Macaques Immunized with HLA-DR Are Protected from Challenge with Simian Immunodeficiency Virus

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Received 27 September 1994/Accepted 6 February 1995

Macaques immunized with uninfected human cells have been shown to be protected from challenge with simian immunodeficiency virus (SIV) propagated in human cells. To identify the potential antigens involved in this protection, macaques were immunized with uninfected human cells, sucrose density gradient-purified culture fluid from uninfected human cells (mock virus), beta-2 microglobulin (β 2M), immunoaffinity-purified HLA class I and class II proteins from these human cells, and adjuvant. Although all macaques immunized with β 2M and HLA class I developed high antibody titers to β 2M, these animals were not protected from a subsequent challenge with infectious SIV grown in human cells. In contrast, the macaques immunized with class II protein (HLA-DR) and mock virus developed antibodies to class II protein and were protected from the intravenous infectious virus challenge. The class II protein- and mock virus-immunized animals which were protected from challenge were given boosters of the appropriate antigen and challenged with the same SIV propagated in macaque cells. All animals became infected, indicating that the protection seen with human class II protein did not extend to protection from infection with SIV containing macaque class II proteins. Since the virus released from SIV-infected macaque cells would contain macaque class II proteins, our results suggest that the initial SIV infection was completely prevented. In addition, the lack of protection from challenge with SIV propagated in macaque cells provided strong evidence that the protection was due to an immune response to the cellular proteins and not to epitopes cross-reactive between class II proteins and the viral proteins, since the identical virus proteins were present in both challenge stocks. These results are the first demonstration that immunization with a purified cellular protein can protect from virus infection.

Vaccination has been the most effective intervention for the prevention of disease spread, especially the spread of viral diseases. The immunogens of almost all viral vaccines currently available for human use are either inactivated virus or attenuated viruses. In anticipation of the development of killed human immunodeficiency virus (HIV) vaccine for humans, several investigators have evaluated inactivated simian immunodeficiency virus (SIV) as an immunogen for the prevention of infection by SIV challenge (9, 15, 18-20, 22, 30-32, 36, 39-41, 44-46, 48, 49, 53, 54, 56-58). Stott (55) tested glutaraldehyde-inactivated SIV-infected human cells as a potential immunogen for protection from SIV infection. As a control, macaques were immunized with uninfected human cells. Upon challenge with SIV propagated in human cells, two of the four macaques immunized with uninfected human cells were protected. This finding raised the possibility that immune responses to cellular antigens associated with the killed viral vaccines could protect from infection with immunodeficiency viruses.

We have reported that major histocompatibility complex (MHC)-encoded cellular antigens (HLA-DR and HLA class I) were present in sucrose gradient-purified preparations of HIV type 1 (HIV-1), HIV-2, and SIV (1). Antisera to these proteins

precipitated intact HIV-1, indicating that these cellular proteins were physically associated with immunodeficiency virus. To determine if any of these virus-associated cellular proteins were responsible for protection in vivo, we purified HLA-DR and HLA class I proteins from human cells and immunized macaques with these proteins as well as with beta-2 microglobulin (β 2M), mock virus, glutaraldehyde-treated human cells, and adjuvant. Mock virus was used as an immunogen because this preparation contains additional cellular antigens that may contribute to immune responses seen when inactivated virus is used as an immunogen. These animals were then challenged with SIV propagated in human cells, and the animals that were protected from infection were challenged with SIV grown in macaque cells to determine which human proteins provided protection.

MATERIALS AND METHODS

Virus. SIV/Mne clone E11S is a single-cell clone of HuT 78 cells infected with SIV/Mne. Cell culture supernatants were prepared from an early-passage vial of this clone as previously described (4, 7) and stored frozen in aliquots of 10-fold dilutions over liquid nitrogen in the repository at the National Cancer Institute-Frederick Cancer Research and Development Center.

