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

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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, 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 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 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 I179–182 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 + RIBI adjuvant; monkeys J138–141 were injected subcutaneously four times with 100 μg formalin-fixed SIVmac 251 + RIBI; monkeys I217–220 and J68–71 were injected subcutaneously with two doses, respectively, of 2 × 10⁶ SIVmac 251–infected C8166 cells fixed with glutaraldehyde + Quil-A adjuvant; and monkeys J72–75 were injected subcutaneously with two doses of 2 × 10⁶ C8166 cells fixed with glutaraldehyde + Quil-A adjuvant. All the animals were challenged intravenously with 10 50% monkey infectious doses (MID$_{50}$) 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 × 10⁶) were labeled for 6 h with 0.5 mCi [³⁵S]methionine in methionine-free RPMI containing 10% FCS, lysed, and radioimmune 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 10⁶ 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 (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 OKT11 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 preprecipitation 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 preprecipitated 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
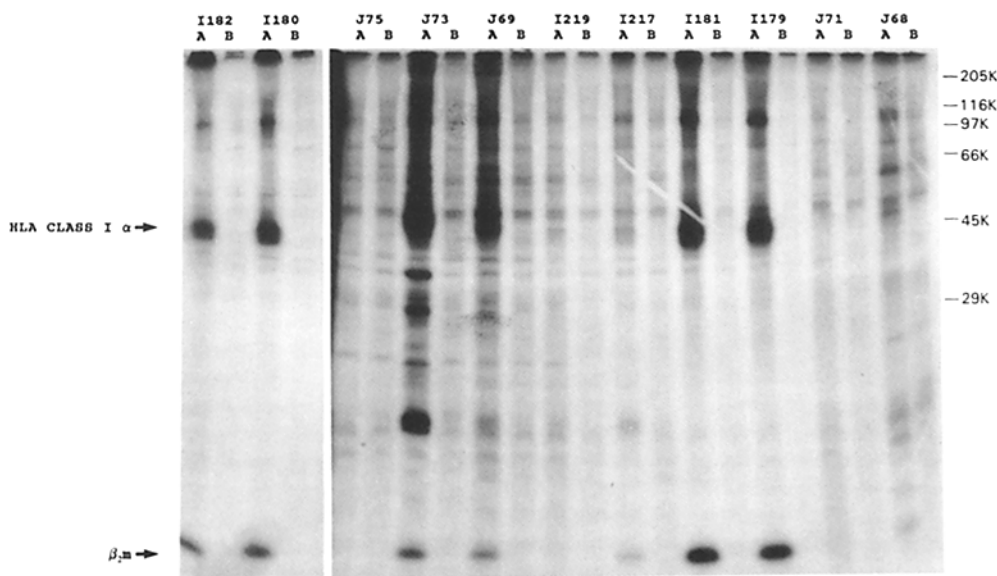

Figure 1. Sera from protected monkeys contain antibodies specific for human HLA class I molecule. [35S]Methionine-labeled C8166 cell lysates were immune precipitated with prebleed (B) and hyperimmune (A) plasma (20 μl) from monkeys vaccinated with inactivated purified SIVmac (I179, I180, I181, I182), fixed SIV-infected C8166 cells (I217, I219, J68, J69, J71), or C8166 cells (J73, J75). With the exception of J71, J75, and I219, all the other monkeys were protected against SIVmac 251 (grown in C8166 cells) challenge infection (reported in references 4 and 5).
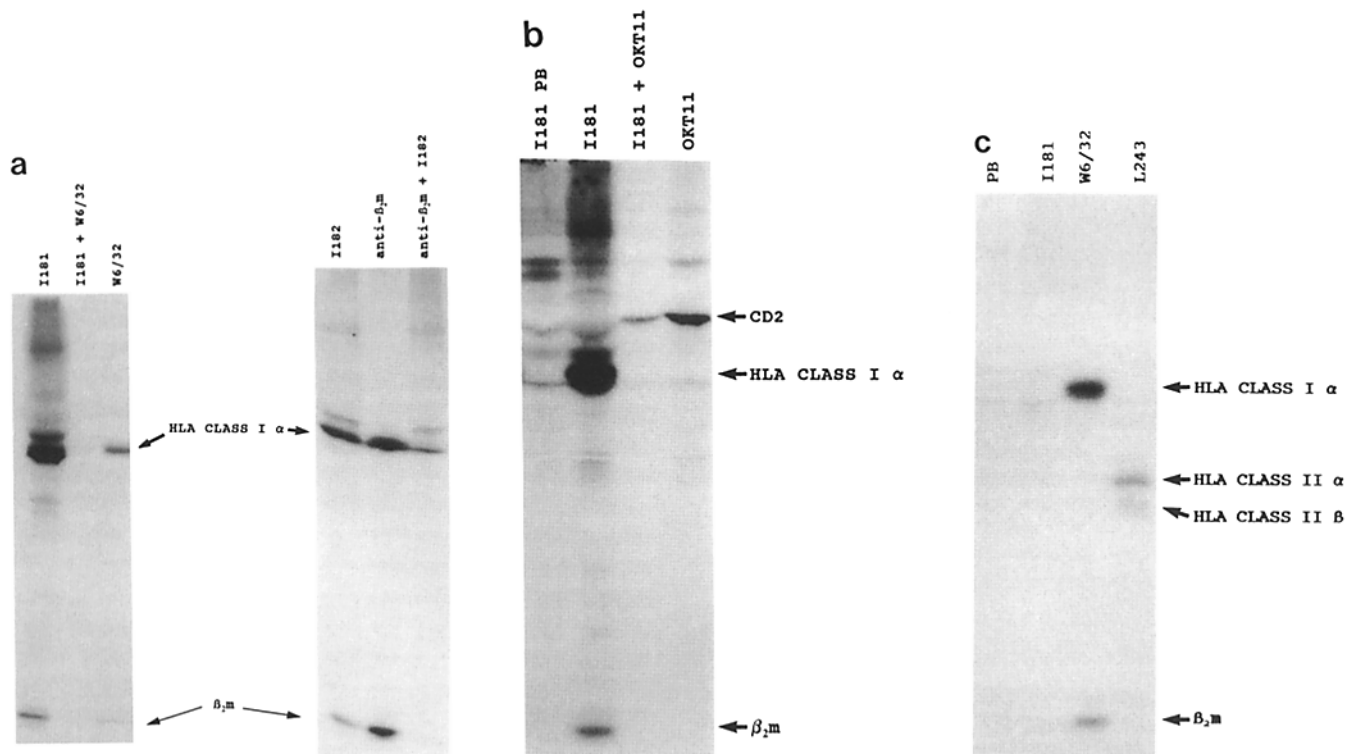
b

