Impaired responses to *Mycobacterium Leprae* antigens in rhesus monkeys experimentally inoculated with simian immunodeficiency virus and *M. leprae*

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Summary Seven of eight rhesus monkeys (RM) coinfected with simian immunodeficiency virus (SIV) and *Mycobacterium leprae* harboured acid-fast bacilli (AFB) at sites of dermal inoculation and/or at disseminated sites at times of humane sacrifice (up to 270 days post-*M. leprae* inoculation) due to SIV-induced debilitation or, in one long term survivor's case, to date over 3 years post-*M. leprae* inoculation. Detectable AFB were cleared in biopsies of inoculation sites of RM inoculated with *M. leprae* alone after 63 days postinoculation; these sites have, so far, remained AFB-negative, thereafter.

Compared to animals infected with *M. leprae* alone, RM coinfected with SIV plus *M. leprae* showed: 1, completely suppressed serum antibody responses to *M. leprae*-specific PGL-I antigen, but strong anti-SIV Gp120 antibody responses; 2, impaired sensitization of blood mononuclear cells (MNC) to *in vitro* recognition of *M. leprae*-specific antigens in blastogenic stimulation assays; 3, impaired *in vitro* responses of blood MNC to nonspecific (ConA) blastogenic stimuli; and 4, early post-*M. leprae* inoculation, there was a significant incremental diminution of percentages of blood CD4+CD29+ T-cells in addition to the existing SIV-induced diminished percentages of CD4+CD29+ T-cells.

The results indicate that humoral and cellular immune responses to *M. leprae* antigens are compromised in *M. leprae*-inoculated RM previously infected with SIV. These results provide an immunologic basis for the demonstration of enhanced *M. leprae* persistence or leprosy susceptibility in SIV-*M. leprae* coinfected RM.

Introduction

It is well established that human immunodeficiency virus (HIV)-positive patients are rendered more susceptible than virus-negative persons to secondary infections with *Mycobacterium tuberculosis*^{1–3} and other opportunistic infectious agents. SIV is very similar to HIV-2 and causes AIDS in RM with fatal symptoms and sequelae essentially identical to AIDS in humans, e.g. lymphadenopathy, diarrhoea, destruction of CD4+ T-cells, a decreased blood MNC CD4:CD8 ratio, and increased susceptibility to lymphomas and opportunistic infections.^{4–8}

We reported that RM infected with both SIV and *M. leprae* are more susceptible to clinical leprosy than RM infected with *M. leprae* alone.^{4,5} This observation led us to predict an increase in the incidence of leprosy worldwide, secondary to the AIDS pandemic.⁴ This prediction has not been consistently confirmed to date, however, for unknown reasons.^{9–14} Data from one field study was consistent with our observation of an increased risk of leprosy in HIV-positive individuals, finding a 4·6-odds ratio in increased multibacillary (MB) leprosy risk (and 8·3 for tuberculosis risk) among HIV-positive patients in Tanzania.⁹ Other reports failed to find a significant association in the aggregate populations studied, ^{10–14} but two of these studies found a significantly increased leprosy relapse rate among HIV-positive African leprosy patients after completion of antileprosy chemotherapy.^{10,14} Another study showed diminished blastogenic, and skin test responses to *M. leprae* antigens among HIV-positive tuberculoid leprosy risk.¹²

The controversial nature of the reported studies suggests that subtle, undetected factors could be disguising the true relationship between AIDS and leprosy in the reported epidemiological studies. Our observations are based on direct disease signs and immunologic determinations in a controlled experimental model system,^{4,5} whereas observations in humans are correlative, based on the presence or absence of antibody to HIV in identifiable leprosy patients.^{9–14} In the present study, we have utilized this model to further study the possibility of clinical and immunologic interactions between SIV and *M. leprae*. The results are consistent with increased *M. leprae* persistence and probable enhanced leprosy susceptibility due to impaired immune responses to *M. leprae* in SIV-coinfected RM compared to those infected with *M. leprae* alone.