A stock of this virus grown in *Macaca fascicularis* peripheral blood mononuclear cells (PBMCs) was prepared as follows: *M. fascicularis* PBMCs (2×10^6 cells per ml of culture medium) were stimulated with 1 μ g of phytohemagglutinin (HA16; Murex Diagnostics, Dartford, England) per ml for 72 h in the presence of RPMI 1640 medium supplemented with 2.0 mM L-glutamine, 100 U of penicillin per ml, 100 μ g of streptomycin per ml, 100 μ g of gentamicin per ml, 20% heat-inactivated fetal calf serum (Advanced Biotechnologies, Inc., Columbia, Md.), and 2 μ g of Polybrene (Sigma Chemical Co., St. Louis, Mo.) per ml at 37°C and 5% CO₂. After 72 h, the cells were centrifuged at 250 $\times$ g for 15 min,

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adjusted to 7×10^5 cells per ml in the presence of fresh culture medium and 10% delectinized interleukin 2 (IL-2) (Advanced Biotechnologies, Inc.), and grown for 2 to 3 days. After that time, 10^7 macaque PBMCs were incubated with 1.0 ml of SIV/Mne CL E11S virus stock for 3 h at 37°C, washed three times, and incubated at 7×10^5 cells per ml in the presence of 10% IL-2. After 6 days, 20×10^6 autologous *M. fascicularis* cells, previously exposed to phytohemagglutinin and IL-2, were added to the culture. Cell culture medium was replaced and the supernatant was harvested 4 days later. The cells obtained at the time of preparation of the final stock were frozen for molecular characterization and as future reference samples.

Virus isolation. To identify macaques infected with SIV, 4.0×10^6 macaque PBMCs, isolated over Histopaque-1077 (Sigma Chemical Co.) as previously described (5), were added to 5×10^6 AA-2 CL 5 cells and both were cultured in RPMI 1640 containing 10% fetal calf serum, 10% IL-2, and 1 µg of phytohemagglutinin per ml. Reverse transcriptase assays were performed on culture fluids every 2 weeks (6); cultures were maintained for 9 weeks before being discarded.

Gel electrophoresis and immunoblotting. Proteins from sucrose gradient-purified SIV/Mne CL E11S grown in HuT 78 cells were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), transferred to Immobilon paper (Millipore Corp., Bedford, Mass.), and processed as previously described (6). Macaque plasma was diluted 1:50 before assay.

PCR analysis. PBMCs were isolated from EDTA-treated whole blood and DNA was prepared as described previously (7). DNA (350 ng) was used as template for a two-step amplification by PCR. A nested set of oligonucleotides specific for the long terminal repeat regions of SIV/Mne were used as primers. For the first step, 5'-TGGAGGGGATTATTACAGTGAAGA-3' and 5'-TCGAGTACCGAGTTGACCAAGGCGG-3' were used. For the second step, the primers were 5'-CCAGATTGGCAGAAATTACACTCGGGACCAGG-3' and 5'-GAGAGATGGGAGCAGACACTGGCTTA-3'. The primers were annealed at 60°C, and templates were amplified at 72°C for 35 cycles. Products from the first amplification step were diluted 100-fold, and aliquots of 1 µl each were used as templates for the second round of amplifications (total reaction volume, 25 µl). The final amplified fragment was 642 bp in length and was detected by ethidium bromide staining of 8% acrylamide gels.

Purification of class I and class II proteins. H9 cells, a clone of HuT 78 cells, were used as a source of class I and class II proteins. Cultures were maintained in RPMI 1640 supplemented with 5% heat-inactivated fetal bovine serum (Flow Laboratories, Inc.), 2 mM L-glutamine, 100 U of penicillin per ml, and 100 µg of streptomycin per ml. The H9 cells were harvested from 850-cm² roller bottle cultures at a cell density of approximately 10^6 cells per ml by filtration against a 0.65-µm-pore-size membrane (Millipore Prostack). The cells were washed once in phosphate-buffered saline (PBS) and pelleted by centrifugation at $2,000 \times g$. The resulting packed cell pellet was frozen at -70°C. Frozen cell pellets were thawed at 37°C and transferred to a Thomas homogenizer. All the remaining steps were performed at 4°C. For each gram of cells, 2.5 ml of lysis buffer (10 mM Tris-HCl, 1 mM dithiothreitol, 0.1 mM phenylmethylsulfonyl fluoride) was added. The cells were then disrupted mechanically by five strokes in the homogenizer. The resulting supernatant was centrifuged and saved, and the pellet was resuspended in lysis buffer and recentrifuged. The collection and washing of the pellet were repeated until the collected supernatant was clear (typically five to six washes). The pooled supernatant (cell membrane material) was then transferred to a Beckman 45 Ti ultracentrifugation tube and centrifuged at $175,000 \times g$ for 40 min at 4°C. The supernatant fraction was then decanted, and the pellet (membranes) was scraped from the tube, transferred to a new tube, and resuspended in 1.9 ml of lysis buffer per g of original cell mass. The resuspended membranes were then further dispersed in the Thomas homogenizer. Nonidet P-40 was then added to bring the final concentration to 4%, and the mixture was rehomogenized and recentrifuged at $175,000 \times g$ for 2 h at 4°C. The supernatant fraction (detergent-soluble membranes) was then collected, and the pellet was discarded.

