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 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,
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 positive correlation of protection
with antibody response to the HLA class I molecule.

Macaques 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 vac-
cines against AIDS (1-3). Recently, we demonstrated that
Cynomolgus macaques vaccinated with either inactivated par-
tially 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 anti-
body 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 mole-
cules. These results suggest that antibodies against HLA class
I molecule and/or other yet undetected antigen(s) may be
useful in immunoetherapy against HIV infection.

Materials and Methods

Animals. Cynomolgus monkeys (Macaca fascicularis) were main-
tained 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-J137 were injected subcutaneously
three times with 500 µg formalin-fixed SIVmac 251 + RJIB adju-
vant; monkeys J138-J141 were injected subcutaneously four times with 100 µg formalin-fixed SIVmac 251 + RJIB; monkeys J217-J220
and J68-J71 were injected subcutaneously with two doses, respec-
tively, of 2 x 10^7 SIVmac 251-infected C8166 cells fixed with
glutaraldehyde + Quil-A adjuvant; and monkeys J72-J75 were in-
jected subcutaneously with two doses of 2 x 10^7 C8166 cells fixed
with glutaraldehyde + Quil-A adjuvant. All the animals were chal-
 lenged intravenously with 10 50% monkey infectious doses
(MIDs0) 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 con-
firmed by immunofluorescence of virus antigen on infected cells using stan-
dard 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 x
10^6) were labeled for 6 h with 0.5 mCi [35S]methionine in
methionine-free RPMI containing 10% FCS, lysed, and radiol
immune 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 (Amer-
sham 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
(P815-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 FACS® 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 (15). 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. Cantrell, 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-human 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 (Tll, 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 1. Sera from protected monkeys contain antibodies specific for human HLA class I molecule. [35S]Methionine-labeled C8166 cell lysates were immune precipitated with prebleed (B) and hyperimmune (A) plasma (20 μl) from monkeys vaccinated with inactivated purified SIVmac (1179, 1180, 1181, 1182), fixed SIV-infected C8166 cells (1217, 1219, 1168, 1269, J71), or C8166 cells (J73, J75). With the exception of J71, J75, and 1219, all the other monkeys were protected against SIVmac 251 (grown in C8166 cells) challenge infection (reported in references 4 and 5).
Figure 2. (a) In lanes $1181 + W6/32$ and $\text{anti-}\beta_2m + 1182$, the radiolabeled lysate was preprecipitated twice with plasma from $1181$ or anti-$\beta_2m$ before subsequent precipitation with mAb $W6/32$ or $1182$ plasma, respectively. (b) Similarly, in lane $1181 + \text{OKTII}$, precipitation with mAb OKTII was subsequent to that with $1181$ plasma. Lane $1181\ PB$ denotes preimmune plasma. (c) $^{[35]}S$-Methionine-labeled cell lysates of a $H.\ papio$-transformed monkey B lymphoblastoid cell line were immune precipitated with 25 $\mu l$ prebleed or hyperimmune plasma from protected monkey $1181$ and 2 $\mu l$ (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>
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<th>Antibody</th>
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<th>HIV-1</th>
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<td>-</td>
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<td>0.014 ± 0.003</td>
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* 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.

The TCR complex, but little or no detectable CD4, CD2, or CD8 molecules. Flow cytometry analysis also shows that sera from protected monkeys recognized a murine cell line (P815) transfected with HLA B27. To control for non-specific binding, the same set of plasma was also used to react with untransfected P815 cells (data not shown). Similar results were obtained with sera from other protected monkeys.

The high levels of anti-human HLA class I antibody induced in protected monkeys vaccinated with purified SIVmac251 (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 SIVmac251 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 antibody response to human HLA class I antigen and protection of monkeys from SIVmac251 (grown in C8166 cells) infection. The mechanism of the possible protection by this antibody 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. However, the anti-human HLA class I antibody does not recognize monkey HLA class I antigen (Fig. 2c). This may explain 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). Although sera from these monkeys also contain high levels of specific anti-SIV antibodies (7, 8, 10, 16, 17, and our unpublished 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 protect 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 undetected antigen(s) may be useful in immunotherapy against HIV infection.

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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.

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