Immunization with recombinant macaque major histocompatibility complex class I and II and human immunodeficiency virus gp140 inhibits simian–human immunodeficiency virus infection in macaques

Gui-Bo Yang,1 Yufei Wang,2 Kaboutar Babaahmady,2 Jørgen Schøller,3 Durdana Rahman,2 Evelien Bunnik,4 Ralf Spallek,5 Chun-Miao Zong,1 Jia-Zhong Duan,1 Chuan Qin,6 Hong Jiang,6 Mahavir Singh,5 Robert Vaughan,2 Lesley A. Bergmeier,7 Hanneke Schuitemaker,4 Yiming Shao1 and Thomas Lehner2

Correspondence
Thomas Lehner
thomas.lehner@kcl.ac.uk
Yiming Shao
yshao08@gmail.com

Genetic, epidemiological and experimental evidence suggest that the major histocompatibility complex (MHC) is critical in controlling human immunodeficiency virus (HIV) infection. The objectives of this study were to determine whether novel recombinant Mamu MHC constructs would elicit protection against rectal challenge with heterologous simian–human immunodeficiency virus (SHIV) strain SF162.P4 in rhesus macaques. Mamu class I and II gene products were linked together with HIV gp140, simian immunodeficiency virus (SIV) p27 and heat-shock protein 70 to dextran. The vaccine was administered to two groups, each consisting of nine macaques, either subcutaneously (SC), or rectally and boosted by SC immunization. The controls were untreated or adjuvant-treated animals. Repetitive rectal challenges with up to ten doses of SHIV SF162.P4 showed a significant decrease in the peak and sequential viral RNA concentrations, and three macaques remained uninfected, in the nine SC-immunized animals, compared with infection in all nine controls. Macaques immunized rectally followed by SC boosters showed a less significant decrease in both sequential and peak viral loads compared with the SC-immunized animals, and all were infected following rectal challenge with SHIV SF162.P4. Plasma and mucosal IgG and IgA antibodies to Mamu class I alleles and HIV gp120, as well as to RANTES (regulated upon activation, normal T-cell expressed, and secreted; CCR5) were increased, and showed significant inverse correlations with the peak viral load. These results suggested that allo-immunization with recombinant MHC constructs linked to HIV–SIV antigens merits further investigation in preventing HIV-1 infection.

INTRODUCTION

Xeno-immunization with inactivated simian immunodeficiency virus (SIV) grown in human CD4+ T-cells consistently prevents SIV infection in ~90% of rhesus
macaques (Arthur et al., 1995; Carlson et al., 1990; Desrosiers et al., 1989; Dormont et al., 1995; Murphey-Coir et al., 1989; Stott et al., 1994). However, attempts to reproduce protection against SIV by allo-immunization with inactivated SIV grown in macaque CD4+ T-cells have been met with limited success or failure (Polyanskaya et al., 1997; Stott et al., 1994). The critical significance of human leukocyte antigen (HLA) in the development and prevention of human immunodeficiency virus type 1 (HIV-1) infection has been widely documented. HIV and SIV replication are significantly related to major histocompatibility complex (MHC) class I alleles (Carrington & O’Brien, 2003; Goulder & Watkins, 2008). Indeed, HLA-B*57 and HLA-B*27 with HIV in humans and Mamu-B*08, Mamu-B*17 and Mamu-A*01 with SIV in macaques control virus replication and progress in the development of AIDS. In contrast, HLA-B*3502 and HLA-B*3503 are associated with rapid progression to AIDS. Genome-wide single-nucleotide polymorphisms have been identified in HLA (International HIV Controllers Study, 2010), with specific amino acids in the HLA-B peptide-binding groove that control HIV infection.

