Major Histocompatibility Complex Class I–associated Vaccine Protection from Simian Immunodeficiency Virus–Infected Peripheral Blood Cells

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Summary

To evaluate the effectiveness of vaccine protection from infected cells from another individual of the same species, vaccinated rhesus macaques (Macaca mulatta) were challenged with peripheral blood mononuclear cells from another animal diagnosed with acquired immune deficiency syndrome (AIDS). Half of the simian immunodeficiency virus (SIV)–vaccinated animals challenged were protected, whereas unprotected vaccinates progressed as rapidly to AIDS. Protection was unrelated to either total antibody titers to human cells, used in the production of the vaccine, to HLA antibodies or to virus neutralizing activity. However, analysis of the serotype of each animal revealed that all animals protected against cell-associated virus challenge were those which were SIV vaccinated and which shared a particular major histocompatibility complex (MHC) class I allele (Mamu-A26) with the donor of the infected cells. Cytotoxic T lymphocytes (CTL) specific for SIV envelope protein were detected in three of four protected animals vs. one of four unprotected animals, suggesting a possible role of MHC class I–restricted CTL in protection from infected blood cells. These findings have possible implications for the design of vaccines for intracellular pathogens such as human immunodeficiency virus (HIV).
munological responses as well as the immunogenetic background of protected and unprotected animals we attempted to gain further insight into the possible mechanisms involved in vaccine protection from PBMCs from another SIV-infected macaque. We report new findings that vaccine protection from infected blood cells was related to sharing of a particular MHC class I allele between the SIV-infected donor and SIV-vaccinated animals protected from infection. These findings suggest the importance of evolving cell-mediated responses in the design of effective HIV vaccine strategies.

Materials and Methods

Immunization, Challenge, and Virological Follow-up of Animals. Twelve captive rhesus macaques (M. mulatta) derived from an outbred pedigreed MHC-typed colony were used for cell-associated challenge. Eight were immunized with two different whole inactivated SIV vaccine preparations and four controls were immunized with two different corresponding measles virus vaccines as described previously (12). Briefly, the SIV vaccines were prepared from whole SIV virions either inactivated with formalin and mixed with the adjuvant muramyl dipeptide (MDP), or inactivated with β propiolactone and incorporated into immune stimulating complexes (ISCOMs). All twelve animals were challenged intravenously with an in vivo titrated, uncultured stock of PBMC taken directly from whole SIV virions either inactivated with formalin and mixed inactivated SIV vaccine preparations and four controls were immunized with two different whole inactivated SIV vaccines except for animal IIM which died at 35 wk post challenge. The percent gp120-specific release is shown as percent specific release on gp120-peptide pulsed targets less percent specific release on control medium pulsed targets at an E/T ratio of 30:1. The following peptide pools were used to sensitize target cells: gp120 EVA 774 1-19; gp120 EVA 774 20-25; and gp120 774 26-49. Peptides were used as overlapping 20 mers based on consensus sequence of the SIVmac251/32H isolate. Peptides 5, 38, and 46 were not available. Effector cells used were cryopreserved macaque PBMC isolated by LSM (Organon Teknika, Oss, The Netherlands) density gradient centrifugation, prepared either by Con A activation (5 µg/ml; Sigma Chemical Co., St. Louis, MO) and IL-2 expansion for 6 d or by cocultivation for 9–13 d with autologous peptide-pulsed feeder cells. Briefly, 5–10 × 10^6 PBMC were plated in 1 ml of RPMI 1640 containing 10% FCS in 24-well plates together with 10–20 × 10^5 2,500 rad irradiated 10-d-old peptide-pulsed autologous Con A blasts. Autologous Con A blasts were prewashed for 2 h with a pool of 46 overlapping gp120 peptides at a concentration of 12.5 µg/ml peptide. On day 3, IL-2 was added to effector cell cultures at a final concentration of 20 U/ml. Cells were maintained for 6 d (Con A) or 9–13 d (peptide pulse) and plated over Ficol before the assay. CTL assays were performed using autologous Herpes papio immortalized B lymphocyte cell lines or Con A blasts labeled with 0.1 mCi Na_2CrO_4 for 1 h, then pulsed with medium (unpulsed controls) or with pools of peptides at 25 µg/ml per peptide for 1 h followed by a 16-h peptide incubation period at 2.5 µg/ml per peptide. Subsequently, target cells were washed twice and plated at 10^5 cells/well in 96-well U-shaped plates at various effector/target ratios. After 5 h at 37°C supernatants were harvested and counted in a gamma counter. Percentages of specific ^51Cr release were calculated as 100 × (experimental release − spontaneous release)/(maximum release − spontaneous release). All experimental values were calculated in duplicate while maximum and spontaneous releases were performed in quadruplicate. Responses of 10% or more above specific lysis of control targets, were scored as positive.