Methods

ANIMALS

Rhesus monkeys (*Macaca mulatta*), 3–4 years old, were born and reared at the Tulane Regional Primate Research Center. Eight RM which had been inoculated with SIV 8–10 months previously and four normal control RM were inoculated with *M. leprae*. At the time of *M. leprae* inoculation, 3 of the 8 SIV-inoculated RM were showing acute AIDS symptoms (L156, J314, and I510, Table 1); the remaining 5 RM were negative for AIDS symptoms. All RM were clinically and immunologically followed longitudinally after *M. leprae* inoculation.

INOCULATIONS

An armadillo previously inoculated with *M. leprae* taken from a sooty mangabey money (SMM) developed progressive, disseminated lepromatous leprosy and was humanely killed.

Coinfected RM*	Histopath DX†	Days PI‡	SAIDS Status§
L156	PB	16	+
M368	MB	49	+
J717	PB	19	+
J314	MB¶	270	+
I510	MB¶	160	_**
I877	_	118	+
J703	PB	119	+
J798	MB [¶]	146	+

Table 1. Clinical results of SIV/M. le prae coinfected RM

* Coinfected RM received SIV 8–10 months prior to *M. leprae*.

†Histopath DX = histopathological diagnosis (PB, paucibacillary; MB, multibacillary) at necropsy (except for J798, which remains alive).

[‡]Days PI, number of days after *M*. *leprae*-inoculation that the animal was euthanized (except for J798—which first was observed to have AFB-positive nasal secretions and inoculation site biopsies on day 146 and continues nasal-positive on day 1137).

§ AIDS status at time of necropsy (except J798 which developed AIDS symptoms approximately 3 years post-*M. leprae* inoculation and is alive).

¶RM J314, I510 and J798 had nasal secretions strongly positive for acid-fast bacilli (AFB), indicating systemic dissemination of leprosy.

** I510 was euthanized on day 160 PI due to complications secondary to an unrelated condition; I510 had AFB at dermal sites of inoculation with MB histopathology and had AFB-positive nasal secretions, but was had no evidence of AIDS at necropsy.

M. leprae was harvested from its spleen. All armadillo procedures, including inoculation, husbandry and organ harvests were performed by Dr Richard B Truman at the Research Branch, National Hansen's Disease Center, Baton Rouge, LA, USA. Contaminating SIV from SMM does not cause detectable AIDS symptoms in and does not survive passage through armadillos (Gormus, BJ, Murphey-Corb, M., unpublished observations). The spleen was obtained aseptically, minced and homogenized in cold phosphate buffered saline using a Dounce homogenizer with a 40 Ml mortar and Teflon pestle (Wheaton Scientific, Millville, NJ, USA), passed through gauze and centrifuged at $200 \times G$ for 5 min at 4°C. The AFB in the supernatant were counted and morphologic indices (MI) determined by the method of Shepard & McCrae.¹⁵ RM were inoculated with *M. leprae* suspensions by combined intradermal (ID) and intravenous (IV) routes using 2 ID sites per ear, the tip of the nose, lateral forearms and lateral calves. IV inoculations were made via the saphenous vein. The inoculum contained $2\cdot24 \times 10^8$ AFB/Ml with an MI of 7%. A total of $1\cdot5$ Ml ($3\cdot4 \times 10^8$ AFB) was inoculated by the ID route and $2\cdot0$ Ml ($4\cdot5 \times 10^8$ AFB) by the IV route, for a total of $7\cdot8 \times 10^8$ ($5\cdot5 \times 10^7$ solidly staining) AFB/RM.

 SIV_{Delta} B670 at the standard (5 × 10⁻³) dilution was thawed and inoculated IV via the saphenous vein, as previously reported.^{4,16}

CLINICAL OBSERVATIONS

Animals were observed twice daily and examined in detail monthly or more frequently, depending on the status of the animal. Clinical aspects of AIDS and leprosy were recorded at each time of observation. The Ridley–Jopling system was used to classify leprosy histo-pathologically,¹⁷ with the exception that classification at the paucibacillary (PB) end of the spectrum differs slightly in RM from humans, as previously noted (Gormus BJ, *et al*, submitted for publication). For purposes of this report, however, leprosy will be described as multibacillary (MB) or PB. MB will include borderline to lepromatous forms on the Ridley–Jopling spectrum;¹⁷ PB includes tuberculoid to borderline tuberculoid forms. AIDS was diagnosed as previously described.^{5–8}