Epidemiological evidence suggests that transmission of HIV from mother to baby occurs more frequently among uniparous women (Kind et al., 1995), and mother-to-child HLA class I concordance increases pre-natal HIV-1 transmission (MacDonald et al., 1998). Slow progression is significantly associated with protective HLA-B alleles, especially when these are not shared between mother and child (Thobakgale et al., 2009). Furthermore, systemic allo-immunization of women has revealed that HIV replication ex vivo in CD4+ T-cells is inhibited, and this was correlated with a significant increase in CCR5 antibodies and CC chemokines and downmodulation of the CCR5 co-receptors (Leith et al., 2003; Wang et al., 1999, 2002). Unprotected heterosexual and homosexual intercourse also elicits allogeneic responses in both partners, which may contribute to protection against HIV infection (Kingsley et al., 2009; Peters et al., 2004). Allo-immunization as an AIDS vaccine was proposed early in vaccine development (Lehner et al., 2000; Shearer et al., 1993).

We have recently developed novel recombinant HLA class I and II proteins linked to dextran molecules (Schøller et al., 2010). These have been combined with trimeric HIV gp140, SIV Gag p27 and heat-shock protein (HSP) 70 on dextran backbones and mixed with TiterMax adjuvant to immunize rhesus macaques (Mörner et al., 2011). Intravenous challenge with a single large dose of simian-human immunodeficiency virus (SHIV) strain SF162.P4 resulted in a significant decrease in viral load or in prevention of infection.

The objectives of this study were: (i) to establish whether the allogeneic Mamu constructs were immunogenic in rhesus macaques; (ii) to establish whether protection could be induced by subcutaneous (SC) administration of the recombinant Mamu vaccine candidate and challenging macaques rectally with repeated doses of SHIV SF162.P4; and (iii) to attempt rectal mucosal-primed and SC booster immunization with the Mamu vaccine and to monitor the viral load following rectal challenge with SHIV SF162.P4. The results suggested that systemic allo-immunization with the Mamu vaccine candidate elicited significant protection against rectal challenge with SHIV SF162.P4.

RESULTS

Vaccine preparation and viral load in SC-immunized macaques

The purity of class I Mamu-A*01, trimeric HIV gp140 and the C-terminal fragment of HSP70 (HSP359-609) were determined by Western blotting (Fig. 1a–c). A diagrammatic illustration of the structure of the vaccine components bound to a dextran backbone is shown in Fig. 1(d), with the CD8 molecule of the CD8+ T-cell binding to Mamu class I, and T-cell receptors to the antigens of the construct. Systemic SC immunization (three times) with the Mamu vaccine candidate and adjuvant was followed by repeated doses (up to ten times) of rectal challenge with 25 TCID50 SHIV SF162.P4 (group 1; Fig. 1e). The sequential viral load [measured as log10 RNA equivalents (ml plasma)−1] following challenge with SHIV SF162.P4 showed a significant decrease of up to 3 logs in the immunized macaques (P<0.01 to P<0.0001) compared with the untreated controls (group 4, Fig. 2a). The corresponding data with control group 3a treated with HSP359-609+dextran+TiterMax showed a less significant decrease in viral load (P<0.05) (Fig. 2b). The vaccine prevented SHIV infection in three out of nine macaques, and a significant decrease in the peak viral load after infection (2.8±0.7) in the immunized compared with the untreated (3.96±0.22, P=0.048; group 4, Fig. 2a) or adjuvant-treated (4.22±0.3, P=0.046; group 3a, Fig. 2b) controls. However, analysis of the six infected animals (3.2±0.4) in comparison with control group 3a (4.2±0.3) showed a decrease of 1 log but failed to reach the 5% level of significance, probably because of the small number of animals (n=6 and n=4, respectively). The peak viral load in the protected group 1 macaques at week 2 reached the same level as the untreated controls (group 4), but the viral load in the latter group increased by another log by week 3, whereas, at the same time, viral load in the immunized animals decreased by 1 log (Fig. 2a). This was not observed with the treated controls (group 3a), which reached the peak value at the same time as the immunized group 1 but with a 1 log higher viral load (Fig. 2b). The sequential viral loads for each animal in the three groups are shown in Fig. 2c–e. Thus, it appeared that treatment with dextran + HSP70 + TiterMax exerted a limited non-specific protection against SHIV SF162.P4 compared with the untreated controls.
Viral load in rectally immunized animals boosted by SC immunization

