Latent Infection as a Source of Disseminated Disease Caused by Organisms of the \textit{Mycobacterium avium} Complex in Simian Immunodeficiency Virus–Infected Rhesus Macaques

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Whether infection with \textit{Mycobacterium avium} complex (MAC) among patients with acquired immune deficiency syndrome results from recent exposure to virulent strains or reactivation of latent infection acquired years earlier is unknown. To address this question, tissue samples from 47 simian immunodeficiency virus (SIV)–infected and 63 SIV-uninfected rhesus macaques were cultured. MAC was cultured from 14 SIV-uninfected macaques (22.2%) and 32 SIV-infected macaques (68.1%); median bacterial burdens were 33.3 and 998.7 cfu/g, respectively. Genetically distinct strains of MAC were identified for 13 SIV-uninfected macaques (20.6%) and 15 SIV-infected macaques (31.9%). A genetically identical MAC strain (K128A) was identified for 25 SIV-infected macaques (53.2%) and 1 SIV-uninfected macaque (1.6%). Multivariate analysis identified infection with SIV/DeltaB670, diagnosis of an SIV-related tumor or opportunistic infection, and birth on site as risks for MAC infection. SIV-uninfected and SIV-infected macaques yielding unique strains of MAC were considered to have latent and reactivation infection, respectively, whereas animals infected with strain K128A were considered to have recent infection, demonstrating that both mechanisms occur among rhesus macaques.

Disseminated disease caused by organisms of the \textit{Mycobacterium avium} complex (MAC) represents a major cause of morbidity among persons with AIDS. Before the introduction of highly active antiretroviral therapy and specific prophylactic regimens, MAC infection was reported to occur in as many as 40% of patients by the time of death [1, 2]. The widespread use of highly active antiretroviral therapy has resulted in a coincident decrease in the incidence of MAC infection [3]. Despite highly active therapies, however, many patients continue to progress to end-stage disease and die of opportunistic infections, including infection with MAC [4].

Studies have sought to define the environmental reservoirs of invasive strains of MAC. Two large prospective epidemiological studies examined the risk of disseminated disease associated with exposure to potential environmental sources of MAC [5, 6]. Attempts to culture MAC from sources identified as representing risk...
Table 1. Epidemiologic risks and prevalence of Mycobacterium avium complex (MAC) infection in simian immunodeficiency virus (SIV)-infected and SIV-uninfected macaques.

<table>
<thead>
<tr>
<th>Clinical factor</th>
<th>SIV infected</th>
<th>SIV uninfected</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MAC positive</td>
<td>MAC negative</td>
</tr>
<tr>
<td></td>
<td>(n = 32)</td>
<td>(n = 15)</td>
</tr>
<tr>
<td></td>
<td>MAC positive</td>
<td>MAC negative</td>
</tr>
<tr>
<td></td>
<td>(n = 14)</td>
<td>(n = 49)</td>
</tr>
<tr>
<td>Age, median months (range)</td>
<td>42 (36–123)</td>
<td>78 (28–114)</td>
</tr>
<tr>
<td></td>
<td>98 (37–308)</td>
<td>102 (16–308)</td>
</tr>
<tr>
<td>Comorbidity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diarrhea</td>
<td>15 5</td>
<td>1 4</td>
</tr>
<tr>
<td>Wasting</td>
<td>13 4</td>
<td>0 0</td>
</tr>
<tr>
<td>Colitis</td>
<td>5 3</td>
<td>3 15</td>
</tr>
<tr>
<td>Cytomegalovirus infection</td>
<td>6 0</td>
<td>0 0</td>
</tr>
<tr>
<td>Campylobacter coli colitis</td>
<td>3 2</td>
<td>0 4</td>
</tr>
<tr>
<td>Cryptosporidiosis</td>
<td>5 0</td>
<td>0 0</td>
</tr>
<tr>
<td>SIV pneumonia</td>
<td>4 1</td>
<td>0 0</td>
</tr>
<tr>
<td>Tumor</td>
<td>4 0</td>
<td>0 0</td>
</tr>
<tr>
<td>SIV encephalitis</td>
<td>1 1</td>
<td>0 0</td>
</tr>
<tr>
<td>Amyloidosis</td>
<td>1 1</td>
<td>1 10</td>
</tr>
<tr>
<td>Any opportunistic infection or tumor</td>
<td>19 2</td>
<td>0 0</td>
</tr>
<tr>
<td>SIV strain&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>J943 or B670</td>
<td>27 3</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>5 12</td>
<td></td>
</tr>
<tr>
<td>CD4 cell count, median cells/mm&lt;sup&gt;3&lt;/sup&gt; (range)</td>
<td>25 (0–60)</td>
<td>30 (0–56)</td>
</tr>
<tr>
<td>Housing</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Born at Tulane Regional Primate Center</td>
<td>31 12</td>
<td>12 36</td>
</tr>
<tr>
<td>Born at other facility</td>
<td>1 3</td>
<td>2 13</td>
</tr>
</tbody>
</table>

