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***Pneumocystis* Colonization in Immunocompetent and Simian Immunodeficiency Virus–Infected Cynomolgus Macaques**

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Abstract

Pneumocystis (Pc) colonization is common among human immunodeficiency virus (HIV)–infected subjects, although the clinical consequences of Pc carriage are not fully understood. We examined the frequency of asymptomatic carriage in healthy and simian immunodeficiency virus (SIV)–infected cynomolgus macaques by use of polymerase chain reaction (PCR) and assessment of changes in the serologic response to a recombinant fragment of the Pc protein kexin (KEX1). Anti-KEX1 antibodies were detected in 95% of healthy monkeys. To create a model of natural transmission of Pc, SIV-infected monkeys were cohoused with macaques coinfecting with SIV and Pc. Pc colonization occurred when the CD4⁺ T cell count decreased to <500 cells/μL, despite anti-Pc prophylaxis with trimethoprim-sulfamethoxazole. Increases in anti-KEX1 antibody titers preceded detection of Pc DNA in bronchoalveolar lavage (BAL) fluid samples by use of PCR. These results demonstrate the usefulness of recombinant KEX1 in serologic studies of Pc colonization and will improve the understanding of Pc transmission and clinical consequences of Pc colonization in HIV-infected patients.

Pneumocystis (Pc) pneumonia (PCP) remains a common serious infection in immunocompromised individuals, particularly those with AIDS [1,2]. Although anti-Pc prophylaxis and highly active antiretroviral therapy (HAART) have resulted in a significant decrease in the frequency of PCP, PCP remains the most common AIDS-defining opportunistic infection in the United States [1–3].

A clear understanding of the route of Pc transmission is lacking because of an inability to culture the organisms in vitro. Several hypotheses exist regarding transmission of Pc among immunocompromised persons, including reactivation of latent infection acquired during childhood, active acquisition of infection from environmental exposure, and person-to-person transmission [3]. Airborne transmission has been demonstrated in animal models and has been used experimentally to initiate infection in healthy rodents and in immunocompromised rodent models of PCP [4,5]. PCP has been documented in experimental macaque models of AIDS [6,7], but a naturally occurring model of transmission and colonization in primates has not been described.

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Evidence from experimental systems and serologic studies of humans suggests that exposure to Pc stimulates a humoral response and that antibodies play a significant role in host defense against PCP [8–14]. Clinical application of Pc serologic findings has been of limited use in understanding the transmission and progression of Pc infection resulting from the frequency of antibodies in both healthy and immunocompromised hosts [15,16]. Recent studies using the major surface glycoprotein (MSG) of Pc have shown a high frequency of serum antibodies among healthy and immunosuppressed subjects [17–20], and changes in MSG-specific antibody levels were detected in serially obtained serum samples, in response to Pc infection and treatment [21]. These studies suggest that recombinant Pc antigens may be useful in distinguishing past from current Pc exposure.

Highly sensitive techniques, such as polymerase chain reaction (PCR) analysis, have been used to detect very low levels of Pc DNA in asymptomatic persons [22–24]. Detection of Pc DNA in asymptomatic individuals by use of PCR has been defined as colonization or subclinical carriage [2], although the clinical consequences of Pc colonization are unknown. Pc colonization may be important for several reasons: it may increase the risk for progression to PCP in susceptible individuals, carriers may transmit infection, and organism persistence may induce chronic inflammation resulting in lung damage. Pc colonization is not common in immunocompetent adults, but it can be detected in individuals with mild to severe immunosuppression [23,25]. In HIV-infected subjects, the frequency of Pc colonization has been reported to be 5%–69% [26]. We previously reported that experimental, intrabronchial inoculation of Pc in simian immunodeficiency virus (SIV)–infected rhesus macaques could lead to persistent, subclinical Pc colonization and PCP. In this model, Pc colonization before PCP development resulted in a chronic inflammatory response in the lungs that was characterized by prominent CD8⁺ T cell infiltration and cytokine production [27]. A shortcoming of this model is the lack of purity of the inoculum, which results from an inability to culture Pc in vitro and which may induce a transient, nonspecific inflammatory response.

In the present study, we aimed to determine the antibody response and frequency of Pc colonization in a cohort of healthy monkeys, to establish a model of airborne transmission of Pc colonization in SIV-infected macaques, and to evaluate the dynamics of the anti-Pc antibody response in peripheral blood and bronchoalveolar lavage (BAL) fluid samples during colonization of SIV-infected macaques.