Rectal mucosal immunization was carried out with the Mamu vaccine (twice) but exchanging TiterMax for the CpG-C mucosal adjuvant, followed by SC immunization (twice) with the vaccine + TiterMax. After challenge (up to ten times) rectally with SHIV SF162.P4, all immunized and untreated control macaques became infected (Fig. 3c). However, a significant decrease in the sequential viral load (up to 2 logs) was observed between the immunized and untreated controls ($P < 0.05$ to $P < 0.001$; Fig. 3a). The peak viral load was also significantly lower in the immunized than in the untreated animals ($2.91 \pm 0.2$ vs $3.9 \pm 0.22$; $P = 0.011$), but this was not found in the adjuvant-treated control animals (Fig. 3a, b). Surprisingly, in the five control macaques in group 3b treated with CpG-C + dextran + HSP70 (twice) followed by TiterMax + dextran + HSP70 (twice), one animal was uninfected and no difference in sequential or peak viral load was recorded (Fig. 3c, d). Hence, rectal mucosal followed by SC immunization resulted in a significant decrease in viral load but none of the animals was free of the viral infection, and four treatments with HSP70, dextran, CpG-C or TiterMax elicited some non-specific protection.

Acquisition of infection

The number of rectal challenges with 25 TCID$_{50}$ SHIV SF162.P4 required to infect the SC-immunized and two
Allo-immunization and inhibition of SHIV infection

control groups of macaques is presented in Table S1 (available in JGV Online). The first three challenges failed to show a significant difference between the immunized (three out of nine) and the combined controls (one out of nine; \( P=0.271 \)), but they were higher in group 1 than in the combined controls. However, the next three challenges (immunizations 4–6) resulted in a log-rank combined controls. However, the next three challenges failed to show a significant difference between the immunized (one out of nine; \( P=0.0095 \)). All nine controls were infected but only five of the nine immunized animals were infected after six challenges; one of the remaining four uninfected animals became infected by week 7, leaving three uninfected animals out of nine by the end of the experiment at week 14. We have not excluded the possibility that the rectal and related lymphoid tissue may have harboured the virus.

Plasma IgG and IgA antibodies to Mamu class I antigens and HIV gp120

All antibodies detected were from the plasma or mucosal fluids collected just before immunization and 4 weeks after the last immunization, just before the animals were first challenged rectally with SHIV SF162.P4. Antibodies to the recombinant Mamu-A*01 and Mamu-A*08 were assayed by ELISA and analysed by analysis of variance (ANOVA) with the Bonferroni multiple comparison test. Antibodies to Mamu-A*01 in the two immunized and two adjuvant-treated control groups were highly significantly different \((F=28.55, P<0.0001 \) or \( P=0.0002 \); Fig. 4a, b). The SC-immunized animals (group 1) showed significantly higher IgG antibody levels [measured as area under the curve (AUC): \( 12.3 \pm 1.2 \)] than the rectal/SC-immunized (group 2, \( 5.9 \pm 0.62 \); \( P<0.05 \)) (Fig. 4a), whilst the control group 3a and untreated controls showed no change in antibody levels. Rectal/SC-immunized (group 2) animals (\( 5.9 \pm 0.62 \)) also showed significantly higher serum IgG antibody levels than the control group 3b (\( 1.34 \pm 0.2 \)). The results of antibodies to Mamu-A*08 were similar to those of Mamu-A*01, except that no significant difference was found between groups 1 and 2 (Fig. 4c). The pre-immunization absorbance values were negligible. A similar analysis of IgA antibodies also showed highly significant ANOVA results for both Mamu-A*01 (\( F=10.03, P=0.0002 \)) and Mamu-A*08 (\( F=17.6, P<0.0001 \)), with higher antibody levels in the immunized groups compared with the corresponding control groups \((P<0.05 \) Fig. 4b, d), but there was a negligible difference between groups 1 and 2 to Mamu-A*01 (Fig. 4b). As with Mamu, IgG and IgA antibodies to HIV (strain YU2) gp120 showed a significant ANOVA result \((P<0.0001 \) and higher IgG and IgA antibody levels were found in group 1 than in group 2 immunized macaques (Fig. 4e, f). Altogether, these data suggested that rectal immunization followed by SC immunization was less effective than SC immunization alone in eliciting IgG and IgA antibodies to the allo-antigens and to HIV gp140. Antibodies to SIV p27 were elicited but failed to show significant differences in antibody levels between the
groups and were not analysed further (data not shown). There was no obvious difference in IgG or IgA titre to the allo-antigens or to HIV gp120 between the three protected and six infected macaques in group 1.