**NOTE.** Data are no. of animals, unless otherwise indicated.

<sup>a</sup> SIV/Delta<sub>B670</sub> and SIV/Delta<sub>J943</sub> represented SIV strains originally isolated from sooty mangabeys [16], used to infect 2 macaques (B670 and J943), and then expanded in vitro in peripheral human lymphocytes (SIV/Delta<sub>B670</sub>) or in herpesvirus-transformed B cell lines from lymphoma tissue (SIV/Delta<sub>J943</sub> [17]). Other SIV strains included SIV/Fred (n = 5), SIV/Delta<sub>B670</sub> variants (n = 3), SIV/17E (n = 2), SIV/mac239 [14] (n = 2), SIV/BK44 (n = 2), SIV/BK28 (n = 1), and SIV/F965 (n = 1). For 1 animal, infection occurred as result of a bite wound, and the viral strain was unknown.

The epidemiology of MAC infection among SIV-infected and SIV-uninfected rhesus macaques was investigated as a model for MAC infection in humans. In general, captive primates are maintained within a well-defined environment and are exposed to a small number of potential sources of mycobacteria. Therefore, we studied SIV-infected and SIV-uninfected rhesus macaques to explore the hypothesis that reactivation of latent infection represents a source of invasive disease for primates.

**MATERIALS AND METHODS**

**Animals and housing.** All study animals were housed at the Tulane Regional Primate Center (TRPC; Covington, LA), and most were bred on site (table 1). Detailed clinical histories, dates of birth and death, dates of SIV infection, and strains used for SIV inoculation were retrospectively reviewed for each animal. At the time of death, necropsies were done on all animals. Histopathologic examination of tissues was done for most animals.

The majority of animals were housed outdoors in 1012-m² factors were generally unsuccessful [5, 6], and when MAC was isolated, patient and environmental strains differed [5]. For a small number of patients, hospital water was shown to represent a source of infecting isolates [7]; however, this represented <5% of at-risk patients (authors’ unpublished data).

Implicit in the aforementioned studies was the assumption that, among immunodeficient persons, clinical disease with MAC develops soon after exposure to pathogenic strains [8]. The inability to identify the source for infecting strains suggested a possible alternative hypothesis: that, for some patients, MAC infection may represent reactivation of latent infection acquired years before clinical disease manifests.

Simian immunodeficiency viruses (SIVs) are lentiviruses with close homology to human immunodeficiency virus (HIV) in genomic organization [9, 10] and biological properties [11]. The SIV-infected rhesus macaque has been shown to be an excellent model for HIV infection with regard to disease pathogenesis and development of opportunistic infections [11–13], including infection with MAC and other mycobacteria of low virulence [14, 15].
corrals; the remainder were housed indoors. The corrals were
open at the top, were bordered by chain-link fencing, and had
grain and dirt flooring. Corrals may have housed different spe-
cies of primates in different years. Detailed housing records
were maintained for each animal. Animals obtained from other
facilities were maintained in quarantine for 2 months before
being introduced into the resident population. Water was sup-
plied to the facility via an 869 m–deep well dug in 1990.

