Mucosal and Systemic Antibody Responses in Humans Infected with Simian Foamy Virus

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Simian foamy virus (SFV) infection and the subsequent immune response are not well characterized. Blood plasma, saliva, and urine were obtained from four humans and nine chimpanzees persistently infected with chimpanzee-type SFV for an unknown length of time. SFV-specific immunoglobulin G (IgG) antibodies, but not IgA antibodies, against the Gag and Bet proteins were detected, by Western blotting, in all sample types from infected humans and chimpanzees. Overall, chimpanzee samples had higher anti-SFV IgG titers than humans. These results provide a first comparative evaluation of SFV-specific host mucosal humoral immunity in infected humans and chimpanzees that is characterized by a predominant IgG response and a virtually absent IgA response.

Cross-species transmission of simian foamy viruses (SFVs) to humans by nonhuman primates (NHPs) or by exposure to their body fluids has been documented (4, 10, 21, 24–26, 29). There is currently no evidence of human-to-human transmission of SFV; however, only a few cases (n = 6) have had a short clinical follow-up (3, 10, 26). Although SFV is more easily transmitted among captive NHPs than among humans, questions remain regarding the epidemiology and the natural history of these infections. While a variety of viral and host factors may contribute to the lack of pathogenicity or transmissibility of SFVs in natural hosts and infected humans, the host immune response may play a role in keeping these viruses persistent yet benign (17, 18).

Even though seroreactivity to SFV proteins has been documented in natural hosts (2, 8) and infected humans (4, 10, 19, 21, 26), SFV-specific immunity has not been characterized. A strong plasma antibody response primarily against the SFV Gag doublet and the nonstructural Bet viral proteins was documented in infected primates (8, 17, 19, 25) and humans (4, 9, 10, 21, 26, 29). Seroreactivity to the Gag doublet is consistently detected in plasma and considered to be a diagnostic marker of infection (8, 11, 25, 26). Although seroreactivity to SFV proteins is persistent, it is unknown whether differences in the nature and type of antibody responses in NHPs and humans play a role in virus persistence or in modulating virus transmission.

In the present study, the mucosal and systemic immunoglobulin G (IgG) and IgA immune responses in humans infected with SFV from chimpanzees (SFVcpz) (cases 6, 7, 9, and 10) were evaluated and compared to those of naturally infected chimpanzees. The cases were enrolled in a Centers for Disease Control and Prevention long-term follow-up study to characterize the clinical course of SFV infection (26). The duration of first seropositivity predates the current study by 10 to 24 years; therefore, their dates of infection could not be determined (26). Matched blood plasma, parotid saliva, and urine samples were collected at defined intervals during the study. Longitudinal samples, obtained 27 to 45 months apart, were available from cases 6, 7, and 10.

For comparison, blood plasma and saliva were collected on an opportunistic basis from four naturally infected chimpanzees (CPZ 1 to 4) (26). Blood plasma, whole saliva, and urine samples were collected from five additional chimpanzees (CPZ 5 to 9) (Yerkes Primate Research Center; Emory University, Atlanta, GA). SFVcpz-specific seroreactivity was confirmed in these five chimpanzees by using a previously described Western blotting (WB) protocol (11). No information was available regarding the length of infection for these chimpanzees.

The WB protocol (11) was modified to detect SFVcpz-specific human or chimpanzee IgG and IgA in plasma and mucosal secretions by using horseradish peroxidase-labeled antihuman IgG or IgA (Jackson ImmunoResearch Laboratories, West Grove, PA). Samples were simultaneously screened for immunoreactivity against proteins in either uninfected or SFVcpz-infected Cf2Th cell lysates. Samples with seroreactivity to the Gag doublet were considered seropositive. All samples containing SFVcpz-specific antibodies were nonreactive against uninfected Cf2Th cell lysates (data not shown).

Plasma from cases 6 and 9 had SFV-specific IgG that reacted equally well to the Gag doublet and Bet proteins, and plasma from cases 7 and 10 had predominant reactivity to the Bet protein (Fig. 1A). Plasma from CPZ 1 and 2 had SFV-specific IgG with predominant reactivity to the Gag doublet, plasma from CPZ 4 had predominant reactivity to the Bet protein, and plasma from CPZ 3 had equivalent reactivity to the Gag doublet and Bet proteins (Fig. 1B).
Saliva from cases 6 and 10 had SFV-specific IgG with predominant reactivity to the Gag doublet, and saliva from case 7 had predominant reactivity to the Bet protein (Fig. 2A). Since a limited amount of saliva from case 7 precluded testing at lower dilutions, WB analysis at higher dilutions (≥1:16) may have missed reactivity to other SFVcpz proteins. Saliva from CPZ 5 and 6 had SFV-specific IgG with equivalent reactivity to the Gag and Bet proteins, and saliva from CPZ 7 had predominant reactivity to the Gag doublet (Fig. 2B).

