 10 rhesus
macaques 17–24 days after intradermal inoculation with an
unspecified number of T. pallidum. In 1957 Turner and Holl-
lander [20] observed primary lesions in all of 6 rhesus ma-
cakes by 17 days after intracutaneous inoculation with 5 ×
10^6 T. pallidum, Nichols strain; most lesions began to resolve
by 28 days. Five of the 6 macaques had detectable serum
antitreponemal antibody by the Eagle flocculation test. Nei-
ther study described development of secondary lesions. De-
spite the limitations of these studies, they demonstrate that

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<th>Control</th>
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<tr>
<td></td>
<td>1</td>
<td>2</td>
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<td>Leukocytes × 10^3/μl</td>
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<td>Hemoglobin (mg/dl)</td>
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<td>Platelets × 10^3/μl</td>
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<td>CD4+ lymphocytes/μl*</td>
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<td>SIV culture of peripheral blood mononuclear cells</td>
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<td>Cerebrospinal fluid leukocytes/μl</td>
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<td>8</td>
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NOTE. NA, not available; ND, not done.
* Normal ranges (for macaques), 660–2160 cells/μl and 22%–30%.
Figure 1. Biopsies. A, Skin chancre from simian immunodeficiency virus (SIV)-infected animal 2 collected on day 14: moderate inflammation by lymphocytes and a few plasma cells (arrow). B, Satellite lesion from SIV-infected animal 1 on day 95: extensive infiltrates containing lymphocytes, macrophages, and plasma cells (arrow). Bars = 20 μm.

Figure 2. Cerebrospinal fluid white blood cell concentration after intradermal inoculation of simian immunodeficiency virus (SIV)-infected and SIV-uninfected macaques with Treponema pallidum.

Figure 3. Serum microhemagglutination assay for Treponema pallidum (MHA-TP) titers after intradermal inoculation of simian immunodeficiency virus (SIV)-infected and SIV-uninfected macaques with Treponema pallidum.

Figure 4. Serum VDRL titers after intradermal inoculation of simian immunodeficiency virus (SIV)-infected and SIV-uninfected macaques with Treponema pallidum.

Figure 5. Immunoblots of serum from simian immunodeficiency virus (SIV)-infected and SIV-uninfected macaques against Treponema pallidum antigens at 4, 8, and 24 weeks after syphilis infection. Lanes 1 and 2, SIV-infected macaques 1 and 2; lanes 3 and 4, controls 1 and 2.
macaques are susceptible to syphilis. Thus the SIV-syphilis model affords a unique opportunity in which to study retrovirus-induced immunodeficiency and *T. pallidum* coinfection in a controlled setting.

The observations that SIV-infected macaques had more severe and prolonged cutaneous lesions and developed only weak and transient serum VDRL responses after *T. pallidum* infection are striking findings of our study. In immunocompetent humans and experimental animals, *T. pallidum* is cleared from early lesions by ingestion and killing by macrophages in the presence of opsonic antibodies [21, 22]. Clearance of treponemes from sites of infection has been shown to be delayed in experimental animals immunosuppressed with cortisone [15] and in HIV-infected rabbits despite lack of immunodeficiency [23]. Individuals with symptomatic and asymptomatic HIV infection have impaired macrophage, T, and B cell function [24]. These deficits could delay bacterial clearance from sites of infection by a number of mechanisms, including inability of antigen-specific T lymphocytes to produce macrophage-activating factors such as interferon-γ, failure to develop appropriate opsonic antibody, or inability of macrophages to ingest and kill *T. pallidum*.

Defects in T and B cell function in retrovirus-infected hosts might explain aberrant serologic responses. A number of studies in patients with the full spectrum of HIV-induced immunosuppression document impaired antibody responses to new and to previously encountered antigens such as influenza [25] and poliovirus vaccines [26]. As expected, more impaired responses are seen in patients with more advanced HIV-related disease.

In our study, SIV-infected animals did not develop consistent reactivity in the VDRL test. While this observation may be explained by SIV-induced immunodeficiency, it is difficult to reconcile it with development of higher-titer MHA-TP responses and normal anti-*T. pallidum* immunoblots in these animals. VDRL antigen and *T. pallidum* should be neoantigens, and synthesis of both VDRL antibody and specific treponemal antibodies is T cell-dependent [27]. While the higher-titer antitreponemal antibody could represent an anamnestic response to shared antigens found on previously encountered treponemes, perhaps as part of the oral or gastrointestinal flora, the animals in this study had no evidence of treponemal antibody by immunoblot or MHA-TP before syphilis infection.

In this pilot study, syphilitic involvement of the central nervous system (CNS) was suggested in the SIV-infected animals by increasing CSF pleocytosis and demonstrable specific treponemal antibody in CSF 12–24 weeks after syphilis infection. CSF mononuclear pleocytosis and intrathecal IgG synthesis are common and nonspecific findings in individuals with HIV infection [28] and presumably reflect viral infection of the CNS. Thus, the changes we observed in CSF of the SIV-infected animals may have been due to SIV infection alone or due to superimposed CNS invasion by *T. pallidum*. This diagnostic dilemma is analogous to that experienced in evaluating CSF abnormalities in HIV-infected patients with syphilis. *T. pallidum* can be isolated from CSF by rabbit inoculation in 30% of patients with primary or secondary syphilis, with or without HIV infection [29]. It is impossible to determine whether the failure to demonstrate *T. pallidum* in CSF in this study simply reflects the small sample size or differences in the frequency of CNS invasion between humans and macaques.

We are aware of only one other animal model for examining the course of syphilis in conjunction with retroviral infection. Tseng et al. [23] demonstrated delayed healing of primary chancres in 6 of 8 HIV-infected rabbits compared with controls, even though the rabbits had no measurable HIV-induced immunologic deficit. Our study confirms and expands on these findings, using a model in which retroviral infection caused immunodeficiency and clinical disease more analogous to HIV infection in humans. The observations provided by this model support the contention that the immunodeficiency caused by HIV results in delayed clearance of *T. pallidum* from sites of infection and may impair the non-treponemal humoral immune response.

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**References**