SIV-uninfected animals maintained for breeding would be
euthanized because of old age, illness, or trauma. Animals se-
lected for SIV-related studies were not housed separately from
other animals during infancy and adolescence. At the time that
such animals were assigned to SIV-related studies, they were
relocated to a dedicated biosafety level 3 (BSL3) building and
maintained there until death. Animals were maintained in
stainless steel mesh cages (1 animal/cage) that were physically
adjoined, with a common tray to capture excrement. Animals
were fed sterile laboratory feed, as well as washed fresh fruits
and vegetables twice weekly. Fresh vegetable choices occasion-
ally included potatoes and other root vegetables. The water
source used to clean the caging was derived from the supply
also used as a potable water source. All animals used in SIV-
related research were euthanized at study end, or earlier if they
manifested signs of severe illness or wasting.

Tissue samples. Tissue samples from rhesus macaques
were prospectively collected. For the present study, excess tissue
was obtained at the time of necropsy from animals that died
or were euthanized. Animals were selected only on the basis of
SIV infection status. Tissue from SIV-infected animals was
kindly supplied by M. Murphey-Corb (TRPC). No animal was
euthanized solely for the purposes of inclusion in this study.

Necropsy samples were done in a closed room with laminar airflow.
Samples (∼0.5–1 g) of liver, spleen, colon, and mesenteric and
paratracheal lymph nodes were obtained aseptically for culture
before tissue was collected for histopathologic examination.
Instruments were flame-sterilized immediately after each organ
was sectioned and then were cleaned and sterilized after use.
One set of instruments was used for each animal. Tissue spec-
cimens were frozen and maintained at −80°C before culture.

Sample processing. A small piece of tissue (∼50–200 mg)
was cut with a sterile scalpel, placed in a sterile microcentrifuge
tube, and weighed. Tissue was decontaminated with NaOH by
a protocol modified from Torrini et al. [18]. In brief, 200 μL
of decontamination solution (2% NaOH and 1.45% sodium
citrate) was added to the microcentrifuge tube, and the tissue
was manually disrupted with a handheld grinder (Kendall).
Normally sterile specimens (lymph nodes, liver, and spleen)
were incubated at ambient temperature for 5 min, and non-
sterile specimens (colon) were incubated at ambient tempera-
ture for 10 min. After decontamination, HCl was added to
raise the pH to 7.0. Each specimen was then inoculated onto
7H10 agar and incubated at 37°C for up to 8 weeks.

Environmental cultures. Water from faucets at various
sites throughout the TRPC was collected in sterile 300-mL con-
tainers and maintained at ambient temperature for processing.
Water was decontaminated with cetylpyridinium chloride (final
concentration, 5 × 10−4%) [7, 19]. After incubation for 30 min
at ambient temperature, the water samples were passed through
a 0.45-μm analytical filter (Millipore HABG) held in a Nalgene
vacuum filter holder (Fisher Scientific), and the filters were
washed with 600 mL of sterile water. The filters were transferred
to mycobacterial environmental medium [19] by use of sterile
forceps and incubated at 37°C for up to 8 weeks.

Soil was collected from the outdoor corrals, shipped at am-
bient temperature, and stored at −20°C until processing. Soil
was decontaminated with cetylpyridinium chloride as described
above or with [14C]carboxypropylbetaine as described elsewhere
[20]. Sediments were inoculated onto 7H11 medium and
incubated at 37°C for up to 8 weeks.

Molecular epidemiological analysis. DNA was isolated in
situ in agarose plugs as described elsewhere [21, 22]. Asf re-
striction fragments were resolved in 1% agarose gels by pulsed-
field gel electrophoresis (PFGE) in 0.5× Tris-borate-EDTA buf-
ner in a CHEF DRII apparatus (BioRad) at 200 V for 22 h,
with switch times ramped linearly between 1 and 40 s. Isolates
with ≥3 differences in banding patterns were considered to be
distinct strains [23].