Results and Discussion

After challenge, all control animals vaccinated with measles virus became plasma antigen positive at 2 wk post challenge

give positive results. Wells were scored on a scale from 1 to 6. Scores >5 were considered positive.

MHC Analysis and CTL Responses. Mamu-specific allosera were used for MHC typing of the outbred pedigreed MHC-typed colony (13). Within our rhesus colony, at least 13 Mamu-A alleles can be identified with the following frequencies: Mamu-A2 (0.036), -A11 (0.100), -A13 (0.074), -A14 (0.029), -A17 (0.068), -A18 (0.069), -A20 (0.015), -A24 (0.097), -A25 (0.031), -A26 (0.244), -A29 (0.059), -A31 (0.013), and -A32 (0.155). At least 13 Mamu-B alleles have been identified with the frequencies of Mamu-B1 (0.029), -B3 (0.030), -B5 (0.038), -B6 (0.195), -B9 (0.155), -B10 (0.146), -B19 (0.103), -B21 (0.004), -B22 (0.048), -B23 (0.073), -B27 (0.007), -B28 (0.045), and -B33 (0.029). The B null alleles have a frequency of 0.099. It should be noted that there is no correlation between the nomenclature of the various HLA and Mamu-A and -B alleles since the numbering of both systems is arbitrarily chosen. One-dimensional isoelectric focusing was used to compare MHC class I gene product isoelectric point differences with serotyping (our manuscript in preparation). MHC sequence analyses were performed as described (14). Gp120 directed cytotoxic T cell activity of SIV-challenged macaques was detected as reported (15) with the following modifications. Briefly, CTL activity against three pools of overlapping env peptides was measured prechallenge and at 4–11 wk post challenge. The percent gp120-specific release is shown as percent specific release on gp120-peptide pulsed targets less percent specific release on control medium pulsed targets at an E/T ratio of 30:1. The following peptide pools were used to sensitize target cells: gp120 EVA 774 1-19; gp120 EVA 774 20-25; and gp120 EVA 774 26-49. Peptides were 10 aa overlapping 20 mers based on consensus sequence of the SIVmac251/32H isolate. Peptides 5, 38, and 46 were not available. Effector cells used were cryopreserved macaque PBMC isolated by LSM (Organon Teknika, Oss, The Netherlands) density gradient centrifugation, prepared either by Con A activation (5 µg/ml; Sigma Chemical Co., St. Louis, MO) and IL-2 expansion for 6 d or by cocultivation for 9–13 d with autologous peptide-pulsed feeder cells. Briefly, 5–10 × 10^6 PBMC were plated in 1 ml of RPMI 1640 containing 10% FCS in 24-well plates together with 10–20 × 10^5 2,500 rad irradiated 10-d-old peptide-pulsed autologous Con A blasts. Autologous Con A blasts were prewashed for 2 h with a pool of 46 overlapping gp120 peptides at a concentration of 12.5 µg/ml peptide. On day 3, IL-2 was added to effector cell cultures at a final concentration of 20 U/ml. Cells were maintained for 6 d (Con A) or 9–13 d (peptide pulse) and placed over Ficol before the assay. CTL assays were performed using autologous Herpes papio immortalized B lymphocyte cell lines or Con A blasts labeled with 0.1 mCi Na_2CrO_4 for 1 h, then pulsed with medium (unpulsed controls) or with pools of peptides at 25 µg/ml per peptide for 1 h followed by a 16-h peptide incubation period at 2.5 µg/ml per peptide. Subsequently, target cells were washed twice and plated at 10^5 cells/well in 96-well U-shaped plates at various effector/target ratios. After 5 h at 37°C supernatants were harvested and counted in a gamma counter. Percentages of specific ^51Cr release were calculated as 100 × (experimental release − spontaneous release)/(maximum release − spontaneous release). All experimental values were calculated in duplicate while maximum and spontaneous releases were performed in quadruplicate. Responses of 10% or more above specific lysis of control targets, were scored as positive.
Table 1. Vaccine type, VN Titers, Clinical and Virological Status