ELISA

The assays were performed as previously reported.^{4,16,18} Baseline sera were obtained prior to *M. leprae* or SIV inoculations and at intervals after inoculations and were stored frozen for later ELISA evaluations of *M. leprae*-specific anti-PGL-I IgG and IgM^{4,18} and for SIV anti-Gp120 antibodies.^{4,16} Natural ML PGL-I was used as antigen (Ag). PGL-I was provided by Dr Patrick J. Brennan, Colorado State University School of Veterinary Medicine, Fort Collins, CO, USA under NIH contract #1-AI-52582. Anti-PGL-I results are presented for individual monkeys as OD_{490} vs time. Anti-Gp120 was assessed by examining serial 2-fold dilutions from 1:50 to 1:25,600, together with known standard positive and negative sera, as previously reported.^{4,16} Individual RM results from the first four dilutions (1:50–1:400) of anti-Gp120 antibody ELISA's are presented as OD_{590} vs time. Gp120 antigen for ELISA was a recombinant product provided by Dr Ronald Montelero, Department of Microbiology, University of Pittsburgh School of Medicine, USA.

BLASTOGENESIS

Heparinized blood was used to prepare buffy coats which were centrifuged on Ficoll/ Hypaque, washed and suspended in RPMI-1640 containing 20% heat-inactivated human AB serum (HuABS), glutamine and penicillin/streptomycin. The mononuclear cell (MNC) fraction was used at 2×10^6 /MI for *in vitro* blastogenesis studies with or without 100 µg/MI of ML sonicate antigen (MLS) or 1 or 10 µg/MI of Concanavalin A (ConA). U-bottom 96well microtiter plates were used. Two $\times 10^5$ MNC per well were incubated at 37°C in 5% CO₂ in triplicate for 5 days with stimulant or media prior to pulsing for 18 hr with 1 µci of ³Hthymidine/well. Thereafter, cells were washed and harvested on a cell-harvester and quantified by scintillation counting. Results are presented as means +/– one standard deviation (SD) of replicate absolute cpm.

PERIPHERAL BLOOD LYMPHOCYTE (PBL) SUBSETS

Whole EDTA blood was obtained longitudinally, stained with mouse anti-human monoclonal antibodies and examined by flow cytometry, as previously reported.^{19,20} Monoclonal antibodies with the following specificities were used: CD4, CD8, CD2, CD20 and CD29. CD2 and CD20 subsets were chosen to quantify the total T- and B-cells, respectively; CD4 and CD8 were examined to determine the T-helper and T-suppressor percentages, respectively, since these cells are known to be involved in responses to *M. leprae* antigens;

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and the CD4+CD29+ subset was monitored because this group contains the T-helper inducer and T-memory cells and is known to be progressively diminished in RM with advancing AIDS.¹⁹ Results are presented as the group mean % of total PBL at different time points + or -1 standard error of the mean (SEM) vs time.

SIV ANTIGENEMIA ASSAY

SIV antigenemia was detected using a commercially available enzyme-linked immunoassay kit specific for the p26 antigen of SIV (Coulter Immunology, Hialeah, FL), as previously reported.²⁰ The cut-off point for antigenemia was an OD value <0.03. Data from individual RM are plotted as Ng/Ml of blood calculated from a standard curve.

STATISTICAL ANALYSES

A statistical package for Macintosh computer was utilized for data analysis. Mancova was used where possible to compare longitudinally-determined lymphocyte subset percentage changes with time after *M. leprae* inoculation in RM groups infected with *M. leprae* alone or together with SIV. Mancova was made difficult for longitudinal comparisons over an extended period, however, due to the deaths of some RM at various time points, resulting in the complete elimination by the statistic of all prior time points for that animal (i.e. total loss of one value of N at all time points) for each death that occurred prior to the end of the study. Therefore, the unpaired Student's *t*-test was additionally used for PBL subset data analysis to statistically compared group averages. The *t*-test was also used for analysis of blastogenic data.