To purify the HLA proteins, detergent-soluble membrane preparation was processed sequentially through a series of columns as previously described (11, 26, 27). The preparation was processed through three columns in the following order: Sepharose CL-4b-normal mouse serum-Affigel 10, protein A-Sepharose-LB3.1-protein A-Sepharose, and W6/32-protein A-Sepharose. After sample loading, each affinity column was washed twice with Tris-HCl (pH 8.0)-0.1% Nonidet P-40, twice with 20 mM morpholinopropanesulfonic acid (pH 8.0)-140 mM NaCl-0.1% deoxycholic acid (DOC), and then four times with 10 mM Tris-HCl (pH 8.0)-0.1% DOC. Column-bound MHC molecules were eluted with 50 mM glycine (pH 11.5)-0.1% DOC and immediately neutralized to pH 8.0 by the addition of 2 M glycine (pH 2.0). Samples were extensively dialyzed against 10 mM Tris-HCl (pH 8.0)-0.1% DOC and then concentrated by ultrafiltration. All steps were performed at 4°C, and each buffer contained 0.05% sodium azide. Since the monoclonal antibody LB3.1 is pan-HLA-DR reactive, the sample eluted from this column contained all DR molecules present on H9 cells (both HLA-DR4 and HLA-DRw53 molecules). Similarly, since the monoclonal antibody W6/32 is pan-HLA-A/B/C reactive, the sample eluted from this column contained a mixture of the HLA-A1, HLA-B62, and HLA-C3 molecules present on H9 cells.

Mock virus. Mock virus was prepared by harvesting culture fluid from uninfected H9 cells and processing the culture fluid by ultracentrifugation in successive sucrose density gradients as previously described for HIV-1 purification (8).

Briefly, uninfected H9 cells were propagated in roller bottles in RPMI-1640 supplemented as described above. Medium was harvested at 3- or 4-day intervals, and cells were removed by filtration against a 0.65-µm-pore-size membrane (Millipore Prostack). Mock virus was purified from the filtrate by two successive ultracentrifugations in sucrose density gradients. The sucrose gradient was pumped through a dual-wavelength UV monitor, and A_{254} and A_{280} were determined. Fractions were also analyzed for sucrose density with a refractometer. The material producing UV absorption at 280 nm and banding at approximately 1.17 gm/cm³ was collected, diluted to below 20% sucrose with TNE buffer (0.01 M Tris-HCl [pH 7.2], 0.1 M NaCl, 1 mM EDTA), and then rebanded in another sucrose gradient. Mock virus-containing fractions were pooled, diluted approximately 1:3 with TNE buffer, and centrifuged at 30,000 rpm in a Beckman type 45 Ti rotor for 1 h to pellet the mock virus. The resulting pellet was resuspended in TNE buffer at a final concentration of $1,000 \times$ or $5,000 \times$ relative to the cell culture supernatant.

Animals. Cynomolgus macaques (*M. fascicularis*) used for immunization and challenge were housed in pairs, on the basis of the immunogen received, in stainless steel cages in a biological safety level 3 facility at the University of Washington Regional Primate Research Center. This primate center is accredited by the American Association for Accreditation of Laboratory Animal Care. The animals were sedated with ketamine hydrochloride (10 mg/kg of body weight) intramuscularly as needed for immunizations, virus inoculations, and venipuncture.

Cell typing. The HLA class II genotype of HuT 78 and H9 cells was determined from genomic DNA by PCR amplification of the polymorphic regions of the second exon of the DQA1, DQB1, and DRB1 loci. Analysis of the products was conducted by single-stranded conformation polymorphism techniques as previously described (16).

Immunogens. MHC class I (HLA-A1, -B62, and -C3) and class II (DR4 and DRw53) molecules purified from H9 cells by sequential immunoaffinity chromatography as described above were used as immunogens. Since this technique has been used to purify HLA-DR1 molecules for crystallographic studies (11), it was considered to be the least denaturing procedure available. Glutaraldehyde-fixed HuT 78 cells were prepared by pelleting the cells at $400 \times g$ for 5 min and resuspending them at 10^7 cells per ml in Earle's balanced salt solution. Glutaraldehyde was diluted to 0.15% with Earle's balanced salt solution, and an equal volume was added to the cells. After 5 min of incubation, an equal volume of 0.1 M glycine in Earle's balanced salt solution was added and the cells were immediately pelleted at $400 \times g$ for 5 min. Supernatant was discarded, and the cells were resuspended at 10^7 /ml in RPMI 1640-20% fetal calf serum with 10% dimethyl sulfoxide. All steps in the fixation of cells were conducted at 4°C. The glutaraldehyde-fixed cells were then frozen and maintained in the vapor phase of a liquid nitrogen freezer until used in immunizations.

