Protection in Simian Immunodeficiency Virus-vaccinated Monkeys Correlates with Anti-HLA Class I Antibody Response

By Woon Ling Chan, Angela Rodgers, Robert D. Hancock, Frank Taffs, Peter Kitchin, Graham Farrar,* and F. Y. Liew†

From the National Institutes for Biological Standards and Control, South Mimms, Potters Bar, Hertfordshire EN6 3QG; the *Division of Pathology, Public Health Laboratory Services Centre for Applied Microbiological Research, Porton Down, Salisbury SP4 0JG; and the †Department of Immunology, Western Infirmary, University of Glasgow, Glasgow G11 6NT, United Kingdom

Summary

Our earlier reports demonstrated that Cynomolgus macaques vaccinated with either inactivated partially purified simian immunodeficiency virus (SIV), fixed SIV-infected C8166 (a human T lymphoblastoid cell line) cells, or even fixed uninfected C8166 cells can be protected against a challenge infection with the 32H isolate of SIVmac 251 (grown in C8166) (Stott, E. J., W. L. Chan, K. H. G. Mills, M. Page, F. Taffs, M. Cranage, P. Greenway, and P. Kitchin. 1990. Lancet. 336:1538; Stott, E. J., P. A. Kitchin, M. Page, B. Flanagan, L. F. Taffs, W. L. Chan, K. H. G. Mills, P. Silvera, and A. Rodgers. 1991. Nature [Lond.]. 353:393). Protection is correlated with the levels of antibody response to cellular antigens in the human cells from which the virus immunogen was grown. However, the mechanism of protection is unclear. We report here the analysis of sera from these protected monkeys and demonstrate that there is a positive correlation of protection with antibody response to the HLA class I molecule.

Macques infected with the simian immunodeficiency virus (SIV) develop a disease similar to that produced by HIV in humans. This simian model is therefore widely used in Europe and the United States for the development of vaccines against AIDS (1-3). Recently, we demonstrated that Cynomolgus macaques vaccinated with either inactivated partially purified SIV, fixed SIV-infected C8166 cells, or even fixed uninfected C8166 cells can be protected against a challenge infection with the 32H isolate of SIVmac 251 grown in C8166 cells (4–6). Protection is correlated with the levels of antibody response to cellular antigens in the human cells (5, 7–10). In the present study, we demonstrate that protection is directly correlated with antibody response to the HLA class I molecules. These results suggest that antibodies against HLA class I molecule and/or other yet undetected antigen(s) may be useful in immunotherapy against HIV infection.

Materials and Methods

Animals. Cynomolgus monkeys (Macaca fascicularis) were maintained in accordance with Guidelines for the Housing and Care of Laboratory Animals Used in Scientific Procedures (1989; Home Office, UK). They were vaccinated as described previously (4, 5). Monkeys 1179–182 were injected subcutaneously four times with 500 μg formalin-fixed SIVmac 251 (32H isolate; 11/88 pool) + SAF-1 adjuvant; monkeys J134–137 were injected subcutaneously three times with 500 μg formalin-fixed SIVmac 251 + RJBI adjuvant; monkeys J138–141 were injected subcutaneously four times with 100 μg formalin-fixed SIVmac 251 + RJBI; monkeys J127–220 and J68–71 were injected subcutaneously with two doses, respectively, of 2 × 10^6 SIVmac 251-infected C8166 cells fixed with glutaraldehyde + Quil-A adjuvant; and monkeys J72–75 were injected subcutaneously with two doses of 2 × 10^6 C8166 cells fixed with glutaraldehyde + Quil-A adjuvant. All the animals were challenged intravenously with 10 50% monkey infectious doses (MIDs) SIVmac 32H isolate, 11/88 pool (grown in C8166 cells) 1 wk after the final boost. Protection was determined using the previously described method of PCR for SIVmac proviral DNA with gag, pol as primers (11), as well as by virus isolation using direct cocultivation of monkey PBMC with C8166 cells. Virus was detected by the appearance of cytopathic effects (CPE) and confirmed by immunofluorescence of virus antigen on infected cells using standard methods. Cultures that did not show CPE were maintained for at least 28 d before being discarded as negative.