Statistical analysis. Data manipulation and statistical cal-
culations were done with Stata 7 (StataCorp). A 2-sample t
test, in which equality of variances was assumed, was used to
test the equality of means, and a nonparametric k-sample test
was used to test the equality of medians. The calculated cri-
fer for the median test was Pearson’s χ² statistic (corrected
for continuity). A retrospective case-control study design was
assumed in attempting to identify risk factors for infection with
MAC organisms. Logistic regression was used to estimate odds
ratios (ORs). A preliminary univariate screen of all exposures
was undertaken to select those that would be included in the
final multivariate analysis. For categorical variables, the signifi-
cance of univariate associations was determined with either
Pearson’s χ² test or the likelihood ratio χ² statistic. For con-
tinuous variables, we used Student’s t test to compare means.
All exposures associated with MAC in the univariate analysis
at P<.20 were selected for inclusion in the multivariate logistic
regression analysis. P<.20 was chosen to avoid a too-rigorous
initial selection among variables. The components of the mul-
tivariate model were determined by a combination of pur-
opositive backwards model selection and an automated stepwise
backwards model selection process. The criterion for accepting
the null hypothesis, and therefore deleting the variable from
the model, was that P>.05 for the likelihood ratio χ² statistic.
Finally, clinically plausible interaction terms were defined and included in the main effects model.

RESULTS

Tissue from 110 rhesus macaques was prospectively collected for analysis between January 1995 and January 1997. Sixty-three animals were not infected with SIV, and 47 had been inoculated with various strains of SIV (table 1). SIV-uninfected animals were significantly older than SIV-infected animals, with a mean age of 130 months and a median age of 102 months (range, 16–308 months) versus a mean age of 57 months and a median age of 43 months (range, 28–123 months; for the mean, \( t = 5.8 \) and \( P < .0001 \); for the median, \( \chi^2 = 17.4 \) and \( P < .0001 \)). The age differences reflected the fact that animals were typically selected for SIV-related research at 24 months of age and were euthanized 6–12 months later. In contrast, SIV-uninfected animals were typically euthanized only if they had sustained injuries as a result of trauma (i.e., fighting within the corrals), illness, or infirmity related to advanced age.

Culture of tissues derived from SIV-uninfected animals yielded MAC for 14 animals (22.2%). MAC was detected in a single organ for 12 animals (85.7%) and in 2 organs for 2 animals (14.3%). Ten animals had MAC isolated from \( \geq 1 \) sterile organ, including the liver (4 animals), mesenteric lymph nodes (4 animals), and paratracheal lymph nodes (3 animals). MAC was cultured only from the colon for 4 animals. Bacterial burdens were typically low, with a median of 33.4 cfu/g of tissue (range, 2–120 cfu/g of tissue).

Culture of tissue derived from SIV-infected animals yielded MAC for 32 animals (68.1%). Twenty-two animals (68.8%) were infected at \( \geq 3 \) sites, and 10 (31.3%) were infected at a single site. Infection of the spleen and mesenteric lymph nodes was observed for 21 animals; of the paratracheal lymph nodes, for 20 animals; of the liver, for 16 animals; and of the colon, for 12 animals. For 10 animals, MAC was cultured from a single organ. This included the paratracheal lymph nodes for 5 animals, the mesenteric lymph nodes for 2 animals, the colon for 2 animals, and the spleen for 1 animal. Bacterial burdens ranged from 5.0 cfu/g to \( > 15,841 \) cfu/g, with a median of 998.7 cfu/g. For some animals, a >1-log difference was observed in bacterial burdens between organs. Typically, the spleen and mesenteric lymph nodes yielded the highest bacterial counts (data not shown). Animals with infection that was limited to a single organ had lower colony counts (median, 111.1 cfu/g; range, 5.0–2835.5 cfu/g) than did animals with multiorgan infection (median, 1295.1 cfu/g; range, 7.1–15,841 cfu/g).