Immunization. Two *M. fascicularis* macaques each were immunized with immunoaffinity-purified, detergent-solubilized HLA class I or class II proteins. In addition, two animals each were immunized with glutaraldehyde-fixed HuT 78 cells, mock virus, purified β2M, or adjuvant only. The macaques were immunized at weeks 0, 4, 8, and 12 with 50 µg of the appropriate protein, 500 µg of mock virus, or 10^7 glutaraldehyde-fixed cells at each inoculation. The initial immunization was formulated in complete Freund's adjuvant and delivered at multiple sites subcutaneously; subsequent doses were in incomplete Freund's adjuvant and were administered intramuscularly. Control macaques (92151 and 92155) received adjuvant in PBS.

RIAs. Radioimmunoassays (RIAs) were conducted as previously described (2). Macaque sera were titrated against HLA-DR (purified as described above) and β2M (Valley Biomedical Products, Inc.; lot no. 92008) that were iodinated by the chloramine T method (28). Antibodies were detected by incubating 15,000 to 20,000 cpm of radiolabeled antigen with 50 µl of threefold serially diluted serum in RIA buffer (0.05 M Tris-HCl [pH 7.8], 0.15 M NaCl, 0.4% Triton X-100, 0.5% bovine serum albumin, 300 mg of phenylmethylsulfonyl fluoride per liter, 0.1% sodium azide) for 2 h at 37°C and then overnight at 4°C. Formalin-treated *Staphylococcus aureus* (20 µl of 5% [wt/vol] *S. aureus* in RIA buffer) was added to each sample to facilitate precipitation of the antigen-antibody complexes. Incubation was continued at room temperature for 15 min, and 500 µl of rinse buffer (0.01 M Tris-HCl [pH 7.8], 0.1 M NaCl, 1.0 mM EDTA, 0.1% Triton X-100) was added to each tube. Pellets were collected at $1,500 \times g$ for 30 min. Radioactivity remaining in the pellet was determined with a gamma counter. Serum titers were recorded as the highest dilutions of sera that precipitated greater than or equal to 3 and 10 times background in the HLA-DR and β2M assays, respectively.

RESULTS

We have previously described the specific association of certain cellular proteins with sucrose gradient-purified preparations of HIV-1 and SIV. In order to determine which of these proteins might be involved in protecting macaques from an in vivo SIV infection, we immunized macaques with purified HLA class I, β2M, and HLA class II, since these are the predominant proteins present in SIV and HIV viral prepara-

FIG. 1. Protein from sucrose gradient-purified mock virus and from HIV-1 (MN) and HIV-1 (IIIB) separated by SDS-PAGE and stained with Coomassie blue. The regions that contain actin and the α - and β -chains of HLA-DR are indicated.

tions grown in HuT 78 cells. To determine if additional cellular proteins might be responsible for protection, we also immunized macaques with disrupted human cells (HuT 78) and with the sucrose-banded supernatant fluid from uninfected H9 cells (mock virus), prepared as described in Materials and Methods.

Mock virus. Membrane vesicles from uninfected cells band in sucrose gradients at approximately the same density as retroviruses. Sucrose gradient-purified mock virus from uninfected H9 cells, as well as two strains of HIV-1 grown in H9 cells, was run on SDS-PAGE gels, and the proteins were stained with Coomassie blue as shown in Fig. 1. The HIV-1 Gag-encoded viral proteins (p24, p17, p7, and p6) were not found in mock virus preparations. Actin was found in purified HIV-1 as well as mock virus preparations. Two darkly staining bands of protein which migrated between p24 and actin were

seen in the virus preparations in the region corresponding to α - and β -chains of HLA-DR. There were also proteins of the molecular size of HLA-DR and HLA class I that were readily apparent in the SDS-PAGE of mock virus. In fact, HLA-DR and HLA class I have been demonstrated to be present in mock virus preparations by immunoblot analysis and competitive RIAs (data not shown).

