Protection of macaques against SIV infection by subunit vaccines of SIV envelope glycoprotein gp160

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Abstract:

Simian immunodeficiency virus (SIV) is a primate lentivirus related to human immunodeficiency viruses and is an etiologic agent for acquired immunodeficiency syndrome (AIDS)--like diseases in macaques. To date, only inactivated whole virus vaccines have been shown to protect macaques against SIV infection. Protective immunity was elicited by recombinant subunit vaccines. Four Macaca fascicularis were immunized with recombinant vaccinia virus expressing SIVmne gp160 and were boosted with gp160 produced in baculovirus-infected cells. All four animals were protected against an intravenous challenge of the homologous virus at one to nine animal-infectious doses. The results indicate that immunization with viral envelope antigens alone is sufficient to elicit protective immunity against a primate immunodeficiency virus. The combination immunization regimen, similar to one now being evaluated in humans as candidate human immunodeficiency virus (HIV)--1 vaccines, appears to be an effective way to elicit such immune responses.

Full Text:

THE SPREAD OF AIDS AND HIV INfection has become a global concern [1]. Development of a safe and efficacious vaccine against HIV is an important component in control of this disease. Major advances made in recent years include the demonstration that inactivated whole virus vaccines protect macaques from infection by SIV [2-4], which is a lentivirus closely related to HIV [5]. However, concerns about insufficient inactivation and inadequate animal models for safety testing confound the use of whole inactivated HIV in seronegative humans. Most efforts in HIV vaccine development to date therefore have been focused on subunit vaccines [6]. Recently, Stott et al. [7] reported that protection against SIV infection appears to correlate with antibodies against cellular components, rather than viral antigens. These findings not only complicate interpretations of earlier vaccine studies but also raise questions about subunit approaches to vaccine development. In the present study, we sought to demonstrate protective immunity in the SIV-macaque model by immunization with recombinant viral subunit vaccines.

The approach we undertook was a combination immunization regimen that included the use of a live recombinant vaccinia virus for priming and a subunit immunogen for boosting. We have shown that rodents immunized with this combination regimen generated greater HIV-specific antibody responses than those that received either live recombinant virus or HIV-1 gp 160 alone [8]. The target antigen we chose for the present work is the envelope glycoprotein gp160 of SIV. Other studies have indicated partial protection in macaques immunized with either an envelope-enriched virion protein preparation [3, 9] or fusion proteins containing SIV envelope peptide sequences [10].

Using described methods [11, 12], we inserted the entire SIV env into the genome of either vaccinia virus (New York City Board of Health strain) [13, 14] or baculovirus (Autographa californica) [15]. The env we used was derived from an infectious molecular clone of SIVmne (clone 8), which was originally isolated from a pig-tailed macaque suffering from lymphoma [16]. This gene encodes a full-length surface glycoprotein, gp120, and a truncated form of the transmembrane protein, gp32 [17]. Both forms of the glycoproteins, as well as the precursor gp160, were expressed by the recombinant vaccinia virus, v-SE5, under the control of the early-late 7.5K promoter [18]. On the other hand, the SIV gp160 expressed in recombinant baculovirus (Ac-SE5) infected insect cells (Spodoptera frugiperda, Sf9) was not efficiently processed [19], as was observed previously in HIV-1 gp160

expressed by recombinant baculovirus [12]. The SIVmne gp160 expressed by Ac-SE5 was partially enriched by lentil lectin affinity chromatography to approximately 25 to 30% purity as determined by Coomassie staining of an SDS-polyacrylamide gel following electrophoresis [19].

Four macaques (M. fascicularis) were first immunized with v-SE5 by skin scarification in two inoculations 12 weeks apart; each inoculation was 1 x [10.sup.8] plaque-forming units per animal. After the primary immunization with v-SE5, all animals showed localized lesions ("takes"), between 7 and 14 days after inoculation, which reached maximal sizes between 1.5 to 2.5 cm in diameter. All lesions were healed by week 3, and no untoward effects of vaccination was observed. All animals seroconverted with low-titered antibodies to SIVmne gp120 and gp32. Their antibody titers increased after the second immunization with v-SE5 (Fig. 1A), despite minimal "takes". Total antibody titers declined 50 to 90% over 20 weeks after the peak level was reached (2 to 4 weeks after the second v-SE5 inoculation) but was still detectable by enzyme-linked immunosorbent assay (ELISA) [18] and by Western blot (Fig. 1) a year later. We also observed that animal 87210 (and 87217 to a lesser degree) developed reactivity to p28 after immunization (Fig. 1B). A similar phenomenon has been observed by Shafferman et al. [10] and was most likely due to antibodies generated against an epitope within gp32 that shares sequence homology with the gag antigen p28. Only low levels of neutralizing antibody were detected to the homologous virus (SIVmne CL E11S) and by a different assay to a closely related isolate, SIVmac251 (Fig. 2B).