MATERIALS AND METHODS

Animals

The cynomolgus macaques (*Macaca fascicularis*; age range, 3.5–4.5 years) used in the present study were housed in an American Association for Accreditation of Laboratory Animal Care–accredited biosafety level 2+ primate facility at the University of Pittsburgh (Pittsburgh, Pennsylvania). Before initiation of the study, all animal experiments were approved by the Institutional Animal Care and Use Committee of the University of Pittsburgh.

Study design

Seventy-four healthy, non-SIV-infected monkeys were tested to determine the frequency and the magnitude of anti-Pc antibody titers by use of Pc recombinant protein kexin (KEX1) ELISA, as described below. Five monkeys were subsequently infected with SIV and used to examine the natural transmission of Pc colonization and the development of a humoral response to Pc. Before SIV infection, monkeys had BAL performed, and the samples obtained were evaluated for the presence of Pc by microscopic examination of the Giemsa-stained samples and by Pc-specific nested PCR [27]. In addition, serologic analysis was performed at baseline to detect anti-KEX1 antibodies (as described below). Monkeys were then infected with a

pathogenic strain of SIV (SIV/Delta B670) [28], and clinical evaluation and plasma viral level determination were performed as described elsewhere [27].

At the time of SIV infection, monkeys received daily treatment with trimethoprim-sulfamethoxazole (TMP-SMZ; 20 mg/kg [TMP] and 100 mg/kg [SMZ]), given in 2 divided doses, to clear any preexisting Pc colonization, and they were cohoused with SIV macaques that had not received anti-Pc treatment. When plasma antibody titers decreased to background levels (4 months after SIV infection), the TMP-SMZ dose was reduced to 10 mg/kg TMP and 50 mg/kg SMZ given 3 times weekly, on alternate days, to approximate a prophylactic regimen [29]. Treatment and prophylactic doses were based on the American Academy of Pediatrics “Red Book” recommendations for Pc [29]. On the basis of previous studies of experimental Pc infection in SIV-immunosuppressed macaques, we predicted that monkeys would be susceptible to natural Pc colonization when peripheral blood CD4⁺ T cell levels decreased to <30% of the total T cell count [27,30]. Thus, TMP-SMZ prophylaxis was discontinued when CD4⁺ T cell counts decreased below this level (~500 CD4⁺ T cells/ μ L), to allow natural Pc colonization to occur. Discontinuation of TMP-SMZ occurred at 9 months after SIV infection for monkeys 133, 157, and 158 and at 15 months for monkeys 143 and 156.

BAL fluid and blood sample collection

Plasma, peripheral blood mononuclear cell (PBMC), and BAL samples serially obtained from healthy and SIV-infected monkeys were collected on a monthly basis for longitudinal analyses, as described elsewhere [27]. Aliquots were removed for flow cytometric analysis, microscopic and microbiologic analyses, and Pc-specific PCR. Plasma and BAL fluid samples were heat inactivated (at 56°C for 30 min) and were used in ELISA for the detection of Pc-specific antibodies. BAL fluid samples were normalized based on the plasma urea concentration [31]. Percentages and absolute numbers of CD4⁺ and CD8⁺ T cells were calculated based on flow cytometric analysis of PBMCs [32]. Pc colonization was defined as a 3-fold increase in the anti-KEX1 reciprocal end point titer (RET) over the titer at baseline, and it was confirmed by detection of Pc DNA by nested PCR in BAL fluid, as described below.

Western blot analysis

A partial fragment of the macaque-derived, Pc kexin gene in the pBAD expression vector (provided by C. G. Haidaris, University of Rochester, Rochester, New York; GenBank accession no. EU918304) was used to produce KEX1. In brief, KEX1 expression was induced in *Escherichia coli* Top10 (Invitrogen) containing the pBAD-KEX1 plasmid; cells were lysed and centrifuged, and supernatant was purified by affinity chromatography. Purified protein was subjected to SDS-PAGE, transferred to nitrocellulose, blocked, and incubated with plasma obtained from a macaque with PCP, as well as with plasma obtained from SIV-infected macaques from the present study. Filters were washed, incubated with horseradish peroxidase-conjugated IgG secondary antibody, and developed according to standard protocols (details on supplementary materials and methods are provided in the appendix, which is available only in the electronic version of the *Journal*).