Rectal and vaginal IgG and IgA antibodies to Mamu class I antigens and HIV gp120

An ELISA of rectal washings showed modest increases in IgG and IgA antibodies to Mamu-A*01 (Fig. 5a, b) and HIV gp120 (Fig. 5c, d), but these were significant only for IgA antibodies to Mamu-A*01 (Fig. 5b). However, vaginal IgG and IgA antibodies to both Mamu-A*01 (Fig. 5e, f; P = 0.01 and P < 0.05, respectively) and HIV gp120 (Fig. 5g, h; both P < 0.001) were significantly raised in SC-immunized (group 1) animals compared with the group 3a controls. In the rectal/SC-immunized (group 2) animals, a significant increase in antibodies was not found.

Plasma and mucosal fluid CC chemokines

Plasma and mucosal fluid CC chemokines were assayed before immunization and 4 weeks after the last immunization and before the first rectal challenge. Three CC chemokines were assayed first in plasma, which showed significant upregulation of regulated upon activation, normal T-cell expressed, and secreted (RANTES; P < 0.05) and macrophage inflammatory protein-1 (MIP-1α; P < 0.05 or P ≤ 0.01) after immunization in groups 1 and 2, but, with the exception of group 3b, in none of the controls (Table 1a, b). Only RANTES was significantly increased in the rectal and vaginal fluid (P < 0.01 and P < 0.05, respectively) (Table 1c, d) in group 1 but not in group 2, although group 3b showed a significant increase in RANTES and MIP-1α. Overall, RANTES was significantly upregulated in all three fluids of the protected group 1 animals but only in the plasma of group 2 animals, showing limited protection. MIP-1α was also increased only in the plasma of both immunized groups of animals, and MIP-1β failed to show a significant increase in any of the animals or fluids.

Correlation between peak viral load and plasma IgG or IgA antibodies to the allo-antigens and HIV gp120

SC immunization elicited a high inverse correlation coefficient (r) between the peak viral load and antibodies to the Mamu-A*01 and Mamu-A*08 allo-antigens (Fig. 6a–d), but only IgG antibodies reached significance (Fig. 6a,
b). Rectal/SC immunization (group 2) also showed high r values with IgG antibodies (−0.48 and −0.4, respectively), but only IgA antibodies to Mamu-A*01 and the peak viral load were significantly different (data not presented). However, the peak viral load failed to show an inverse correlation with plasma anti-HIV gp140 IgG or IgA antibodies after SC or rectal/SC immunization (data not presented).

Correlation between peak viral load and rectal or vaginal IgG and IgA antibodies to the allo-antigens and HIV gp120

SC immunization failed to show any correlation between peak viral load and rectal IgG antibodies to Mamu-A*01 or HIV gp120, unlike the strong trend of inverse correlation with the corresponding IgA antibodies (data not presented). However, vaginal IgG and IgA antibodies to Mamu-A*01 (P=0.01 and P=0.002, respectively; Fig. 6e, g) and HIV gp120 (P=0.003; Fig. 6h) showed a significant inverse correlation with the peak viral load after SC immunization, and a strong trend with IgG to HIV gp120 (P=0.078; Fig. 6f). Rectal/SC immunization (group 2) failed to show any correlation between peak viral load and either IgG or IgA to Mamu-A*01 or HIV gp120 in the two mucosal fluids (data not shown).