Urine from cases 6 and 10 had SFV-specific IgG with equivalent reactivity to the Gag doublet and Bet proteins (Fig. 2C). As observed with saliva, urine from case 7 had reactivity only to the Bet protein (Fig. 2C). Both chimpanzee urine samples (CPZ 8 and 9) had SFV-specific IgG with similar reactivity to the Gag doublet and Bet proteins (Fig. 2D).

In general, human samples (plasma, saliva, and urine) from longitudinal time points had a similar pattern of immunoreactivity to SFV proteins, indicating a persistent humoral response (data not shown). Semiquantitative serial dilutions of plasma and secretions indicated that SFV-specific IgG titers were approximately two- to fourfold higher in chimpanzees than in humans (Figs. 1 and 2).

WBs employing IgA-specific detection reagents were used on the same plasma, saliva, and urine samples that were tested for SFV-specific IgG. No detectable SFV-specific IgA reactivity against the SFV Gag doublet was observed in any of the tested plasma, saliva, and urine samples (Fig. 1A and 2A, human plasma and saliva, respectively; chimpanzee and urine samples not shown). In the few instances in which WB reactivity was observed with the IgA-specific reagents, this reactiv-

FIG. 1. SFVcpz-specific immunoreactivity, by Western blot analysis, in human (A) and chimpanzee (B) plasma samples. IgG reactivity in both human (A, upper panel) and chimpanzee (B) samples is shown. IgA reactivity in the human samples is also shown (A, lower panel). Plasma samples (1:500 dilutions) from an uninfected human (−) and an SFVcpz-infected chimpanzee (+), as controls, are shown. Dilution values are indicated above each lane for the plasma samples.

FIG. 2. SFVcpz-specific immunoreactivity, by Western blot analysis, in human saliva (A and B, respectively) and urine (C and D, respectively) samples. IgA reactivity in the human saliva samples is shown (A, lower panel). Dilution values are indicated above each lane for the saliva samples. Due to a limited amount of saliva, the minimal starting dilution of saliva for case 7 was 1:16.
ity was weak and inconsistent between samples, indicating non-specific reactivity.

To rule out selective IgA deficiency as a cause for undetectable SFV-specific IgA, levels of total IgA or IgG in plasma and saliva samples were measured by enzyme-linked immunosorbent assay as previously described (15) with the following modifications: unlabeled anti-human IgA- or IgG-coating antibodies (BioSource International, Camarillo, CA), a pooled human serum control (The Binding Site, San Diego, CA), and biotinylated anti-human IgA or IgG antibodies (Jackson ImmunoResearch Laboratories, West Grove, PA). All tested samples from SFVcpz-infected humans and chimpanzees contained normal levels of IgA in plasma and saliva (Table 1). IgG levels in chimpanzees were 1.4- to 5.9-fold higher in plasma and 2.1 to 9.4-fold higher in saliva than in human samples (Table 1).

Since physiologic concentrations of plasma IgG may “mask” the activity of IgA (1, 14, 27), GammaBind G Sepharose was used (16) to remove IgG in selected plasma and saliva samples (median depletion of 86.5%) (Table 1). There was minimal loss of IgA in human and chimpanzee plasma (median depletion of 21.5%). Despite minimal loss of IgA in chimpanzee saliva (median depletion of 21%), there was a twofold greater loss of IgA in human saliva (Table 1). Depleted samples were run simultaneously with matched untreated samples in the modified WB protocol. Removal of IgG resulted in a marked decrease in the detection of SFV proteins in depleted plasma (Fig. 3) and saliva (not shown). Importantly, increased detection of SFV-specific IgA was not observed in depleted plasma (Fig. 3) and saliva (data not shown), indicating that IgG was not masking IgA.

This is the first report comparing the presence and type of antibodies in plasma and mucosal secretions from occupationally SFV-infected humans and naturally infected chimpanzees. SFV-specific WB reactivity was restricted to IgG antibodies and IgG titers were generally lower in humans than in chimpanzees. Although normal IgA levels were detected, SFV-specific IgA against the Gag doublet or Bet proteins was not detected in mucosal secretions or plasma samples. Unlike studies in which removal of IgG from plasma or serum resulted in elevated titers of human immunodeficiency virus (HIV)-specific IgA (1, 14, 27), no enhancement of SFV-specific IgA reactivity was observed in IgG-depleted plasma and saliva samples. The lack of an SFV-specific IgA response in the mucosa suggests that an IgG-mediated systemic humoral response predominates in infected humans and chimpanzees. Similar WB profiles between saliva, urine, and plasma in matched human samples suggest that SFVcpz-specific IgG antibodies transude from plasma into mucosal secretions.