Histopathologic examination and acid-fast smears were done for 38 SIV-infected animals (80.9%) and 50 SIV-uninfected animals (79.4%). Mycobacteria and granulomatous disease were detected by histopathologic examination for 8 animals; all were infected with SIV and had positive results of culture for MAC. Granulomas were not detected for smear-negative animals. The median tissue burden of culture-positive organs from smear-positive animals (tissue burdens were determined for 7 animals) was 3886.0 cfu/g (range, 205.1–15,841 cfu/g), compared with 510.7 cfu/g for smear-negative animals (range, 5.0–6667 cfu/g).

Clinical correlates of and risk factors for MAC infection.

The demographic and clinical variables that were tested for inclusion in the final multivariate logistic model are shown in table 1. On the basis of the univariate screen (table 2), age (considered as a continuous variable per year of life; OR, 0.989 for each year), having been born and raised in the TRPC (OR, 4.78), being infected with SIV (OR, 7.47), a diagnosis of diarrhea (OR, 3.26), a diagnosis of wasting (OR, 5.91), or a summary variable denoting any opportunistic infection or invasive complication of SIV infection and SIV-related tumors (OR, 19.93) were included in the initial multivariate model. All other variables, including CD4 cell count, were excluded. Age, wasting, and diarrhea were subsequently dropped after adjustment for the other variables. None of the plausible interaction terms were retained in the final multivariate model; thus, the ORs for each remaining variable can be interpreted directly. The odds that an SIV-infected macaque was infected with MAC were 3.45 times greater than the odds for an SIV-uninfected macaque (with respect to the other covariates in the model; \( P = .013 \); table 2). Similarly, the odds of MAC being detected in any organ for a macaque born and raised in the TRPC were 3.32 times greater than the odds for a similar macaque that was born elsewhere and subsequently brought into the TRPC (\( P = .103 \); table 2). Finally, for an SIV-infected macaque with a diagnosis of an opportunistic infection or invasive complication of SIV infection, including crypto-

Table 2. Relative risks for Mycobacterium avium complex infection in simian immunodeficiency virus (SIV)–infected and SIV-uninfected macaques.

<table>
<thead>
<tr>
<th>Factor or demographic variable</th>
<th>OR (CI)a</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Univariate analysis</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age at time of death</td>
<td>0.989 (0.988–0.990)</td>
<td></td>
</tr>
<tr>
<td>Born at Tulane Regional Primate Center</td>
<td>4.78 (4.02–5.69)</td>
<td></td>
</tr>
<tr>
<td>Diarrhea</td>
<td>3.26 (2.88–3.69)</td>
<td></td>
</tr>
<tr>
<td>SIV-related infection or tumorb</td>
<td>19.93 (15.94–24.92)</td>
<td></td>
</tr>
<tr>
<td>SIV infection</td>
<td>7.47 (6.59–8.46)</td>
<td></td>
</tr>
<tr>
<td>Wasting</td>
<td>5.91 (5.02–6.96)</td>
<td></td>
</tr>
<tr>
<td><strong>Multivariate analysis</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Birth at Tulane Regional Primate Center</td>
<td>3.32 (0.78–14.03)</td>
<td>0.103</td>
</tr>
<tr>
<td>Opportunistic infection or tumor</td>
<td>8.08 (1.53–42.46)</td>
<td>0.014</td>
</tr>
<tr>
<td>SIV infection</td>
<td>3.45 (1.29–9.15)</td>
<td>0.013</td>
</tr>
</tbody>
</table>

**NOTE.** CI, confidence interval; OR, odds ratio.

a For the univariate model, 80% CIs are listed; for the multivariate model, 95% CIs are given.

b SIV-infected animals only.
sporidiosis, SIV pneumonia, SIV encephalitis, and SIV-related
tumors (typically lymphoma or lymphosarcoma), the odds of
MAC infection were 8.08 times greater than the odds for a similar
macaque with no such diagnosis \( (P = .014; \text{table 2}) \). A separate
analysis of the occurrence of MAC in only the SIV-infected group
of macaques showed that MAC infection was associated with
infection with SIV/Delta\(_{\text{J943}}\) or SIV/Delta\(_{\text{J943}}\) (OR, 21.6; 95% con-
fidence interval [CI], 3.66–149.98; \( P < .0001 \)).