I181 PB
I181
I181 + OKT11
OKT11

←CD2

←HLA CLASS I α

←β₂m

a

I181
I181 + W6/32
W6/32

I182
anti-β₂m
anti-β₂m + I182

HLA CLASS I α→

β₂m

c

PB
I181
W6/32
L243

←HLA CLASS I α

←HLA CLASS II α
←HLA CLASS II β

←β₂m

Figure 2. (a) In lanes *I181 + W6/32* and *anti-β2m + I182*, the radiolabeled lysate was preprecipitated twice with plasma from I181 or anti-β2m before subsequent precipitation with mAb W6/32 or I182 plasma, respectively. (b) Similarly, in lane *I181 + OKT11*, precipitation with mAb OKT11 was subsequent to that with I181 plasma. Lane *I181 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 μl prebleed or hyperimmune plasma from protected monkey I181 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*

Antibody		Antigen recognized	OD reading*	
			SIV	HIV-1
None		–	0.06 ± 0.007	0.014 ± 0.003
ADP373	IgG2a	SIV env gp160	**0.344 ± 0.025**	ND
ADP317	IgG3	HIV-1 env gp160	ND	**0.116 ± 0.005**
W6/32	IgG2a	HLA-A,-B,-C	**0.333 ± 0.038**	**0.113 ± 0.012**
OKT3	IgG2a	CD3	**0.314 ± 0.06**	**0.323 ± 0.008**
L203	IgG1	HLA class II	0.017 ± 0.001	0.012 ± 0.004
L227	IgG1	HLA class II	0.017 ± 0.001	0.008 ± 0.001
L243	IgG2a	HLA class II	0.05 ± 0.009	0.058 ± 0.01
nm31	IgG	HLA class II	0.036 ± 0.004	0.032 ± 0.008
OKT11	IgG2a	CD2	0.043 ± 0.009	0.027 ± 0.003
9.3	IgG2a	CD28	0.03 ± 0.002	0.014 ± 0.002
ADP318	IgG1	CD4	0.014 ± 0.002	0.011 ± 0.006
ADP336	IgG2a	CD4	0.058 ± 0.006	0.062 ± 0.002
ADP351	IgG2a	CD4	0.025 ± 0.003	0.020 ± 0.008
ADP356	IgG2b	CD4	0.036 ± 0.002	0.033 ± 0.001
ADP359	IgG	CD4	0.016 ± 0.001	0.007 ± 0.001

* Values (mean ± 1 SEM, n = 6) that were five times higher than the negative control reading wee considered as positive (in bold type).
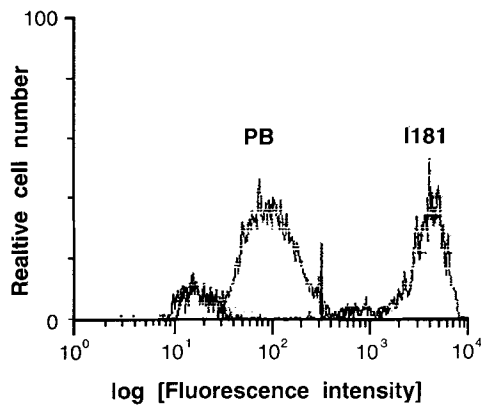
Figure 3. Flow cytometry analysis of anti–human HLA class I reactivity by prebleed and hyperimmune plasma (1:30) from a representative monkey, I181, using mouse P815 cells transfected with HLA B27. To control for nonspecific binding, the same set of plasma was also used to react with untransfected P815 cells (data not shown). Similar results were obtained with sera from other protected monkeys.

molecule. Flow cytometry analysis also shows that sera from protected monkeys recognized a murine cell line (P815) transfected with human HLA class I B27 (Fig. 3); mean antibody titer of protected monkeys was $\log_{10}$ 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 induced 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 antibody response to human HLA class I antigen and protection of monkeys from SIVmac 251 (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. 2 c). 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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