Results

CLINICAL

Seven of the 8 SIV/ML coinfected group were euthanized due to AIDS or, in the case of animal I510 due to unrelated causes, within 270 days postinoculation (PI) with ML (Table 1). Six of the 7 euthanized AIDS-positive RM (L156, M368, J717, J314, I510 and J703) had AFB-positive lesions at necropsy, consistent with leprosy or *M. leprae* persistence at dermal inoculation sites. The 7th euthanized, AIDS-positive RM (I877) had MNC infiltration and nerve involvement at inoculation sites but no readily identifiable AFB were seen. The 8th RM in the coinfected group (J798) developed persistent AFB-positive nasal secretions by 146 days post-M. leprae inoculation, indicative of leprosy dissemination. J798 remains alive with strongly AFB-positive nasal secretions and chronic AIDS more than 3 years PI (Table 1). At necropsy, two of the euthanized coinfected RM (J314 and I510) had AFB-positive inoculation sites as well as AFB-positive nasal secretions, indicative of disseminated leprosy (Table 1). Thus, 7 of the 8 coinfected RM had signs of possible leprosy or AFB persistence at inoculation sites, 3 of the 8 having disseminated leprosy, within 270 days PI. Among the 4 control RM which received only M. leprae, 2 (J446 and M361) had identifiable AFB (PB histopathology) at dermal inoculation sites at 27 and 63 days PI; the other 2 RM (J675 and J265) had PB histopathology at day 27 PI. The lesions regressed after day 27 in J675 and J265 and after 63 days in J446 and M361; there has been no further evidence of M. leprae persistence in any of the 4 control RM to date 1137 days PI.



Figure 1. Longitudinal serum IgG and IgM anti-PGL-1 responses in 8 SIV-*M. leprae* coinfected RM ((a) & (b)) and 4 *M. leprae*-infected control RM (c). The mean +/- one standard error of the mean (SEM) of seven of the coinfected RM are shown in (a); the data for coinfected, long-term survivor RM, J798, is plotted separately (b) from the remaining 7 coinfected RM to emphasize its similarity to the 4 *M. leprae*-only infected controls (c). The X-axis is in reference to the time of *M. leprae* inoculation. The earliest time point is post-SIV inoculation. Time 0 represents the day of *M. leprae* inoculation.

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ANTIBODY RESPONSES TO M. LEPRAE-SPECIFIC PGL-I ANTIGEN

With the exception of J798, over a period of observation spanning approximately 1 year post-*M. leprae* inoculation, none of the 8 SIV/*M. leprae* coinfected group produced any significant serum IgG or IgM antibody response to the ML-specific PGL-I cell-wall antigen (Figures 1(a) and (b)). J798, a long term survivor, and all 4 *M*· *leprae*-only inoculated (control) RM produced significant amounts of anti-PGL-I antibody, the IgG isotype predominating along with lesser, usually significant, quantities of the IgM isotype (Figure 1(b) and (c)). IgG antibody peaked approximately between 100 and 150 days post-*M. leprae* inoculation.

SIV ANTIGENEMIA AND ANTIBODY RESPONSES TO SIV GP120 ENVELOPE ANTIGEN

Over a period including pre-SIV inoculation (-232 to -300 days relative to M. *leprae* inoculation), 8–10 months post-SIV/pre-*M. leprae* inoculation and up to death or 235 days post-*M. leprae* inoculation, anti-Gp120 responses were significant in all SIV-inoculated RM after the appearance of SIV antigenemia (Figure 2(a)). Only the 1:50 dilution-data are shown. These antibody responses were maintained until death or, in J314 and J798, up to 235 days post-*M. leprae* inoculation, the last time point studied. The first detectable antigenemia peak appeared within approximately 2 weeks post-SIV inoculation, but disappeared thereafter, in all 8 SIV infected RM (Figure 2(a)). After *M. leprae* inoculation, SIV antigenemia was again detectable in RM L156, I877 (minimal, but above the cut-off point) and J703 (Figure 2(a), see asterisks). There was, of course, no SIV antigenemia or anti-Gp120 response in control sera from *M. leprae*-only inoculated animals (Figure 2(b)).