Immune responses in macaques. Two macaques each were immunized with HLA-DR, HLA class I, β 2M, mock virus, HuT 78 cells, and adjuvant. Humoral immune responses to the immunogens were measured by precipitation of 125 I- β 2M and 125 I-HLA-DR. After the initial immunization, the animals were given boosters at 4, 8, and 12 weeks. The highest antibody titers to β 2M were found in sera of animals immunized with β 2M (Table 1). In fact, the titer of one of the β 2M-immunized animals (no. 92112) against β 2M was 145,800 at the time of challenge. The two animals immunized with class I proteins had titers of 600 and 1,800 at the time challenge. Antibodies to 125 I- β 2M were not detected in the macaques immunized with HLA-DR or adjuvant. Titers to 125 I-HLA-DR were found in animals immunized with HLA-DR and in animals immunized with mock viruses (Table 2). Animal 92123, which was immunized with mock virus, produced a better immune response to both β 2M and HLA-DR than did the mock virus-immunized animal 92126. Unfortunately, animal 92123 died, an anesthetic-induced death, prior to the SIV challenge, and protection could not be determined. Antibodies to HLA-DR were not detected in animals immunized with HLA class I, β 2M, HuT 78 cells, or adjuvant.

Challenge with SIV propagated in human cells. All macaques were challenged intravenously with 20 animal infectious doses of SIV that had been propagated in HuT 78 cells. This challenge occurred at 2 weeks after administration of the last booster, i.e., at 14 weeks after the primary immunization. Following this SIV challenge, the animals were monitored by immunoblot analyses for their seroconversion to viral antigens. As shown in Fig. 2, the sera collected prior to any immunizations were negative for all animals by immunoblot analysis with SIV propagated in HuT 78 cells. Macaques immunized with β 2M (92112 and 92113) had detectable antibody to β 2M at time 0, which was the time of challenge. Likewise, animals immunized with HLA class I (92119 and 92122) also had antibodies directed against the α -chain of HLA class I as well as β 2M at the time of challenge. As expected, animals immunized with HuT 78 cells (92127 and 92133) recognized a number of

TABLE 1. Titration of sera from immunized macaques against 125 I- β 2M

Animal no.	Immunogen	Antibody titer at time of immunization (wk 0)	Antibody titers during booster administration (wk) ^a					Antibody titer at time of challenge (wk 14)
			4	6	8	10	12	
92112	β 2M	— ^b	200	48,600	48,600	48,600	48,600	145,800
92113	β 2M	—	—	—	200	1,800	1,800	5,400
92119	HLA class I	—	—	—	—	1,800	200	1,800
92122	HLA class I	—	—	—	—	600	—	600
92127	HuT 78 cells	—	—	—	—	—	200	600
92133	HuT 78 cells	—	—	—	—	—	—	—
92123 ^c	Mock virus	—	—	—	—	—	—	1,800
92126	Mock virus	—	—	—	—	—	—	—
92114	HLA-DR	—	—	—	—	—	—	—
92115	HLA-DR	—	—	—	—	—	—	—
92155	Adjuvant	—	—	—	—	—	—	—
92151	Adjuvant	—	—	—	—	—	—	—

^a Booster immunizations were given at 4, 8, and 12 weeks.

^b —, the serum scored negative at a 1:200 dilution.

^c Died on day of challenge (anesthetic-induced death).

TABLE 2. Titration of sera from immunized macaques against ¹²⁵I-HLA-DR

Animal no.	Immunogen	Antibody titer at time of immunization (wk 0)	Antibody titers during booster administration (wk) ^a					Antibody titer at time of challenge (wk 14)
			4	6	8	10	12	
92112	β2M	— ^b	—	—	—	—	—	—
92113	β2M	—	—	—	—	—	—	—
92119	HLA class I	—	—	—	—	—	—	—
92122	HLA class I	—	—	—	—	—	—	—
92127	HuT 78 cells	—	—	—	—	—	—	—
92133	HuT 78 cells	—	—	—	—	—	—	—
92123 ^c	Mock virus	—	—	540	540	540	14,580	>43,740
92126	Mock virus	—	—	—	—	540	60	540
92114	HLA-DR	—	—	60	60	4,860	4,860	>43,740
92115	HLA-DR	—	—	1,620	540	4,860	540	1,620
92155	Adjuvant	—	—	—	—	—	—	—
92151	Adjuvant	—	—	—	—	—	—	—

^a Booster immunizations were given at 4, 8, and 12 weeks.
^b —, the serum scored negative at a 1:20 dilution.
^c Died on day of challenge (anesthetic-induced death).