Immune Precipitation. Actively dividing C8166 cells (20–30 × 10^6) were labeled for 6 h with 0.5 mCi [35S]methionine in methionine-free RPMI containing 10% FCS, lyzed, and radiimmune precipitated as previously described (12). The washed immune complexes were reduced and subjected to electrophoresis in a 12.5% acrylamide gel. Gels were treated with Amplify (Amersham Corp., Arlington Heights, IL), dried, and exposed to Kodak X-Omat RP film at -70°C.

Flow Cytometry Analysis. 100 μl of a suspension of 10^6 P815 or P815 cells transfected with human HLA class I molecules B27...
in RPMI 1640 containing 10% FCS was incubated with 100 μl of serial fourfold dilutions (1/30–7,680) of monkey plasma (from day of challenge or prebleed as control) containing 0.1% sodium azide for 30 min at 4°C. The cells were washed thrice in RPMI with 10% FCS and sodium azide before a further 30-min incubation with 100 μl of 1/100 of rabbit antibody to human Ig conjugated to FITC (Dako Corp., Santa Barbara, CA). The cells were washed as before and resuspended in PBS containing 1% formaldehyde. The percentage and peak channel fluorescence were analyzed on a FACStar® Consort 30 (Becton Dickinson & Co., Mountain View, CA). The end-point titer was taken as the dilution where ≥20% of the cells were positive.

ELISA. The 32H cognate isolate of SIVmac 251 and the HIV-1 isolate, GB8, were grown in C8166 cells and partially purified by gel exclusion chromatography to minimize loss of envelope glycoprotein (13). Both virus preparations were inactivated with formalin and dialyzed into PBS before use. For the ELISA, 50 μl of SIVmac (2 μg/ml) or HIV-1 (16 μg/ml) diluted in 0.1 M carbonate buffer, pH 9.6, was added to each well of a 96-well microtiter plate (Maxi Sorb; Nunc, Roskilde, Denmark) and all subsequent steps were carried out as previously described (14). mAbs and rabbit antibody to human Ig conjugated to horse radish peroxidase (1:100; Dako Corp.) were diluted in PBS containing 0.05% Tween 20 and 10% heat-inactivated newborn calf serum (HI NCS). Dilutions of mAbs (50 μl) used were 1:100 for ascites fluid, 20–50 μg/ml for purified Ig, and neat spent tissue culture supernatant. All washes were with PBS containing 0.05% Tween 20. The substrate used was 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (Sigma Chemical Co., St. Louis, MO) and the OD was measured at 405 nm. The mAbs used were obtained from the AIDS Directed Programme, Medical Research Council, UK (ADP373, ADP317, ADP318, ADP336, ADP351, ADP356, ADP359); the American Type Culture Collection, Rockville, MD (W6/32, L203, L227, L243, OKT3); Professor J. Lamb, St. Mary's Hospital, London and Bristol-Myers Squibb, Seattle, WA (9.3); and OKTll and nm31 were kind gifts from Dr. D. Cantreil, Imperial Cancer Research Fund, Lincoln's Inn Field, London, and Prof. A. McMichael, Institute for Molecular Medicine, Oxford, respectively. All the mAbs used have been checked for activity using flow cytometry (data not shown).

**Results and Discussion**

Radioimmune precipitation of [35S]methionine-labeled C8166 cell lysate with the monkey sera demonstrated that all the sera from protected but not from unprotected monkeys recognize two major protein bands at 12 and 44 kD (Fig. 1). These bands were precipitated by sera from all the protected animals in the vaccine groups studied but not by their preimmune sera. Blocking experiments were carried out with [35S]methionine-labeled C8166 cell lysate by precipitation with serum from protected monkeys followed by precipitation with mAb W6/32 (specific for a monomorphic determinant on human MHC class I molecule HLA-A, -B, -C). Alternatively, the lysate was precipitated with rabbit anti-β2m (the β chain of HLA class I molecule) followed by precipitation with serum from protected monkeys. Results shown in Fig. 2 demonstrate that the 12- and the 44-kD bands are β2m and the heavy (α) chain of HLA class I molecule, respectively.