BLASTOGENESIS

There was a statistically significant decrease in ConA responses (p = .0015, t-test) in the SIV/*M*· *leprae* coinfected group, compared to a significant increase (p = 0.01) in ConA responses in the *M*. *leprae*-only inoculated group pre- vs 15 weeks post-*M*. *leprae* inoculation (Figure 3). There were no statistically significant responses to MLS in the ISV/*M*. *leprae* coinfected group before or 15 weeks after *M*. *leprae* inoculation except for the long-term survivor, J798, which gave a significant response to MLS 15 weeks post-*M*. *leprae* inoculation (Figure 3). MLS responses increased significantly (p = 0.02) in the *M*. *leprae*-only infected control group 15 weeks PI compared to preinoculation responses (Figure 3).

LYMPHOCYTE SUBSETS

Within the first 300 days post-*M. leprae* inoculation, the following statistically significant changes were observed in the lymphocyte subsets examined: 1, an increase in the % CD2+ subset at 13 and 26 days (p = 0.009 & p = 0.04, respectively, *t*-test) post-*M. leprae* inoculation in the *M. leprae*-only inoculated (control) group (Figure 4(a)); 2, a decrease in % CD4+ T-cells in the coinfected group compared to an increase in CD4+ cells in the *M. leprae*-only inoculated group during the first 109 days post-*M. leprae* inoculation time period (p = 0.007, Mancova) (Figure 4(b)); and 3, there was a significant incremental decrease in the already depleted CD29+ subset of CD4+ T cells during the 62 days post-*M. leprae* inoculation period in the SIV/*M. leprae* coinfected group, compared to the *M. leprae*-only inoculated group over the same time period (p = 0.0496, Mancova) (Figure 4)



Figure 2. Longitudinal serum antibody responses (1:50 dilution) to SIV envelope antigen Gp120 and SIV antigenemia in 8 SIV-*M. leprae* coinfected RM (a) and 4 *M. leprae*-infected control RM (b). The earliest time points (-230 to -316 days) are pre-SIV inoculation in coinfected RM. Time 0 represents *M. leprae* inoculation. Asterisks show SIV antigenemia results at time points where no anti-Gp120 ELISA's were done.

S

L156

M368











Figure 2a continued

1877

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Figure 3. In vitro blastogenic responses to ConA (upper) and to MLS antigens (lower) in 8 SIV-M. leprae coinfected RM (left panels) and 4 M. leprae-infected control RM (right panels). The mean cpm +/- 1 standard deviation of the mean are shown for triplicate wells containing antigen or mitogen after subtraction of media-only controls.

2000

0

1510

Pre-ML

2000

0

1510

1877 J703 J798 L156 M368 J717 J314

Coinfected

J675 J446

J265 M361

ML only

15 Weeks Post-ML

dead

J314 J675 J446

J265 M361

ML only

1877 J703 J798 L156 M368 J717

Coinfected



Figure 4. Longitudinal PBL subset analysis (mean +/-1 SEM) by flow cytometry of CD2+ (a), CD4+ (b), CD4+ CD29+ (c) and CD8+ (d) T-cell subsets in 8 SIV-*M. leprae* coinfected RM and in 4 *M. leprae*-infected control RM. The *X*-axis is in reference to the time of *M. leprae* inoculation; the *Y*-axis represents the % of total PBL; the earliest time point (approximately -300 days) is pre-SIV; and time 0 represents the day of *M. leprae* inoculation.

4(c)). There was no change in the % CD8+ subset in the SIV/*M. leprae* coinfected vs an increase in this subset in the *M. leprae*-only inoculated group during the 62 days post-*M. leprae* inoculation period (Figure 4(d)); this difference was not significant. There was a transient increase in CD20+ B-cells in the SIV/*M. leprae* coinfected group and a decrease of longer duration in CD20+ B-cells in the *M. leprae*-only inoculated group early after *M. leprae* inoculation, but this difference was also not statistically significant.