Molecular analysis of infecting isolates. Isolates from 13
(92.9%) of 14 SIV-uninfected and 31 (96.9%) of 32 SIV-in-
fected animals were available for analysis. Chromosomal DNA
of 5 colonies (as available) from each organ yielding MAC was
analyzed by PFGE (figure 1).

For SIV-uninfected animals, a single infecting strain was
identified for 12 (92.3%) of 13 animals, and 2 different infecting
strains were identified for 1 animal. Analysis of the isolates
from SIV-infected animals revealed a single infecting strain for
20 (64.5%) of 31 animals; 2 strains, for 9 animals (29.0%); and
3 unique strains, for 2 animals (6.5%). In many cases of pol-y-
clonal infection, 2 strains were cultured from the same organ.

PFGE polymorphisms of infecting strains were compared
between animals. Each SIV-uninfected animal was infected with
a different (i.e., unique) strain of MAC (figure 1). For the SIV-
infecting animals, a total of 20 unique strains of MAC were
identified. One strain, K128A, was identified in samples from
25 animals (80.7% of 31 animals with a positive result of culture
for MAC and 53.2% of the 47 SIV-infected animals). Nine SIV-
infecting animals were infected with MAC strain K128A and
\( \geq 1 \) other unique strain. Six animals were infected with a strain
of MAC other than K128A, including 2 animals infected with
3 unique strains other than K128A. K128A was also identified
as one of the strains infecting an SIV-uninfected animal. The
latter animal had been housed within the SIV BSL3 facility and
had MAC cultured from the paratracheal lymph nodes. Except
for the epidemic strain (K128), no other strain was found to
infect more than a single animal.

An attempt to derive an estimate of the relative virulence of
the epizootic strain K128A versus enzootic MAC strains was
qualitatively estimated for animals infected with either SIV/
Delta\(_{\text{J943}}\) or SIV/Delta\(_{\text{J943}}\). The median age at euthanasia for the
10 animals also infected with MAC strain K128A was 42 months
(range, 36–50 months); the median age at euthanasia was 40
months (range, 38–45 months) for the 5 animals infected with
MAC strains other than K128A and 40 months (range, 36–52
months) for the 9 animals infected with both K128A and other
MAC strains. Similarly, there were no observed differences in

Figure 1. Pulsed-field gel electrophoretic analysis of Mycobacterium avium complex (MAC) isolates from simian immunodeficiency virus
(SIV)–uninfected and SIV-infected macaques. AseI restriction macrorestriction patterns for strains from 2 water samples, 3 SIV-uninfected (−) macaques,
and 5 SIV-infected (+) macaques are shown. Lane 1, Smal-digested DNA from Staphylococcus aureus strain ATCC 8325 as molecular weight marker
[24]. Lanes 2 and 3. Strains EW1 and EW2, respectively, from samples of environmental water. Strain EW2 represents the dominant strain from water
that was cultured at Tulane Regional Primate Center. Strains from SIV-uninfected animals N282, J738, E799, and F116 are shown in lanes 4–6 and
8; those from SIV-infected animals I335, H523, L904, L845, and K019 are shown in lanes 7 and 9–12; and strains shown in lanes 13–15 are all from
SIV-infected animal N499. SIV-uninfected macaque J738 (lane 5) and the SIV-infected macaques H523 and L904 (lanes 9 and 10) were infected with
MAC strain K128A. All remaining macaques were infected with unique strains of MAC; macaque N499 (lanes 13–15) was infected with 3 unique
strains.
tissue burdens for the 3 categories of animals (data not shown). Five of the 8 animals with acid-fast bacilli detected on histopathologic examination had been inoculated with SIV/DeltaB670 or SIV/Delta nabat. All 5 were coinfected with epizootic MAC strain K128A, including 3 that were also infected with unique enzootic strains. These data do not clearly suggest a difference in virulence between K128A and other MAC strains.

**Molecular analysis of environmental samples.** Exposures to sources of MAC included soil in the outdoor corrals, water, and surfaces contaminated with fecal material from birds or infected macaques. The commercial feed was not considered to be a likely source of MAC. Although fresh fruits and vegetables could conceivably represent a source of MAC, previous studies have not supported this hypothesis [6, 25].