End point antibody titer determination

Microtiter plates (Immunolon 4HBX; Thermo Fisher Scientific) were coated with 5 μ g/mL purified KEX1 in Na₂CO₃ (pH 9.5). Heat-inactivated plasma samples were diluted 1:100 in blocking buffer (PBS; 5% nonfat milk). A total of 50 μ L of plasma was plated onto KEX1-coated wells, and serial dilutions were produced to determine end point titers. Goat anti-monkey immunoglobulin-conjugated horseradish peroxidase (dilution, 1:10,000 [for IgG] and 1:2000 [for IgM]) (Nordic Immunology) was used for detection, and plates were developed using standard methods. Plasma samples obtained from a healthy (i.e., uninfected and Pc-negative) macaque were used as a negative control, and plasma samples obtained from a

monkey with PCP were used as a positive control (details on supplementary materials and methods are provided in the appendix, which is available only in the electronic version of the *Journal*). The RET was calculated as the highest dilution at which the optical density values for the test sample were equal to or less than the optical density values for the sample obtained from the healthy macaque.

Nested PCR of BAL fluid samples

BAL fluid cell lysate samples were analyzed for the presence of Pc DNA by nested PCR analysis of the mitochondrial large-subunit rRNA gene, as described elsewhere [27,33]. Nested PCR was performed on 5 μ L of the first-round product, by use of primers P1 and P2 [34]. PCR for the detection of β -globin was also performed on BAL samples, to control for DNA quality [32].

Flow cytometric analysis

Mouse anti-monkey CD3–fluorescein isothiocyanate (clone SP34), mouse anti-human CD8–Cy-Chrome (clone SK1), and mouse anti-monkey CD4–allophycocyanin (clone L200) were purchased from BD Pharmingen. Cells were isolated from whole blood and were stained as described elsewhere [32].

RESULTS

Western blot analysis of KEX1

After confirming the reactivity of purified recombinant KEX1 to plasma obtained from a macaque with microscopically confirmed PCP (data not shown), plasma samples obtained from the SIV-infected monkeys were examined by Western blot analysis; a sample obtained from one animal is shown in figure 1. SDS-PAGE analysis of purified KEX1 demonstrates purity achieved from a typical protein preparation in figure 1A. Western blot analysis demonstrated that plasma reacted with KEX1 at the expected molecular mass of 30 kDA but not with proteins encoded by the vector only, demonstrating the specificity of the antigen (figure 1B).

Anti-KEX1 serologic testing and Pc colonization in healthy cynomolgus macaques

The majority ($n = 74$ [95%]) of healthy cynomolgus macaques had detectable RETs (>100). Of those, 59% had a RET of 100–4800, and the remaining 41% had a RET of >6400 .

We observed transient Pc colonization in 2 healthy monkeys, as was indicated by an increase in the antibody titer and by detection of Pc DNA in the BAL fluid sample. At study entry, 1 monkey had no detectable Pc DNA in the BAL fluid sample and a plasma anti-KEX1 IgG antibody reciprocal titer of 100. Six weeks later, this animal had detectable Pc DNA (as determined by nested PCR) in the BAL fluid sample and a 32-fold increase in the anti-KEX1 antibody end point titer. At subsequent monthly time points, the plasma Pc-specific reciprocal IgG titer decreased to 400, and Pc DNA could no longer be detected in BAL fluid samples. Transient colonization was also detected in another monkey by means of nested PCR analysis of Pc DNA in BAL fluid at entry into the study, but it was not found to be positive by nested PCR performed 4 weeks later.

Natural Pc colonization of SIV-infected macaques

To evaluate Pc colonization status at baseline before SIV infection, nested PCR was performed on BAL fluid samples, and plasma and BAL supernatant samples were analyzed for anti-KEX1 antibodies. Pc DNA was not detected in experimental monkeys before infection with SIV, although 4 of 5 monkeys (monkeys 133, 156, 157, and 158) had detectable anti-KEX1 IgG antibody reciprocal titers, which ranged from 600 to 1600 (figures 2 and 3). Monkeys were

treated with TMP-SMZ, and antibody titers decreased to background levels. BAL fluid samples obtained on a monthly basis had negative results of nested PCR, except for the results obtained at a single time point (3 months) for monkeys 156 and 157 (figure 2). Treatment doses were then decreased to the level of prophylactic doses, and, within 3 months of being cohoused with Pc-infected monkeys, all monkeys experienced a serial increase in anti-KEX1 antibody titers, and Pc DNA was detected in BAL fluid samples obtained from 3 of 5 monkeys, suggesting reemergence or secondary colonization by Pc, despite the use of anti-Pc prophylaxis (figure 2).