It is unknown whether these findings with SFVcpz-infected humans are representative of what occurs in individuals infected with other SFV variants. HIV type 1 and simian immunodeficiency virus, retroviruses that infect across the mucosa, are also inefficient at inducing an IgA response but induce a strong IgG response in the genitourinary (28) and gastrointestinal tracts (12, 22, 23, 30). In humans lacking HIV-specific IgA, normal influenza-specific IgA levels were detected, suggesting an intact common mucosal immune system (30). Al-

### Table 1. Depletion of IgG from plasma and saliva

<table>
<thead>
<tr>
<th>Sample from indicated case or chimpanzee</th>
<th>IgG Pre</th>
<th>IgG Post</th>
<th>% IgG depletion</th>
<th>IgA Pre</th>
<th>IgA Post</th>
<th>% IgA depletion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma (mg/ml)*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Case 9</td>
<td>5.66</td>
<td>0.497</td>
<td>91</td>
<td>0.817</td>
<td>0.610</td>
<td>25</td>
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<tr>
<td>Case 7</td>
<td>10.2</td>
<td>1.73</td>
<td>83</td>
<td>2.11</td>
<td>1.39</td>
<td>34</td>
</tr>
<tr>
<td>Case 10</td>
<td>5.30</td>
<td>0.392</td>
<td>93</td>
<td>0.900</td>
<td>0.945</td>
<td>0</td>
</tr>
<tr>
<td>CPZ 1</td>
<td>31.3</td>
<td>18.8</td>
<td>40</td>
<td>4.56</td>
<td>3.75</td>
<td>18</td>
</tr>
<tr>
<td>CPZ 2</td>
<td>16.9</td>
<td>4.05</td>
<td>76</td>
<td>6.38</td>
<td>4.45</td>
<td>30</td>
</tr>
<tr>
<td>CPZ 4</td>
<td>14.3</td>
<td>1.40</td>
<td>90</td>
<td>3.00</td>
<td>3.15</td>
<td>0</td>
</tr>
<tr>
<td>Saliva (µg/ml)*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Case 6</td>
<td>0.560</td>
<td>0.004</td>
<td>99</td>
<td>153</td>
<td>118</td>
<td>23</td>
</tr>
<tr>
<td>Case 7</td>
<td>1.53</td>
<td>0.004</td>
<td>99</td>
<td>64.5</td>
<td>38.5</td>
<td>40</td>
</tr>
<tr>
<td>Case 10</td>
<td>2.16</td>
<td>0.040</td>
<td>98</td>
<td>23.3</td>
<td>12.1</td>
<td>48</td>
</tr>
<tr>
<td>CPZ 7</td>
<td>4.57</td>
<td>0.200</td>
<td>96</td>
<td>31.3</td>
<td>34.6</td>
<td>11</td>
</tr>
<tr>
<td>CPZ 5</td>
<td>5.25</td>
<td>0.311</td>
<td>94</td>
<td>62.2</td>
<td>50.3</td>
<td>19</td>
</tr>
<tr>
<td>CPZ 6</td>
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<td>0.294</td>
<td>96</td>
<td>94.9</td>
<td>84.1</td>
<td>11</td>
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</tbody>
</table>

* IgG and IgA levels before (Pre) and after (Post) removal of IgG.
though other IgA-specific responses were not tested in this study, it is likely that these individuals have intact IgA immunity to other mucosal pathogens. While poor HIV-specific IgA responses may be due to low-dose tolerance (7) or HIV-specific T-helper cell anergy (5), it remains unclear why virus-specific IgA responses are not induced. Since a lack of HIV-specific IgA antibodies in the mucosa may facilitate HIV replication in mucosal lymphoid tissues (30), a similar paucity of SFV-specific mucosal IgA antibodies may explain SFV shedding into saliva (2) and replication in the oral mucosa (6, 13). However, differences in plasma and mucosal viral loads could be more important determinants of virus transmission than the presence of SFV-specific antibodies, as is the case for HIV type 1 (20). Further, mechanisms of transmission in humans differ substantially from those of chimpanzees that involve biting, scratching, and fighting behaviors.

The results from this study provide an initial comparison of host mucosal humoral immunity and SFV-host interactions in humans and chimpanzees. Further study of SFV-specific host mucosal immunity is necessary to determine the contribution to viral persistence in the infected host and differences in transmission between chimpanzees and humans.

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REFERENCES