Correlation between CC chemokines and viral load

Significant inverse correlations were found between peak viral load and plasma MIP-1α levels in groups 1 (P=0.01) and 2 (P<0.05) (Fig. 7c, d), but not with RANTES (Fig. 7a, b) or with MIP-1β (results not shown). Vaginal fluid showed a significant inverse correlation between peak viral load and RANTES (P=0.003) only in group 1 (Fig. 7e) and not with MIP-1α. Rectal fluid failed to show a significant correlation (data not presented). These results are consistent with RANTES predominately blocking the CCR5 coreceptors in the vaginal mucosa and plasma, preventing HIV-1 entry into CD4+ T-cells in the SC-immunized animals.
Neutralizing activity

Complement-dependent and -independent neutralizing activity was tested by a T2M-bl-based assay. Neutralizing activity was not detected in control animals after the last immunization. Although a low-titre, complement-independent, neutralizing activity greater than the IC₅₀ using SHIV SF162.P4 grown in rhesus PBMCs (passaged in a macaque expressing Mamu-A*01 and DRB*W1/W2), was found in four out of nine group 1 immunized animals after the last immunization, this was also seen in the pre-immunized animals (data not presented) and was not considered to be significant.

Mamu alleles

Mamu class I and II alleles were determined, and the protective Mamu class I lineage Mamu-A*01 or Mamu-A*08 was not found in these Chinese macaques (Goulder & Watkins, 2008). However, seven animals showed Mamu-B*17, of which two were in the immunized group 1, three in the adjuvant-treated group 3a and one in the untreated group 4. Of the three immunized and completely protected animals in group 1, only one expressed Mamu-B*17, whilst another was infected. Surprisingly, three out of four of the adjuvant-treated group 3a animals expressed Mamu-B*17 and, although they were all infected, the viral load was lower than that in the untreated animals, consistent with the Mamu-B*17 allele having some protective effect. Mamu-B*17 was also found in one of the group 2 animals and one in group 3b adjuvant-treated control, but none was protected. All five macaques in the untreated group, one of which expressed Mamu-B*17, were infected and the viral load was not decreased. As the animals expressed neither Mamu-A*01 nor Mamu-A*08, it is unlikely that self-tolerance had been broken by immunization with the Mamu-A*01 and Mamu-A*08 antigens.

DISCUSSION

Following protection or inhibition of SHIV infection elicited by xeno-immunization with a recombinant HLA class I and II HIV–SIV antigens and HSP70 dextramer

<table>
<thead>
<tr>
<th>Group</th>
<th>Mamu-B*17</th>
<th>Protected</th>
</tr>
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<tbody>
<tr>
<td>Group 1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Group 3a</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Group 3b</td>
<td>1</td>
<td>0</td>
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<tr>
<td>Group 4</td>
<td>0</td>
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**Fig. 5.** Comparative rectal (a–d) and vaginal (e–h) IgG and IgA anti-Mamu-A*01 (a, b, e and f) and anti-HIV gp120 (c, d, g and h) antibody levels in macaques SC immunized with the vaccine (group 1) or rectal/SC-immunized (group 2) using CpG-C in the mucosal and TiterMax in the SC immunizations. Control group 3a animals were treated with HSP₃₅₉–₆₀₉ + dextran + TiterMax and group 3b animals were treated rectally using CpG-C instead of TiterMax and SC boosted with the adjuvant used in group 3a. The results are expressed as means (±SEM) of the AUC of absorbance after the last immunization, 1 or 2 days before challenge with SHIV SF162.P4.
Table 1. Analysis of RANTES, MIP-1α and MIP-1β in plasma and in rectal and vaginal fluid in immunized and control macaques

The concentrations of RANTES, MIP-1α and MIP-1β in plasma (a, b) and in rectal (c) and vaginal (d) fluid of SC-immunized group 1, its control (group 3a), rectal/SC-immunized group 2, its control (group 3b) and the untreated control (group 4). Fluids were collected before and after the last immunization. Concentrations were determined before (Pre) and after (Post) immunization. NS, Not significant.