cellular proteins. All of these animals, including the two adjuvant controls (92151 and 92155), seroconverted to various Gag and Env antigens, indicating that they had become infected with SIV. In marked contrast, the two macaques immunized

with HLA-DR (92114 and 92115) and the surviving macaque immunized with mock virus (92126) did not seroconvert to viral proteins after intravenous challenge with SIV. At the time of challenge, the HLA-DR-immunized animals clearly had an-

FIG. 2. Immunoblot analyses of antibodies to SIV/Mne and cellular antigens in control and immunized macaques that were challenged with SIV propagated in human cells (HuT 78). Macaque sera (diluted 50-fold) were reacted with SDS-PAGE-separated proteins from disrupted, sucrose gradient-purified SIV (Mne) CL E11S grown on HuT 78 cells as described previously (6). Immunogens are indicated under the gels, and the molecular masses of these antigens are listed in parentheses. The arrows indicate the presence of antibodies to the cellular antigens in some of the macaques on the day of SIV/Mne challenge (week 0). Antibodies to the SIV/Mne envelope surface (gp120) and transmembrane (gp32) proteins; to the Gag proteins p28, p16, and p6; and to the X-ORF protein, p14, were detected in infected animals at various times after SIV challenge. Sometimes sera from uninfected macaques show transient reactivity in Western blots (immunoblots) to a protein which comigrates with p28. This reaction could be seen in the prebled sample from macaque 92126.

TABLE 3. Detection of SIV infection after challenge of immunized macaques^a

Animal no.	Immunogen	Result for PBMCs isolated at the following time (wk) after SIV challenge (virus isolation/PCR) ^b :						
		0	2	4	8	12	16	20
92112	β2M	-/-	+/+	+/+	-/+	-/+	-/-	-/NT ^c
92113	β2M	-/-	+/+	+/+	+/+	+/+	+/+	-/-
92114	HLA-DR	-/-	-/-	-/-	-/-	-/-	-/- ^d	-/-
92115	HLA-DR	-/-	-/-	-/-	-/-	-/-	-/- ^d	-/-
92119	HLA class I	-/-	-/-	-/-	+/+	+/+	-/-	+/+
92122	HLA class I	-/-	-/-	+/+	-/+	-/+	-/-	-/NT
92123 ^e	Mock virus	NT	NT	NT	NT	NT	NT	NT
92126	Mock virus	-/-	-/-	-/-	-/-	-/-	-/- ^d	-/-
92127	HuT 78 cells	-/-	+/+	+/+	+/+	-/+	+/+	+/NT
92133	HuT 78 cells	-/-	+/+	+/+	+/+	+/+	+/+	+/NT
92151	Adjuvant	-/-	+/+	+/+	+/+	+/+	-/+	-/NT
92155	Adjuvant	-/-	-/-	+/+	+/+	+/+	-/+	+/NT

^a Virus isolation and PCR detection of SIV sequences in macaques challenged with SIV. PBMCs were isolated from EDTA-treated blood at the indicated numbers of weeks after challenge as described previously (5). PBMCs (4×10^6) were cultured with 5×10^6 AA-2 CL5 cells. The presence of SIV was detected by reverse transcriptase assays performed on culture fluids (6); all cocultivations were maintained for 9 weeks before being discarded. Proviral sequences were detected by a two-step PCR amplification with a nested set of primers specific for the long terminal repeat region of SIV/Mne (7, 35).

^b +, virus was detected in PBMCs by cocultivation or by PCR; -, no virus was detected, and PBMCs were PCR negative.

^c NT, not tested.

^d At week 16, lymph nodes from these three animals were also examined for the presence of virus by PCR and cocultivation; no SIV was detected.

^e Died on day of challenge (anesthetic-induced death).

tibodies to the α-chain of HLA class II and animal 92114 showed a high level of antibody to the β-chain as well. As expected, the animal immunized with mock virus showed immune reactivity to numerous proteins.

Virus infection. To analyze for SIV infection, PBMCs from each animal were cocultivated with AA-2 CL5 cells and the cultures were monitored for virus expression by reverse transcriptase assays. In addition, DNA obtained from PBMCs was examined for SIV sequences by PCR. As shown in Table 3, at various times after SIV challenge, virus was isolated from PBMCs and/or SIV viral DNA was detected by PCR for all the animals immunized with β2M, HLA class I, HuT 78 cells, or adjuvant, indicating that no protection was achieved with these immunogens. In contrast, virus was not recovered and SIV DNA was not detected for either animal immunized with HLA-DR or for the surviving animal immunized with mock virus. Lymph nodes obtained from the HLA-DR- and mock virus-immunized animals at week 16 after challenge were also examined for the presence of SIV by PCR and virus isolation and were found to be negative. These results reveal that an immune response to antigenic epitopes on the HLA-DR molecule could provide protection from challenge with SIV propagated in human cells.