Discussion

The clinical results are consistent with our previous suggestions derived from experimental studies in RM that SIV/ML coinfection enhances the susceptibility of RM to leprosy.⁴ Seven of 8 SIV/*M. leprae* coinfected RM developed signs of leprosy or *M. leprae* persistence that were sustained until necropsy or, in one surviving case, to date 37 months post-*M. leprae* inoculation. Control RM, inoculated with *M. leprae* only cleared AFB from dermal *M. leprae*

inoculation sites by 27 or 63 days PI and have remained AFB-negative to date, 1137 days after *M. leprae* inoculation.

We have previously observed that the appearance of M. leprae in nasal secretions is an early indication of systemic dissemination of clinical leprosy in experimentally inoculated monkeys.¹⁸ Three of the 8 coinfected animals studied herein developed strongly AFB-positive nasal secretions. Thus, the clinical data show an increased susceptibility towards persistence of M. leprae at dermal sites and an increased tendency to disseminate systemically in SIV coinfected compared to M. leprae-only infected animals.

Only one (I510) of the 8 coinfected RM failed to develop clinical AIDS, but it was prematurely sacrificed due to an unrelated medical condition 160 days post-*M.leprae* inoculation. This animal had MB leprosy at an inoculation site and AFB-positive nasal secretions at necropsy. Six of the 7 AIDS-positive RM were necropsied due to terminal AIDS within 270 days post-*M.leprae* inoculation.

The 7th AIDS-positive animal, the long-term survivor, remains alive with disseminated MB leprosy. The long term survivor has strongly AFB-positive nasal secretions first observed by 160 days PI, but has continued to show AFB-negative dermal biopsies. This RM displayed essentially normal immune responsiveness. The exact reason for it's uniqueness among the 8 coinfected RM is not known with certainty, but approximately 25% or RM experimentally infected with SIV_{B670} fall into this AIDS-slow progression category (M. Murphey-Corb, unpublished observations). To our knowledge, this RM had never been previously exposed to M. leprae. It is important to note that this latter RM, #J798, and 2 other animals with AFBpositive nasal secretions (J314 and I510) presumably shed large numbers of *M. leprae* into the environment as a result of the copious AFB-laden nasal exudates, although these 3 RM showed only minimal visible leprosy signs. The only evidence of leprosy in these 3 RM was the identification of AFB in nasal secretions and/or evidence seen in biopsies routinely taken from M. leprae inoculated monkeys. J798 has had an AFB-positive nasal status for approximately 3 years, but began to show symptoms of chronic AIDS 1137 days post-M. leprae inoculation and approximately 1350 days post-SIV infection. It is doubtful that humans with clinical characteristics similar to J314, I510 or J798 would have been recognized as leprosy patients. An ambulatory human patient similar to J798 might have exposed hundreds of contacts with *M. leprae* during such a time period while having few, if any, gross clinical symptoms of AIDS or leprosy. The possible implications of this observation for the future of leprosy among human populations remain to be seen.

The immunologic data provide a basis for the clinical observations. The impaired nonspecific (ConA) and *M. leprae*-specific blastogenic responses in *M. leprae*-SIV coinfected RM are in agreement with one study in humans showing abrogated *in vitro* blastogenic responses and skin test responses to *M. leprae* antigens in HIV-positive tuberculoid leprosy patients.¹² We previously reported the progressive loss of *M. leprae* skin test responses to *M. leprae* antigens and *skin test responses to M. leprae* antigens appear to become compromised in blood and skin in leprosy-AIDS coinfected cases even though leprosy lesion histopathology may resemble that present in HIV-negative leprosy cases.¹²

The totally suppressed antibody responses to *M. leprae*-specific PGL-I cell wall antigen in 7 of 8 SIV/ML coinfected RM compared to significant responses in *M. leprae*-only infected RM indicates that SIV abrogates primary antibody responses to this *M. leprae* antigen in all of the coinfected RM except the long term survivor (J798). The impaired antibody responses appear to effect the primary, and not the secondary or memory compartment, as suggested by

the long-term presence of undiminished levels of anti-Gp120 antibodies beyond the known half-life of immunoglobulins. Unfortunately, in the design of this study we did not anticipate the need for testing for responsiveness to unrelated antigens. This will be included in a future protocol.