When the CD4⁺ T cell count decreased to <500 cells/ μ L, all monkeys eventually experienced an increase in anti-KEX1 plasma antibody titers (figure 2). The anti-KEX1 IgG reciprocal antibody titer did not increase above the background level in monkey 133; however, by 9 months after SIV infection, serial increases in anti-KEX1 IgM antibody titers were detected, suggesting primary exposure to Pc (figures 2 and 3). After monkeys had experienced seroconversion, Pc DNA was detected by nested PCR in multiple BAL samples obtained on a monthly basis, confirming Pc colonization. During the course of the study, the monkeys with immunosuppression due to SIV were unable to clear Pc colonization.

Anti-KEX1 antibody response in BAL fluid samples

Serially obtained BAL fluid samples were analyzed for the presence of anti-KEX1 IgG, IgA, and IgM antibodies. Increases in anti-KEX1 titers in BAL fluid occurred at approximately the same time points as did such increases in plasma samples (figure 3 and figure 4, the latter of which is available only in the electronic version of the *Journal*), and they followed a similar dynamic with respect to class switching, despite low peripheral CD4⁺ T cell levels in blood.

DISCUSSION

We developed an anti-Pc antibody ELISA to evaluate Pc colonization and established a cohousing model of Pc colonization in SIV-infected macaques. Using these tools, we found that (1) the majority of immunocompetent adult cynomolgus macaques have detectable anti-Pc antibody titers; (2) transient Pc colonization occurs in healthy macaques and can be monitored by anti-KEX1 serologic testing and nested PCR analysis of BAL fluid samples; (3) cohousing of SIV-infected macaques with animals coinfecting with SIV and Pc results in Pc colonization, which is not prevented by anti-Pc prophylaxis; and (4) anti-KEX1 seroconversion precedes detection of Pc by nested PCR analysis of BAL fluid samples.

In the present study, we used a serologic assay based on a recombinant fragment of Pc kexin protein (i.e., KEX1). Pc kexin shares sequence homology with a family of fungal serine endoproteases, and, in *Pneumocystis jirovecii* (human-derived Pc), Pc kexin is encoded by a single-copy gene [35–37]. Thus, as a serologic target, KEX1 does not present the complexity of multiple gene copies and antigenic variation associated with MSG [38,39]. Immune responses to Pc kexin have been associated with control of Pc infection in experimental models [40,41]; therefore, changes in anti-KEX1 antibody titers and antibody isotypes during and after infection may serve as a useful correlate of protection.

Studies of Pc prevalence in humans are limited in their ability to correlate antibody levels with Pc colonization and exposure, because of the difficulty of obtaining serial blood and BAL fluid samples from subjects. The nonhuman primate model enables evaluation of anti-Pc antibody responses in serially obtained samples and correlates results with the detection of Pc DNA in BAL fluid samples obtained from both immunocompetent and immunosuppressed animals. We found that ~95% of healthy macaques had detectable plasma anti-KEX1 antibody RETs, ranging from 100 to >12,800, suggesting that the majority of healthy macaques were exposed to Pc. Similar frequencies of anti-Pc antibodies other than those against KEX1 have been

documented in human epidemiologic studies, with up to 84% of adults having antibodies to Pc [15–20,42]. We also found that healthy monkeys developed transient colonization, as defined by an increase in anti-KEX1 titers, followed by detection of Pc DNA by means of nested PCR analysis of BAL fluid samples. Antibody titers subsequently decreased, and Pc DNA was no longer detectable, indicating that healthy monkeys become transiently colonized and are able to clear the organism. Similar results have been reported for immunocompetent rodents when they are exposed to immunosuppressed rodents with PCP [43,44].

We previously showed that SIV-infected macaques are susceptible to Pc infection when they are intrabronchially inoculated with macaque-derived Pc [27]. Because Pc cannot be cultured *in vitro*, one drawback of earlier studies was the use of Pc derived from BAL fluid samples obtained from SIV-infected monkeys with fulminant PCP for initiation of infection. Although inocula were enriched for Pc, the introduction of alloantigen and SIV as a component of the Pc inocula could not be excluded and increased the likelihood of transient, nonspecific inflammatory responses. In addition, the inoculum used in previous studies was a high dose of Pc, which facilitated progression to PCP in these monkeys.