The challenge stock of SIV was grown in HuT 78 cells, and the HLA antigens were purified from H9 cells, a clone obtained from HuT 78. Although both H9 and HuT 78 cells have been previously shown to type serologically as DR4, we examined whether the class II DRB1 alleles involved in the protection from the SIV challenge in those two cell lines were indeed identical. The DRB1 genotype was examined by single-stranded conformational polymorphism and compared with the DRB1 genotypes present in control cells homozygous for various DRB1*04 alleles. The results, shown in Fig. 3, reveal that the HLA-DRB1 genotypes in H9 and HuT 78 cells are both DRB1*0401.

FIG. 3. Identification of the HLA-DRB1 genotype of the HuT 78 and H9 cell lines. The first six lanes from the left show the pattern of single-stranded conformational polymorphism of the PCR products from control cells homozygous for various DRB1*04 alleles. The right two lanes show the results obtained with DNA from the HuT 78 and H9 cell lines. ATCC, American Type Culture Collection.

Challenge with SIV propagated in macaque cells. As described above, xenoinmunization (human HLA-DR immunization) was protective when the monkeys were challenged with SIV propagated in human cells. To determine if this xenoinmunization could protect from challenge with the same virus grown in monkey cells, the two macaques immunized with human HLA-DR and the one immunized with mock virus were given boosters of the appropriate antigen and challenged 2 weeks later with 30 animal infectious doses of SIV propagated in macaque PBMCs. At the time of challenge, sera from all three animals were tested in the immunoprecipitation assay for reactivity to HLA-DR. The titers at the time of challenge for the HLA-DR-immunized animals 92114 and 92115 were 1:14,580 and 1:1,620, respectively. The titer for the mock virus-immunized animal was 1:180. As shown in Fig. 4, the HLA-DR-immunized animals (92114 and 92115) had antibodies to the α-chain of HLA-DR and both animals had detectable responses to the β-chain. The mock virus-immunized macaques developed antibodies to a number of cellular proteins. However, all three animals became clearly positive for antibodies to gp120 by 4 weeks postchallenge, indicating that they had become infected after challenge with SIV propagated in macaque cells. Infection was also verified by virus isolation and PCR analyses (data not shown).

DISCUSSION

Utilization of cellular metabolic machinery by viruses for synthesis of the viral components is well established and has been the subject of numerous studies. In contrast, the incorporation of cellular proteins into viruses and the function of these virus-associated cellular proteins have been the subject of relatively few reports, even though in 1933 Gye and Purdy (29) utilized virus neutralization assays to show that antisera to cellular antigens could neutralize infection with a "filterable" fowl tumor agent, indicating that cellular antigens were physically associated with the virus. Additional studies have shown that cellular antigens associated with retroviruses include the following: H2 antigenic determinants with Friend leukemia

FIG. 4. Immunoblot analyses of antibodies to SIV/Mne and cellular antigens in control and immunized macaques that were challenged with SIV propagated in macaque cells. Procedures were as described in the legend to Fig. 2. Immunogens are indicated under the gels, and the molecular masses of these antigens are listed in parentheses. The arrows indicate the presence of antibodies to the cellular antigens in some of the macaques on the day of SIV/Mne challenge (week 0). Antibodies to the SIV/Mne envelope surface (gp120) and transmembrane (gp32) proteins, to the Gag proteins p28 and p16, and to the X-ORF protein, p14, were identified in infected animals at various times after SIV challenge.

virus (12, 17), IL-1 receptor with human T-cell leukemia virus type I (38), HLA antigens with feline leukemia virus (3), CD4 with avian leukosis virus (59), and Thy-1 glycoprotein with murine leukemia virus (13). We previously reported the presence of HLA-DR proteins in sucrose gradient-purified HIV-1 (33) and subsequently showed that HLA-DR and class I proteins were physically associated with HIV-1, HIV-2, and SIV particles (1). Utilizing solid-phase capture assays, immunoelectron microscopy, and fluorescence-activated cell sorter (FACS) analysis, other investigators have also demonstrated the association of class I and class II proteins, as well as other virus-associated cellular proteins, including D71, CD63, CD43, CD8, LFA-1 (CD11A/CD18), CD44, CD54, CD3, CD5, CD25, and CD30, with immunodeficiency virus (14, 23, 34, 42, 43, 47, 50).