Water samples were collected in July 1995, June 1996, and October 1996 from the SIV BSIS facility and from other indoor and outdoor sites. A total of 4 strains of MAC were identified in the water (figure 1). One strain was observed in samples of water at each time period and was the dominant strain in all samples. The isolates cultured from water were compared with the clinical isolates, but no matches were identified.

Soil was collected from 28 corrals at 2 time points, September 1995 and November 1996. These included samples from all birth corrals for study animals infected with unique strains of MAC. Unfortunately, the majority of corrals at the TRPC were covered with fresh topsoil in early September 1995, before the first sampling. Although soil was preferentially selected from the uncovered perimeters of the corrals, contamination from “new” soil could not be excluded. For 3 corrals, there was no uncovered area, and therefore “new” soil was cultured. A total of 30 strains were identified. Three strains were identified in 2 corrals each. Comparison of soil isolates with clinical strains showed no matches.

The catch pan under the monkey cage used to collect waste material was cultured subsequent to the identification of an epidemic strain in the colony. This sample yielded MAC strain K128A. Other strains were not identified.

**DISCUSSION**

We investigated the epidemiology of MAC infection among rhesus macaques. Our main hypothesis was that rhesus macaques could manifest latent infection with MAC organisms and that latent MAC infection could reactivate and cause clinical disease among immunosuppressed animals. By comparison of the rates of infection among SIV-infected and SIV-uninfected macaques, it should be possible to test this hypothesis. The presumption was that MAC infection rates should be equal in the 2 groups. Moreover, through a careful survey of environmental sources of MAC, it may be possible to determine the etiology of infecting isolates.

Our hypothesis that rhesus macaques may be latently infected with organisms of MAC is proven by the observation that MAC was cultured from sterile sites (liver, spleen, and mesenteric lymph nodes) from 19.1% of SIV-uninfected animals. Latently infected animals did not demonstrate granulomatous disease, even though such disease was specifically sought as part of this study. Our initial expectation that SIV-infected macaques would manifest a prevalence of infection similar to that in SIV-uninfected animals was not met; the actual prevalence was >3 times higher (68.1%). Molecular analysis of infecting isolates, however, demonstrated 2 patterns of infection among the SIV-infected animals. An epidemic pattern of infection involving a single common strain (K128A) was identified for 53.2% of SIV-infected animals. Unique strains other than K128A (i.e., “endemic” infection) were identified for 34.9% of SIV-infected animals, a rate more similar to that for SIV-uninfected animals. Clinical disease and the level of infection (colony counts per organ) were independent of MAC strain. Of note was that K128A was also cultured from 1 of the SIV-uninfected animals.

By combining molecular analysis of infecting isolates with the results of culture, we showed that for both SIV-infected macaques and SIV-uninfected macaques, exposure to an invasive strain of MAC could result in clinical or subclinical infection in relation to the level of immune suppression. Moreover, our second hypothesis, that reactivation of latent infection could cause disease among immunosuppressed macaques, is supported by the observation of “endemic” infection among SIV-infected animals.

A multivariate model was constructed to investigate the potential risks associated with MAC infection. Univariate analysis showed an association between the detection of MAC infection and birth at the TRPC, diarrheal illness, wasting, SIV coinfection, and, for SIV-infected animals, the diagnosis of an opportunistic infection (other than MAC) or an SIV-related tumor. Other clinical factors and CD4 cell counts were not associated with MAC infection. Three variables were retained after multivariate analysis: SIV infection, birth at the TRPC, and an opportunistic infection or SIV-related tumor.