| Vaccine   | Macaque | VN titers | Pl Ag wk | Clinical status | VI PBMC | PCR | Transfusion recipients* |
|-----------|---------|-----------|----------|----------------|---------|-----|------------------------|
|           |         |           |          |                |         |     |                        |
| SIV-iscom |         |           |          |                |         |     |                        |
| 8653      | 905     | -         | Diarrhea | +              | +       | +   |                        |
| 4097      | 34      | -         | AIDS (122w†) | +           | +       | +   |                        |
| 8668      | 269     | -         | Protected | -            | -       | -   | -                      |
| 8730      | 453     | -         | Protected | -            | -       | -   | -                      |
| SIV-mdp   |         |           |          |                |         |     |                        |
| 8645      | 538     | -         | Protected | -            | -       | -   | -                      |
| 8649      | 190     | -         | Protected | -            | -       | -   | -                      |
| 1IM       | 190     | -         | AIDS (35w†) | +           | +       | +   | +                      |
| KP        | 80      | -         | AIDS (54w†) | +           | +       | +   | +                      |
| Controls  |         |           |          |                |         |     |                        |
| MV-iscom  |         |           |          |                |         |     |                        |
| 8672      | 2       | 2 => AIDS (39w†) | + | + |
| 8679      | 2, - , 27 => AIDS (101w†) | + | + |
| MV-mdp    |         |           |          |                |         |     |                        |
| 2CA       | -       | 2        | Asymptomatic | +           | +       |     |                        |
| 1YH       | -       | 2        | AIDS (80w†) | +           | +       |     |                        |

Plasma antigen (Pl Ag) is shown as the week post challenge in which animals had detectable virus antigen in plasma and persistent levels thereafter (indicated as =>). VN titers on the day of challenge are shown. VI results on PBMCs at week 6 post challenge are shown, and tests were performed at routine intervals post challenge. All animals positive at week 6 were consistently positive thereafter except for no. 2CA who has become periodically virus isolation negative but remains PCR positive. The absence of infection of protected animals was confirmed by blood transfusion to naive recipients* who remained negative by all criteria, in contrast to the transfusion recipient from one vaccinated but unprotected animal. PCR results from naive recipients 14 wk after blood transfusion from protected vaccinated donors and 11M, an unprotected animal, are shown. Clinical status is described as protected (uninfected), asymptomatic (infected), and (wt) = week of death post challenge.

and SIV could be isolated from PBMC at 6 wk and time points thereafter (Table 1). Of the eight SIV vaccinates challenged intravenously with SIV-infected rhesus macaque PBMC, animals nos. 8668, 8637, 8645, and 8649 remained negative by all criteria (Table 1). SIV vaccinates nos. 8653, 4097, 11M, and KP, remained plasma antigen negative, possibly due to vaccine-induced anti-SIV antibodies, but after 6 wk, SIV could be isolated and at 12 wk provirus could be detected in PBMCs. Three of four of these animals progressed to AIDS and died. One of these vaccinates progressed faster to AIDS and died before any of the controls (Table 1). As seen in Table 1, the absence of virus in protected animals was confirmed by blood transfusion to naive recipients who remained negative by all criteria, in contrast to the transfusion recipient from one vaccinated but unprotected animal (Table 1). PCR on lymph node and bone marrow biopsies from protected animals 1 yr post challenge failed to demonstrate evidence of virus infection.

To investigate the mechanism of protection observed, we first analyzed humoral immune responses to both SIV and to human cells. Uninfected C8166 cells have been reported to elicit protection from challenge with human cell grown SIV in a group of immunized cynomolgus macaques. In that study, in contrast to neutralizing antibodies, anticell antibodies were found to correlate with protection (9). As seen in Table 1, no association was found between virus neutralizing activity and protection. Moreover, we were unable to find a correlation between protection and the level of total antibodies to the vaccine substrate (C8166) measured either by ELISA or by fluorescent flow cytometry (data not shown).

It has been described that whole inactivated C8166 cell-propagated SIV vaccines induce antibodies that react with HLA class I molecules (17) and in one report these antibodies were found to correlate with vaccine protection from cell-free challenge (16). The presence of MHC proteins bound to lentiviruses propagated on human cell lines has been described (10, 17–19), and antibodies to MHC are reported to inhibit virus infection in vitro (10). The mechanism of protection when both the vaccine and challenge virus are produced on the same xenogenic (human) cell line appear to be due to immune responses to foreign cell components carried by the virus after budding from foreign cells (10, 11). Al-
Figure 1. Antibodies to HLA class I proteins with sera from vaccinated macaques. Vaccines were as follows: SIV-ISCOM; formalin-inactivated SIV-MDP (muramyl dipeptide adjuvant); measles virus (MV)-ISCOM; MV-MDP; and from an SlVm~.32, infected, unvaccinated macaque (INF). The positive control consisted of a human class I dimer-specific mAb W6/32. (U, unprotected; P, protected). Titers to flz-microglobulin are shown above each lane.

though this problem was circumvented by using an in vivo-derived rhesus monkey PBMC challenge, we wished to rule out that antibodies to these foreign cell components including foreign HLA and other cellular antigens found on human cells, were not mediating protection as reported in experiments in which the challenge virus was propagated on human cells (9, 11, 16).