Since the CD4+CD29+ subset contains helper-inducer and memory cells, it is conceivable that the *M. leprae*-specific immunologic impairments may be associated with a pre-existing SIV-induced diminution of CD4+ and CD4+CD29+ T-cell subsets that is observed in most SIV-positive RM.^{4,19,20} The longitudinal PBL subset data indicate that, in SIV-positive RM, an early additional small, but statistically significant loss occurs in the CD4+CD29+ T-cell subset after *M. leprae* inoculation. The CD4+CD29+ subset deficit is known to correlate with rapid AIDS progression in RM (19), but further investigations will be required to determine whether this specific defect contributes to the enhanced susceptibility to *M. leprae* persistence and dissemination in coinfected RM.

Previously, we reported that RM inadvertently coinoculated with SIV simultaneously with *M. leprae* (prior to our knowledge that captive sooty mangabey monkeys, the source of the M. leprae inoculum, carry SIV asymptomatically) developed AIDS and leprosy and produced anti-PGL-I IgG antibody responses.⁴ This is in contrast to the observations herein which revealed a complete inhibition of anti-PGL-I responses in 7 of 8 coinfected RM. A probable explanation for this discrepancy is twofold: 1, RM in the former study most likely received extremely low doses of SIV, inadvertently present as a contaminate in the *M. leprae* inoculum, compared to known lethal doses of cryopreserved SIV experimentally given in the present study; and 2, RM in the former study⁴ were simultaneously infected with ML and SIV, whereas in the present study, RM were inoculated with SIV 8-10 months prior to *M. leprae.* Surprisingly, the clinical results of an additional study now in progress suggest that lethality is greater when SIV is given to RM simultaneously with M. leprae compared to SIV being given prior to *M*. leprae (Gormus, BJ et al, unpublished observations). Thus, it appears that the effect of coinfection with the two agents depends, among other things, on the doses given and the relative timing of the two infections. Experiments are planned to examine in greater detail the effects of relative timing of the 2 infections, including *M. leprae* infection soon after SIV inoculation. Studies are now in progress to examine the effects of SIV infection on leprosy reactivation in leprosy-quiescent *M. leprae* infected animals.

The present data do not permit us to explain with certainty why studies from the field fail to consistently detect an interaction between HIV and leprosy among human populations. The results suggest that susceptibility to clinical leprosy is enhanced in leprosy cases coinfected with the AIDS virus. It is difficult to absolutely prove this point due to the generally observed rapid lethality of the combined infections observed in RM.

The rapid lethality observed in most SIV/*M. leprae* coinfected RM is a possible partial explanation for the failure of field studies to consistently detect an interaction between HIV and *M. leprae*. If a significant percentage of HIV-coinfected leprosy patients with a secondary HIV infection were to succumb early, prior to leprosy recognition (and/or, perhaps, prior to recognition of AIDS), it could conceal the true effect of coinfection.

If it is hypothesized that visible leprosy clinical symptoms are largely the result of the immune response to *M. leprae*, another possible explanation, suggested by the present immunologic results, is that clinical symptoms fail to appear in coinfected cases due to the AIDS virus-induced suppression of the immune response to *M. leprae* antigens. If so, coinfected patients would tend to be systematically excluded from study populations. Taken together, our clinical and immunologic data present a strong argument that some

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systematic factor is being overlooked in field studies that have failed to find an association between AIDS and increased leprosy susceptibility.¹⁰⁻¹⁴

The specific effect of SIV on the course of *M. leprae* infection is dependent on several variables and is deserving of continued study. The possible implications of our observations for future resurgence of increased numbers of leprosy cases worldwide would seem to demand a very through investigation of and resolution to questions we have raised. Our studies are direct, not depending on epidemiological correlations, using a nonhuman primate model that is phylogenetically very similar to humans to reach our conclusions. The results imply that it may not be readily possible to determine the true epidemiologic relationship between HIV and *M. leprae* infections by studying human populations. Our conclusions are disturbing because they continue to portend a possible drastic increase in future leprosy cases in AIDS/leprosy-endemic areas without detection before it is too late to take effective preventative measures.

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