(a) Plasma

<table>
<thead>
<tr>
<th></th>
<th>RANTES (ng ml⁻¹)</th>
<th>MIP1-α (pg ml⁻¹)</th>
<th>MIP-1β (pg ml⁻¹)</th>
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<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
<td>Pre</td>
</tr>
<tr>
<td>Group 1</td>
<td>18.5±2</td>
<td>24.5±1.3*</td>
<td>83.5±11.7</td>
</tr>
<tr>
<td>Group 2</td>
<td>16.5±2</td>
<td>21.3±1.1*</td>
<td>89.9±12.5</td>
</tr>
<tr>
<td>Group 3a</td>
<td>18.9±2.1</td>
<td>13.2±3.6</td>
<td>92.3±12.1</td>
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<tr>
<td>Group 3b</td>
<td>15.8±1.2</td>
<td>21±1.5†</td>
<td>63.8±11.9</td>
</tr>
<tr>
<td>Group 4</td>
<td>11.9±1.3</td>
<td>12.7±3.2</td>
<td>67.4±10.4</td>
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</table>

(b) ANOVA of the post-immunization groups followed by the Bonferroni’s multiple comparison test

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<tr>
<th></th>
<th>RANTES</th>
<th>MIP-1α</th>
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<tbody>
<tr>
<td></td>
<td>F=</td>
<td>7.254</td>
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<tr>
<td></td>
<td>P=</td>
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<th>P</th>
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<tr>
<td>Group 1 vs 3a</td>
<td>3.954</td>
<td>&lt;0.01</td>
<td>2.545</td>
<td>NS</td>
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<tr>
<td>Group 1 vs 4</td>
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<td>&lt;0.001</td>
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<td>0.1223</td>
<td>NS</td>
<td>0.2238</td>
<td>NS</td>
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<td>Group 2 vs 4</td>
<td>3.258</td>
<td>&lt;0.05</td>
<td>0.2238</td>
<td>NS</td>
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(c) Rectal fluid

<table>
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<tr>
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<th>MIP-1α (pg ml⁻¹)</th>
<th>MIP-1β (pg ml⁻¹)</th>
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<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
<td>Pre</td>
</tr>
<tr>
<td>Group 1</td>
<td>38±3.6</td>
<td>56.1±3.1†</td>
<td>12.1±1.5</td>
</tr>
<tr>
<td>Group 2</td>
<td>39.8±8.4</td>
<td>54.7±3.1</td>
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<tr>
<td>Group 3a</td>
<td>40±5.9</td>
<td>42.5±8.1</td>
<td>9.3±4</td>
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<tr>
<td>Group 3b</td>
<td>36.2±10.5</td>
<td>40.4±6.8</td>
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<tr>
<td>Group 4</td>
<td>51.6±4.1</td>
<td>48.8±3.1</td>
<td>11.2±2.4</td>
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(d) Vaginal fluid

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<th>MIP-1α (pg ml⁻¹)</th>
<th>MIP-1β (pg ml⁻¹)</th>
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<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
<td>Pre</td>
</tr>
<tr>
<td>Group 1</td>
<td>15.2±3.2</td>
<td>46.1±12.6*</td>
<td>11.6±2.6</td>
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<tr>
<td>Group 2</td>
<td>15.4±2.9</td>
<td>20.1±3.7</td>
<td>9.5±4.3</td>
</tr>
<tr>
<td>Group 3a</td>
<td>21±5.3</td>
<td>15±1.1</td>
<td>9.2±2.2</td>
</tr>
<tr>
<td>Group 3b</td>
<td>18.3±3.2</td>
<td>26.1±3.3*</td>
<td>17.4±4.4</td>
</tr>
<tr>
<td>Group 4</td>
<td>14.7±4.4</td>
<td>12.9±2</td>
<td>13.3±2.7</td>
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*P<0.05, compared with pre-immunized samples.
†P≤0.01, compared with pre-immunized samples.