The intensity and mass of the bands around the 44-kD region precipitated by sera from some protected monkeys (Fig. 1) suggest that other T cell surface proteins with similar molecular masses, such as CD28 (44 kD), CD2 (T11, 50 kD), and actin (44 kD), may also be recognized. However, the sera from protected monkeys vaccinated with purified SIVmac 251 virus do not contain anti-CD2 (Fig. 2 b) or anti-CD28 antibodies (data not shown). The sera from protected monkeys did not precipitate the α chain or the β chain of the class I molecule of [35S]methionine-labeled, *Herpes papio*-transformed monkey B lymphoblastoid cell lines (Fig. 2 c), suggesting that the anti-human class I antibodies in the sera are directed at polymorphic regions of the human HLA class I
Figure 2. (a) In lanes 1181 + W6/32 and anti-β2m + 1182, the radiolabeled lysate was preprecipitated twice with plasma from 1181 or anti-β2m before subsequent precipitation with mAb W6/32 or 1182 plasma, respectively. (b) Similarly, in lane 1181 + OKT11, precipitation with mAb OKT11 was subsequent to that with 1181 plasma. Lane 1181 PB denotes preimmune plasma. (c) [35S]Methionine-labeled cell lysates of a H. papio-transformed monkey B lymphoblastoid cell line were immune precipitated with 25 μl prebleed or hyperimmune plasma from protected monkey 1181 and 2 μl (ascites fluid) of mAbs W6/32 or L243 (specific for nonpolymorphic determinants of human class II molecule HLA-DR). Similar results were obtained with sera from other protected monkeys.

Table 1. Detection of Cellular Antigens on SIV and HIV-1 Virions by ELISA

<table>
<thead>
<tr>
<th>Antibody</th>
<th>antigen recognized</th>
<th>OD reading*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>SIV</td>
</tr>
<tr>
<td>None</td>
<td></td>
<td>0.06 ± 0.007</td>
</tr>
<tr>
<td>ADP373 IgG2a</td>
<td>SIV env gp160</td>
<td>0.344 ± 0.025</td>
</tr>
<tr>
<td>ADP317 IgG3</td>
<td>HIV-1 env gp160</td>
<td>ND</td>
</tr>
<tr>
<td>W6/32 IgG2a</td>
<td>HLA-A,-B,-C</td>
<td>0.333 ± 0.038</td>
</tr>
<tr>
<td>OKT3 IgG2a</td>
<td>CD3</td>
<td>0.314 ± 0.06</td>
</tr>
<tr>
<td>L203 IgG1</td>
<td>HLA class II</td>
<td>0.017 ± 0.001</td>
</tr>
<tr>
<td>L227 IgG1</td>
<td>HLA class II</td>
<td>0.017 ± 0.001</td>
</tr>
<tr>
<td>L243 IgG2a</td>
<td>HLA class II</td>
<td>0.05 ± 0.009</td>
</tr>
<tr>
<td>nm31 IgG</td>
<td>HLA class II</td>
<td>0.036 ± 0.004</td>
</tr>
<tr>
<td>OKT11 IgG2a</td>
<td>CD2</td>
<td>0.043 ± 0.009</td>
</tr>
<tr>
<td>9.3 IgG2a</td>
<td>CD28</td>
<td>0.03 ± 0.002</td>
</tr>
<tr>
<td>ADP318 IgG1</td>
<td>CD4</td>
<td>0.014 ± 0.002</td>
</tr>
<tr>
<td>ADP336 IgG2a</td>
<td>CD4</td>
<td>0.058 ± 0.006</td>
</tr>
<tr>
<td>ADP351 IgG2a</td>
<td>CD4</td>
<td>0.025 ± 0.003</td>
</tr>
<tr>
<td>ADP356 IgG2b</td>
<td>CD4</td>
<td>0.036 ± 0.002</td>
</tr>
<tr>
<td>ADP359 IgG</td>
<td>CD4</td>
<td>0.016 ± 0.001</td>
</tr>
</tbody>
</table>

* Values (mean ± 1 SEM, n = 6) that were five times higher than the negative control reading were considered as positive (in bold type).
p < 0.0005.