For these reasons and to investigate the earliest points after Pc colonization, we examined natural acquisition of Pc during SIV infection. Pc colonization status was evaluated before SIV infection, and, although the results of nested PCR analysis of BAL fluid samples were negative for all monkeys, 4 of 5 monkeys had detectable anti-KEX1 IgG titers ranging from 600 to 1600 (figures 2 and 3). Detection of anti-KEX1 antibodies in monkeys without detectable PCR products in BAL fluid samples may reflect previous Pc exposure or current colonization below the limit detectable by nested PCR. To clear potential Pc colonization at the start of SIV infection, all monkeys received treatment doses of TMP-SMZ for 4 months, which resulted in undetectable antibody titers and negative results of nested PCR analysis of BAL fluid samples for the detection of Pc DNA. Monkeys then continued receiving a prophylactic dose of TMP-SMZ to prevent Pc colonization, until the CD4⁺T cell count in peripheral blood decreased to a level predicted to allow colonization. An increase in anti-KEX1 antibody titers subsequently occurred in all SIV-infected monkeys, followed by detection of Pc DNA in BAL samples, indicative of newly acquired Pc colonization. In addition, the serial increase in anti-KEX1 antibody titers occurred before detection of Pc DNA in BAL fluid samples, suggesting that the serial KEX1 ELISA is a more sensitive indicator of early Pc colonization in this model. Interestingly, we observed a serial increase in anti-Pc antibody titers and positive PCR results in some animals during the period of TMP-SMZ prophylaxis, indicating that this regimen did not adequately prevent Pc colonization. These results support human epidemiologic studies showing that the risk of Pc colonization was not associated with prophylaxis [23, 26]. Although Pc prophylaxis may result in a reduction in organism burden that is sufficient to prevent active PCP, the results suggest that Pc colonization may persist or recur during prophylaxis.

These results indicate that cohousing of SIV-infected primates is an efficient method to establish colonization and will allow us to examine the long-term consequences of Pc colonization in a model of AIDS. Development of this model is important, because a large number of HIV-infected persons appear to be colonized with Pc, and because the effects of such colonization are unknown [23,26]. Given the high rate of seropositivity among healthy monkeys in the present study, it is likely that Pc colonization of SIV-infected macaques denotes secondary exposure and that the antibody response is a secondary response rather than a primary response. This is likely the case for HIV-infected subjects; therefore, this model may be used to address questions regarding the effect of HIV on the development and strength of memory responses to opportunistic pathogens. Although the predominant antibody responses were IgG antibody responses, one monkey had an IgM antibody response (figures 2, 3, and 4, the latter of which is available only in the electronic version of the *Journal*). Because this

monkey had detectable anti-KEX1 IgG titers at baseline, IgM production may reflect exposure and response to a new Pc strain.

Although all SIV-infected monkeys became colonized with Pc, and although colonization appeared to persist, none of the SIV-infected monkeys developed PCP during the course of this study (>15 months), despite the fact that none of the monkeys received treatment or prophylaxis for Pc after becoming colonized. These results contrast with results from a previous study by Board et al. [27] that used intrabronchial inoculation of Pc in SIV-infected macaques, in which most monkeys developed PCP. This discrepancy may be caused by the larger inoculum used in previous studies, compared with the low level of Pc exposure that likely occurs during cohousing. In addition, it may be that the duration of the study was insufficient to allow for development of PCP from low-level colonization. Other investigators have shown that the development of PCP in SIV-infected, co-housed macaques was associated with the duration of SIV infection [6]. Although Pc colonization was detected at necropsy in monkeys infected with SIV for <2 years, development of PCP was significantly higher in monkeys infected for 2–4 years [6]. Likewise, our laboratory and others have reported the development of active PCP in an SIV model [7,27,32,45–47], although development of Pc colonization in SIV-infected macaques has not been addressed.