Mamu constructs administered SC (three times) either prevented SHIV infection in three of nine macaques or significantly decreased sequential and peak viral loads in the animals when challenged rectally by up to ten repeated doses of heterologous SHIV SF162.P4. However, rectal mucosal immunization followed by SC immunization failed to prevent SHIV SF162.P4 transmission, although the peak viral load was significantly decreased, and one of
the five adjuvant-treated controls remained uninfected. The lack of complete protection might be ascribed to the significantly lower serum IgG antibody levels to the Mamu class I and HIV gp120 antigens compared with systemic immunization alone. This was less evident with serum IgA and was not evident with rectal IgG or IgA antibodies. Mucosal allo-immunization has been attempted previously only in one macaque study, applying unmatched mono-nuclear cells to rectal or vaginal mucosa (Bergmeier et al., 2005). A significant decrease in SIV infectivity was elicited with CD4⁺ T-cells ex vivo, but the animals were not challenged in vivo.

It is unlikely that protection and/or a decrease in viral load would have been achieved without either HLA class I and II constructs or HIV and SIV antigens, as HLA xeno-immunization has clearly established that both HLA and HIV/SIV constructs are critical in eliciting protection against SHIV SF162.P4 infection (Mörner et al., 2011). Indeed, no difference from the controls was observed when a group of eight macaques was immunized with the vaccine without the HLA constructs or when another group of eight macaques was immunized without HIV and SIV antigens (Mörner et al., 2011). It is also unlikely that the recombinant HLA constructs exerted significant adjuvant activity, as another control group of six macaques with all vaccine constituents (as in the protected group 1) but without the TiterMax adjuvant showed no protection and poor immune responses. In the early experiments, most macaques were protected from SIV infection if they were immunized with inactivated SIV grown in human CD4⁺ T-cell line and challenged with SIV grown in the same human CD4⁺ T-cell line (Arthur et al., 1995; Carlson et al., 1990; Desrosiers et al., 1989; Dormont et al., 1995; Murphey-Corb et al., 1989; Stott et al., 1994). However, in the more limited studies, protection was greatly decreased if the animals were immunized with human CD4⁺ T-cells alone (Langlois et al., 1992; Stott, 1991). It is noteworthy that, although the control animals (group 3a) treated with HSP₃₅₉₋₆₀₉ + dextran + TiterMax showed a peak viral load significantly higher than that in the immunized animals, the sequential viral load was less significant than that recorded in the untreated group 4 animals. This is likely to be a non-specific adjuvant effect exerted by the three agents, which has often been observed with other adjuvants. Importantly, none of the systemically treated controls showed complete prevention of SHIV infection, unlike three of the nine immunized animals.

The possibility that serum IgG and mucosal IgA antibodies to the viral construct were correlates of protection is greatly enhanced by the significant inverse correlation between peak viral load and serum or mucosal (vaginal) IgG and to a lesser extent by IgA antibodies. However,
Among the CC chemokines, only RANTES (CCL-5) was significantly increased in plasma and in rectal and vaginal fluid in the SC-immunized but not in the rectal/SC-immunized animals. Furthermore, a significant inverse correlation between the peak viral load and RANTES was found only in the vaginal fluid of the SC-immunized animals. MIP-1α showed a significant increase only in plasma and significant inverse correlations with the peak viral load in both group 1 and 2 animals. It is therefore likely that only RANTES enhanced the protection elicited by IgG and IgA specific antibodies.