It is clear that immune responses to antigens encoded by SIV can protect animals from challenge with SIV, as demonstrated by vaccine experiments with viral peptides (51), envelope glycoproteins (35), and attenuated virus (21). In several other laboratories macaques immunized with inactivated SIV propagated in human cells were protected from challenge with SIV grown in human cells (9, 15, 18–20, 22, 30–32, 36, 39–41, 44–46, 48, 49, 53, 54, 56–58). However, macaques immunized with inactivated SIV grown in human cells became infected when challenged with SIV propagated in macaque cells (19, 48, 53). These data, coupled with those from protection experiments using uninfected human cells as an immunogen in macaques (xenoinmunization) (55), strongly suggest that protection was due to an immune response to cellular antigens. The function of these virus-associated cellular proteins is not known, but it could affect infectivity, tropism, and pathogenesis.

In this report, we have shown that macaques immunized with HLA-DR proteins were protected from challenge with SIV propagated in human cells. The animal immunized with mock virus developed antibodies to HLA-DR and was also protected. In contrast, the macaques that had been immunized

with HLA class I, β 2M, uninfected HuT 78 cells, or adjuvant all became infected. It is not clear why the animals immunized with uninfected cells were not protected. In previous experiments only 50% of the macaques immunized with uninfected human cells were protected from a subsequent challenge with SIV grown in the same cells (55). It is possible that the small number of animals used in the present experiment failed to demonstrate a partial protective response. In addition, the macaques immunized with HuT 78 cells did not elicit any antibodies to class II DR antigens, which we have shown to be associated with protection from infection. The protective response elicited by immunization with uninfected cells may thus depend on the particular cell line used and on the relative concentration of class II antigens expressed on the cell surface. These results clearly demonstrate that an immune response to cellular antigens is protective and identify HLA-DR as a cellular component on the surface of SIV involved in this protection. This is the first demonstration of protection from virus challenge by immunization with a purified cellular protein.

To determine if the protection seen with human HLA-DR immunization could extend to macaque class II-associated antigens, the HLA-DR- and mock virus-immunized macaques were given boosters of human HLA-DR and then challenged with SIV propagated in macaque cells. All animals seroconverted to SIV antigens, and SIV could be isolated from all the macaques, showing that they had become infected. Since the same strain of SIV was used in both challenge experiments, the only variable was the propagation in human or macaque cells. Epitopes shared between HIV-1 envelope glycoproteins and MHC-encoded molecules have been reported (10, 24, 25), and it was possible that protection seen with the first challenge (SIV propagated in human cells) could have been due to recognition of these shared epitopes. However, the lack of protection with SIV propagated in macaque cells would argue against this possibility, since the same SIV antigens were present on both xenopropagated and allopropagated challenge viruses. These results clearly show that the immune response(s) to

human class II antigen was responsible for the protection and that the protection was not due to responses to cross-reactive epitopes shared between MHC-encoded proteins and viral envelope glycoproteins. In addition, when sera from macaques immunized with mock viruses and HLA-DR were analyzed by FACS analysis, reactivity to human B cells, but not to macaque B cells, was seen (data not shown), indicating the lack of an immune response to macaque cell surface proteins. Since the human HLA-DR present on the challenge stock of SIV would not be present on SIV budding from macaque cells after infection, our results also strongly suggest that the initial SIV infection was completely prevented (sterilizing immunity). Prevention of the initial infection of a retrovirus is an attractive feature for an AIDS vaccine because it would prevent integration of the viral genome into the host cell.

It has recently been suggested that immunization with alloantigens might serve as a vaccine to protect against HIV infection (37, 52). Our demonstration that immunization with HLA-DR protects from SIV challenge would support this concept. Because of this finding, detailed analyses of the protective nature of an alloimmunization to assist in the design of AIDS vaccines are warranted. If alloimmunization protects from infection, incorporation of certain class II antigens into the HIV-1 viral vaccines should be considered.

ACKNOWLEDGMENTS

We thank R. Imming, M. Grimes, S. Sloane, R. W. Hill, and W. B. Knott (NCI-FCRDC), P. Klimovitsky (Harvard University), and L. Kuller (University of Washington) for technical assistance; P. C. Grove (NCI-FCRDC) for clerical support; and R. C. Gallo (NCI) for HIV-1-infected and uninfected H9 cells.

This research was sponsored, in part, by the National Cancer Institute, DHHS, under contract number N01-CO-74102 with Program Resources, Inc./DynCorp. R.G.U. is supported by the Irvington Institute for Medical Research.

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