Apparent control of Pc infection in these monkeys might also result from the development of a strong IgG response in the majority of monkeys, despite progressive immunosuppression due to SIV. Board et al. [27] previously reported the development of an anti-Pc humoral response in SIV-infected macaques and indicated that the ability to undergo immunoglobulin class switching may contribute to the control of Pc; conversely, a diminished secondary humoral response may be associated with progression to PCP. Although the precise role of antibodies in Pc infection is not well defined, experimental immunization studies involving rodent models of Pc infection indicate that the humoral response plays an important role in the prevention of PCP [8,9,11,14,41,48,49]. Control of experimental Pc infection can be enhanced either by establishment of a Pc-specific antibody response before CD4⁺ T cell depletion sufficient to resolve infection [9–11,40,41,50] or by passive transfer of polyclonal immune serum samples or monoclonal Pc-specific antibodies [14,48]. The immune responsiveness of SIV-infected monkeys to Pc colonization is an important finding, because it suggests that, although monkeys are susceptible to Pc colonization during SIV infection, they are capable of mounting a strong humoral response even with diminished help from CD4⁺ T cells. The KEX1 ELISA is also useful in distinguishing primary and secondary humoral responses and in evaluating the role of a secondary response in preventing PCP in SIV-immunosuppressed monkeys.

In summary, we developed an antibody test for Pc colonization that appears to be more sensitive than PCR. Using this test, we found that the majority of immunocompetent monkeys have detectable anti-Pc antibody titers and that transient Pc colonization occurs in these animals. Furthermore, we established a reliable, efficient model of natural Pc colonization in a simian model of AIDS. Although PCP treatment was able to clear colonization, colonization still developed in these animals despite administration of PCP prophylaxis. These studies have direct clinical relevance to HIV-infected patients, because Pc colonization may occur despite the use of Pc prophylaxis and HAART [23].

Although active PCP has been described in SIV-immunosuppressed monkeys by our laboratory [27,32] and by other investigators [6,45–47], the present model of Pc colonization will be useful in studies examining the role of the immune response in the development and progression of colonization and in examining the long-term effects of colonization on the lung. By defining circumstances associated with early Pc colonization, we will be able to address specific questions regarding transmission, clearance, susceptibility, and clinical consequences of persistence of the organisms in an AIDS model.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. SDS-PAGE and Western blot analysis of recombinant *Pneumocystis* (Pc) recombinant kexin protein (KEX1). A, Coomassie blue-stained SDS-polyacrylamide gel of purified KEX1 (lane 2) and preparation of protein purified from the pBAD expression vector not containing the KEX1 insert (lane 1). Equal amounts of protein (2.5 μ g) were applied to both lanes. The migration of molecular mass markers (expressed in kilodaltons) (left) applies to the Western blot (B) as well. B, Western blot of recombinant KEX1 (lane 2) or proteins from the *Escherichia coli* expression vector (lane 1) using plasma from a Pc-colonized macaque. Equal amounts of protein were applied to both lanes (2.5 μ g).

Figure 2. Natural *Pneumocystis* (Pc) colonization of simian immunodeficiency virus (SIV)-infected macaques. After SIV infection, blood and bronchoalveolar lavage (BAL) fluid samples were collected at monthly intervals. Anti-recombinant kexin protein (KEX1) IgG (for monkeys 143, 156, 157, and 158) or IgM (for monkey 133) (solid black bar) antibody reciprocal end point titers (RETs) of individual monkeys are shown on the left-hand y-axes, and peripheral blood CD4⁺ T cell numbers (solid black line) are shown on the right-hand y-axes. Detection of Pc DNA by nested polymerase chain reaction analysis of BAL fluid samples is denoted by a plus sign. The duration of trimethoprim-sulfamethoxazole (TMP-SMZ) treatment (solid black bracket) or prophylaxis (segmented bracket) is shown. *The RET for monkey 133 is an anti-KEX1 IgM antibody RET.

Figure 3.

Comparison of the anti-recombinant kexin protein (KEX1) antibody response in bronchoalveolar lavage (BAL) fluid supernatant with the anti-KEX1 antibody response in plasma, for 2 representative animals. Blood and BAL fluid supernatant were collected for each animal at monthly time points after simian immunodeficiency virus (SIV) infection. Anti-KEX1 IgM (-*-), IgG (-o-), and IgA (-■-) reciprocal end point titers (RETs) were determined.

Figure 4.

Comparison of the anti-recombinant kexin protein (KEX1) antibody response in bronchoalveolar lavage (BAL) fluid supernatant with the anti-KEX1 antibody response in plasma, for 3 simian immunodeficiency virus (SIV)-infected macaques.