In conclusion, we have presented evidence that only systemic allo-immunization with recombinant Mamu class I and II constructs with HIV/SIV antigens elicits significant protection when challenged with heterologous SHIV SF162.P4, either preventing infection or decreasing the viral load by up to 3 logs. The potential disadvantage of allo-immunization is that it induces allo-antibodies, which might cause allograft rejection, if one were required. However, in HIV-1-endemic regions, the risk-to-benefit ratio of potential allograft failure argues overwhelmingly in favour of allo-immunization. Systemic allo-immunization may prove to be an alternative vaccine strategy, especially as with CC chemokines, suggesting that additional immune factors may have been involved, such as innate antiviral factors or cellular immunity.
as it is independent of HIV mutation and cytotoxic T-lymphocyte or antibody escape. This is the first demonstration that systemic allos-immunization with a recombinant MHC class I and II, HIV gp140 and SIV p27 complex elicits significant inhibition of heterologous SHIV infection in macaques.

METHODS

Selection and preparation of recombinant Mamu alleles. Three Mamu class I and one class II alleles were selected on the basis of the frequency of these alleles found in Chinese rhesus macaques and the appropriate Mamu allele/peptide combinations for efficient folding: (i) Mamu-A*01 (CTPYDINQM), (ii) Mamu-A*08 (KPCVKLTP) and (iii) Mamu-B*17 (IRFPKTFGW). Preparation of these Mamu class I heavy chains and \(\beta_2\)-microglobulin has been described previously (Scholler et al., 2010). The Mamu class II protein (DRB*W602) was purchased from Dr W. Kwok (Benaroya Research Institute, Seattle, WA, USA).

Preparation of trimeric HIV gp140, SIV p27 and HSP\(_{359-609}\). Monobiotinylated HIV strain YU2 gp140 trimer and SIV Gag p27 were prepared as described previously (Mörner et al., 2011). The 28 kDa C-terminal fragment of Mycobacterium tuberculosis HSP70 (HSP\(_{359-409}\)) (Babahmady et al., 2007) was subcloned to enable in vitro biotinylation, as described elsewhere (Mörner et al., 2011). All biotinylated vaccine components were linked to streptavidin-coated divinyl sulfone-activated dextran (Scholler et al., 2010) using concentrations described previously (Mörner et al., 2011).

Animals and immunization and SHIV challenge. Thirty-two rhesus macaques of Chinese origin, serologically negative for SIV, simian retrovirus and simian T-cell leukemia virus, were treated according to the guidelines set out by the Institutional Animal Care and Use Committee at the Institute of Laboratory Animal Science, Chinese Academy of Medical Sciences. Immunized groups of nine macaques each were given the vaccine candidate formulated as an emulsion with TiterMax Gold (Sigma-Aldrich) or CpG-C (Invivogen) and this was carried out up to ten times, after which the appropriate Mamu allele/peptide combinations for efficient folding: (i) Mamu-A*01-DRB*W1/W2 PBMCs. We also thank Drs Gunnel Bethell, Rigmor Thortensson, Andreas Mörner and Marianne Janson of the Karolinska Institute, Stockholm, Sweden, and Dr Richard T. Wyatt of the IAVI Neutralizing Antibody Center at The Scripps Research Institute, La Jolla, CA, USA, for their helpful advice during discussions of the overall project, Dr L. James of the Medical Research Council Laboratory of Molecular Biology, Cambridge, UK, for his advice concerning the mechanism of protection and Mrs Kathy Doyle for her efficient management of the project. The investigation was supported by the Bill and Melinda Gates Foundation grant no. 38608, as part of the Collaboration for AIDS Vaccine Discovery (CAVD) and partly supported by National MEGA Project for Infectious Diseases Control (2008ZX10001-010) to Y.S., a Natural Science Foundation of China (NSFC) grant (no. 30872324) to Y.G.B. and the EU Network of Excellence 'Europrise' (LSHP-CT-2006-307611).

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