To determine if the cell-associated vaccine protection was related to anti-MHC class I antibodies, we performed immunoprecipitation analysis for MHC proteins using lysed C8166 cells with sera from protected and unprotected macaques taken on the day of challenge (Fig. 1). Additionally, antibody titers to flz-microglobulin were determined by ELISA. As can be seen, no correlation with protection from infected cells with flz-microglobulin antibody titers or the ability of sera to immunoprecipitate HLA class I proteins in general was found. Subsequently, we considered whether vaccination with SIV whole virus vaccines propagated on the C8166 human cell line had induced an allospecific humoral response capable of recognizing the 1XC cells used for challenge. Although vaccination induced allospecific antibody responses developed in some of the macaques, the presence of such antibodies did not correlate with protection from challenge with PBMC from macaque no. 1XC (data not shown).

We next asked whether MHC-restricted cellular immunity played a role in vaccine protection. The MHC class I and II types of the challenge donor and of the vaccinated recipients were determined and compared. The MHC system of the rhesus macaque has been designated MhcMamu, and by using alloantisera, a high number of Mamu-A, -B, and

Table 2. MHC Serotype and gp120-Directed cytotoxic T Cell Activity of SIV-vaccinated Macaques between 4 and 11 wk Post Challenge

| Macaque | Vaccine  | MhcMamu- | SIV gp120-directed CTL responses |
|---------|----------|-----------|----------------------------------|
|         |          | A         | B | DR | env peptides |
| Challenge donor | IXC | SIV-iscom | 26, 14 | 10, 10 | 3, 3 | 1-19 | 20-25 | 26-49 |
| Protected | 8668 | SIV-iscom | 26, 11 | 10, 1 | 3, 1 | 9 | 8 | 22 |
|          | 8730 | SIV-iscom | 26, 25 | 19, 6 | 1, 3 | 0 | 0 | 0* |
|          | 8645 | SIV-mdp | 26, 18 | 23, 1 | 5, 4 | 11 | 31 | 7 |
|          | 8649 | SIV-mdp | 26, 26 | 19, 6 | 8, 1 | 9 | 42 | 34 |
| Unprotected | 8653 | SIV-iscom | 13, 14 | 6, 23 | 5, 3 | 0 | 0 | 0 |
|          | 4097 | SIV-iscom | 35, 26 | 10, 1 | 8, 101 | 0 | 0 | 0* |
|          | KP | SIV-mdp | 24, 35 | 10, 19 | 3, 4 | 3 | 0 | 51* |
|          | IM | SIV-mdp | 24, 24 | 6, 23 | 3, 2 | 0 | 0 | 7 |

* Con A blasts; underlined responses were those 10% or more above specific lysis of control targets and scored as positive.
† Macaque no. 4097 was the only Mamu-A26 positive animal originating from another geographically isolated population possessing a Mamu-A26 subtype.
-DR alleles can be detected. Analysis of protected vs. non-protected animals revealed that a MHC class I allele, Mamu-A26, was found to be shared between all protected monkeys and monkey no. 1XC, the SIV-infected challenge donor of A26, was found to be shared between all protected monkeys protected animals revealed that a MHC class I allele, Mamu-DR alleles can be detected. Analysis of protected vs. non-vaccinated controls. SIV vaccination did not appear to prolong survival of SIV-vaccinated animals as compared with measles-infected or vaccinated rhesus macaques may develop MHC class I-restricted CTLs (15). Interestingly, gp120-directed responses were only demonstrated in protected animals after and not before challenge (Table 2). Apparently, vaccination-induced SIV-specific CTL precursor levels were boosted either by antigen presenting 1XC cells, or by undetectable limited virus replication. Consequently, it may be speculated that the mechanism of protection observed in animals nos. 8668, 8645, and 8649 (Tables 1 and 2), depends on an MHC-restricted cell-mediated defence to eliminate infectious 1XC cells. The evidence for the role of MHC class I-restricted CTL in the observed vaccine protection from infected cells is suggested from this study. However, no unique subregion of gp120 was identified as target for CTL (Table 2). It is not unlikely therefore, that, besides Mamu-A26, other alleles were involved in the presentation of viral peptides on autologous infected cells. Alternatively, the Mamu-A26 allotype may have possibly played a role through other MHC-mediated mechanisms such as epitope selection (21). As a control experiment to determine if the Mamu-A26 allotype also played a role in protection from cell-free challenge, macaques immunized with the same SIV vaccines and protected from challenge with human cell grown SIV (12), were rechallenged with monkey cell grown cell-free SIVmac251/32H after revaccination. All of these monkeys became infected, three of which were Mamu-A26 positive (data not shown). Hence, it appears that the mechanism is not Mamu-A26 linked resistance to infection in general, but that it is a specific mechanism that involves protection from infected cells sharing this MHC allele.

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