TCR complex, but little or no detectable CD4, CD2, or
protected monkeys recognized a routine cell line (P815) trans-
molecule. Flow cytometry analysis also shows that sera from
virus preparation may contain HLA class I antigen. The ELISA
titer of protected monkeys was log10 3.1 ± 0.13 (n = 32),
and of the unprotected group was 1.86 ± 0.09 (n = 11),
p < 0.0005.

The high levels of anti-human HLA class I antibody in-
duced in protected monkeys vaccinated with purified SIVmac
251 virus (grown in C8166 cells) suggest that the purified
virus preparation may contain HLA class I antigen. The ELISA
results (Table 1) confirm that the partially purified SIVmac
251 virus preparation used for immunization, and an HIV
vaccine preparation (GB8), contain an HLA class I molecule
and, additionally, CD3, a T cell antigen–forming part of the
TCR complex, but little or no detectable CD4, CD2, or
CD28 antigens. However, sera from protected monkeys were
not able to precipitate any bands depicting the CD3 γ, δ,
ε, ζ, and η chains (26, 20, and 16 kD) (Fig. 1), indicating
that CD3 was poorly immunogenic in these monkeys and
that the level of anti-CD3, if present, is below the limit of
our assay system. In contrast to a previous report (15), we
failed to detect class II antigens in our SIV preparation. This
may be due to the different procedures used in purifying the
viruses.

Our results demonstrate a direct correlation between anti-
body response to human HLA class I antigen and protection
of monkeys from SIVmac 251 (grown in C8166 cells) infec-
tion. The mechanism of the possible protection by this anti-
body is at present unclear. It may be that the antibody reacts
with the human class I antigens in the virus envelope (Table
1) and thereby blocks the interaction of gp120 with the CD4
determinant on the target cells by steric hindrance. How-
ever, the anti–human HLA class I antibody does not recog-
nize monkey HLA class I antigen (Fig. 2 c). This may ex-
plain the observation that the vaccinated monkeys were infected
on subsequent challenge with SIV grown in monkey cells,
even though they had previously been protected against SIV
grown in C8166 cells (8–10, and our unpublished data). Al-
though sera from these monkeys also contain high levels of
specific anti-SIV antibodies (7, 8, 10, 16, 17, and our unpub-
lished data), the lack of protection suggests that neutralizing
antibodies may not be a major protective mechanism in the
present system. However, it does not rule out the possibility
for selection of genetic variants in the virus grown in monkey
cells that are critically divergent from the vaccine virus used,
which was grown in C8166 cells. Such changes could account
for the possible breakdown in immune surveillance by the
existing SIV-specific neutralizing antibodies in the monkeys.
This is evident when recombinant env is used as vaccine and
the specific neutralizing antibody induced could only pro-
tect monkeys against a homologous challenge infection with
cloned SIV (18). Thus, the presence of high levels of SIV-
specific antibody in monkeys not protected against SIV grown
in monkey cells also argues against the notion that antibodies
that crossreact between HLA class I antigens and lentivirus
antigens may play an important role in protection against
SIV infection (19). It is also unlikely that anti-CD4 antibody
plays a significant role in the present system since no anti-
CD4 antibody was detected in the sera of protected monkeys
by flow cytometry (data not shown), nor is CD4 antigen
present in the SIV virus preparation (Table 1). Whatever the
mechanism, the results reported here suggest that antibodies
against human HLA class I molecule and/or other yet un-
detected antigen(s) may be useful in immunotherapy against
HIV infection.

We thank Dr. H. Holmes and the contributors of the MRC AIDS Directed Programme Repository for
the mAbs used; Programme European Vaccine for AIDS for inactivated purified SIVmac 251 and animals
for the E.C. adjuvant experiment; Professor A. McMichael and Dr. E. Weiss for P815-transfected cells;
Drs. G. Schild, K. H. G. Mills, E. J. Stott, and M. Page for comments; and A. Davies for help in preparing
the figures.

This work was supported in part by grants from the Medical Research Council’s AIDS Directed Programme.

Address correspondence to W. L. Chan, Division of Immunobiology, National Institute for Biological
Standards and Control, Blanche Lane, South Mimms, Potters Bar, Herts EN6 3QG, UK.

Received for publication 12 May 1992 and in revised form 9 July 1992.
References