Coinfection with SIV as a risk factor for infection with MAC (OR, 3.45; 95% CI, 1.29–9.15; \(P = .013\)) is consistent with simian and human data showing that host immune status critically determines susceptibility to opportunistic pathogens [8, 14, 15]. In addition, the risk of infection was dependent on the infecting strain of SIV—animals infected with SIV/DeltaB670 or SIV/Delta nabat had a risk of infection 21.6 times greater than that of animals infected with other SIV strains (95% CI, 3.66–149.98; \(P < .0001\))—which is consistent with the results of other studies [14]. We also found that the presence of an opportunistic infection other than MAC or of an SIV-related tumor was highly predictive of MAC infection (OR, 8.08; 95% CI, 1.53–42.46; \(P = .014\)). Although there was no observed
correlation between MAC infection and CD4 cell count, the range of CD4 cell counts was small.

One of the more surprising observations was that animals bred within the open-air environment of the TRPC appeared to have an increased risk for infection, regardless of SIV infection status (OR, 3.32; 95% CI, 0.78–14.03; P = .103). Animals acquired from other facilities were typically >24 months old at the time of transfer, which suggests that exposures early in life may be important for MAC acquisition.

In this study, there were no matches between clinical strains and environmental isolates. The commercially purchased primate diet was not considered to be a likely source of infection. The fresh fruits and vegetables used to supplement the diet were not cultured and could represent a potential, albeit unlikely [6, 25], source of MAC. The water supply appeared to be stably colonized with a limited number of strains, with 1 strain predominating, a result observed for other potable water sources [7]. This water supply was used both as a potable water source and for cage cleaning. Thus, our results suggest that sources other than water were the source of latent infection, in contrast to the experience of researchers at the New England Primate Center [26]. Isolates of MAC cultured from soil demonstrated a high degree of genetic diversity. Therefore, soil would appear to be a logical (albeit unproven) source of latent infection.

We also observed that 25 SIV-infected animals and 1 SIV-uninfected animal were infected with a single strain of MAC, K128A, which demonstrates that exposure to invasive strains can result in clinical infection. We postulate that horizontal transmission of MAC occurred between animals. This hypothesis is supported by the finding that only K128A was cultured from the trough containing fecal material beneath 1 group of cages. This strain was not identified in the water system, making water an unlikely environmental source. Evaluation of this particular strain has shown it to be highly virulent for rhesus macaques [27]. There do not appear to be other episodes of horizontal transmission between animals, because all other strains were unique to individual animals.

Our data have obvious implications for human disease. The existence and prevalence of latent MAC infection among humans can be inferred from a study of autopsy and surgical specimens. MAC was identified in 1%–2% of cervical lymph nodes obtained from children at the time of tonsillectomy [28]. A separate study examined bone marrow specimens from patients with chronic pulmonary disease for the presence of mycobacteria [29]. For patients with MAC detected in sputum and evidence of clinical disease, 44% of bone marrow specimens (8/18) yielded MAC on culture, compared with 17% of patients with sputum cultures positive for MAC and without evidence of clinical mycobacterial infection (4/23) and 8% of persons without pulmonary disease and with sputum cultures negative for MAC (1/12).

Skin-test reactivity to nontuberculous mycobacterial antigens was demonstrated for 10%–25% of humans in a 1969 study of >500,000 Navy recruits [30], more recent US and international studies [31–33], and studies of pediatric populations [34]. In these studies, a positive response was considered to represent past infection [32]. Other studies have demonstrated that skin-test results may be predictive of active infection [35, 36]. More direct evidence that reactivity to nontuberculous skin-test antigens may indicate mycobacterial infection derives from a study of 553 children which showed that the results of skin testing were highly predictive of the species of mycobacteria that could be cultured from clinical specimens [37]. We attempted to correlate reactivity to an M. avium skin sensitin [32] with the results of culture. However, because of the poor skin reactivity of nonhuman primates to skin-test antigens, this was not possible (authors’ unpublished data).

In summary, we were able to demonstrate that latent infection with MAC occurs among SIV-uninfected animals. For SIV-infected animals, we were able to demonstrate that disseminated disease appears to result from reactivation of latent infection, as well as from recent infection with virulent strains. Unfortunately, the inability to identify a source of infection in the water or soil limits absolute proof of our hypothesis that MAC infection among SIV-infected macaques results from reactivation of latent disease due to exposures that predate clinical immune suppression.

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