Despite the weak antibody response, all animals immunized with v-SE5 showed activated SIV-specific helper T cell functions, as indicated by their lymphoproliferative responses to SIV antigens [18]. To enhance specific B cell responses to SIV envelope antigens, we used a subunit antigen boosting regimen shown to be successful in mice immunized with HIV-1 envelope antigens [8]. At weeks 62 and 70, all four v-SE5-immunized macaques were boosted intramuscularly with partially purified gp160 produced in baculovirus-infected insect cells. Two animals received gp160 (0.5 mg per dose of total protein) formulated in incomplete Freund's adjuvant and two in alum. Within 2 weeks of the first gp160 boost, all animals showed a dramatic (30-to 50-fold) increase in antibody response against SIV envelope antigens (Fig. 1A). This increase was also concordant with a significant rise in serum neutralizing activities against both SIVmne and SIVmac251 (Fig. 2). There was no significant difference between the antibody titers in animals that received gp160 formulated in alum (87210, 87221) as compared to those that received antigens formulated in incomplete Freund's adjuvant (87201, 87217). The antibody titers declined five- to tenfold during the next 8 weeks but seemed to stabilize after the second gp160 boost given at week 70.

To determine if the immunity generated was protective, we challenged at week 74 the four immunized animals, together with four control macaques of the same species with an intravenous inoculation of the homologous virus SIVmne. The challenge stock was derived from cell-free supernatants obtained from a single-cell clone of SIVmne-infected HUT-78 cells (CL E11S) [17], the same clone from which the molecular clone was derived. This stock has been titered in vitro and in vivo and has been shown to be infectious and pathogenic in multiple macaque species [20]. The challenge dose contained between 100 to 900 tissue culture infectious doses (TCID), corresponding to one to nine macaque infectious doses [20].

After the challenge, the four control animals seroconverted within 4 to 7 weeks (Fig. 3A). The antibodies developed were directed to the gag (p28, p16, p8, and p6), the x-ORF or sid (p14) [21], and the env (gp120 and gp32) antigens. The intensity of Western blot reactivity increased throughout the 39-week period after challenge. Virus was readily detected in cocultures of lymphocytes from the four animals beginning at 2 to 4 weeks after infection (Table 1). In contrast, all four immunized animals remained virus-negative (longer than 1 year after the challenge) with virus isolation (Table 1) and with polymerase chain reaction analysis (Fig. 4) [18]. An anamnestic response was not observed, consistent with the lack of viral replication (Figs. 2 and 3B). Furthermore, none of the immunized animals developed antibodies to the core antigens of SIV after challenge (Fig. 3B), with

Table 1. Detection of virus in macaques challenged with SIV mne. PBMC were isolated from heparinized blood samples at the indicated number of weeks after challenge. PBMC (4 X [10.sup.6]) were cultured with 5 X [10.sup.6] AA-2 CL1 cells [28]; phytohemagglutinin (1 [microgram]/ml) and interleukin-2 (10%) were present during the first 7 days of cocultivation. Reserve transcriptase assays were performed on culture fluids as described [29]. Number in parentheses indicate of culturing before virus was detected and are an approximate measure of the virus load (number of infected PBMC). (+) = virus isolated, (-) = no virus isolation after 10 weeks in culture. Challenge was with an intravenous injection of the homologous virus, SIV mne (CL E11S), at one to nine animal infectious doses per animal.

Animal	Virus isolation at the following weeks after challenge:								
	0	2	4	7	11	17	22	30	39
				Control					
88033	-	-	+(3)	+(2)	-	-	+(4)	-	-
89079	-	+(2)	+(3)	+(3)	+(2)	-	-	-	-
89134	-	+(2)	+(3)	+(2)	+(3)	+(5)	-	-	+(7)
89152	-	+(2)	+(2)	+(2)	+(3)	+(3)	+(7)	+(7)	+(6)
				Immunized					
87201	-	-	-	-	-	-	-	-	-
87210	-	-	-	-	-	-	-	-	-
87217	-	-	-	-	-	-	-	-	-
87221	-	-	-	-	-	-	-	-	-

the exception of the preexisting cross-reactivity to the gag antigen p28. Finally, no sign of infection was observed in four animals that were inoculated intravenously with lymph node cells (20 x [10.sup.6]) and peripheral blood monocular cells (PBMC) (10 x [10.sup.6]) collected from each of the four immunized animals at 46 weeks after challenge [22]. Taken together, these results indicate that a "sterilizing immunity" against the challenge infection was achieved in the immunized animals.

With few exceptions [9, 10], protection against SIV infection has only been achieved by immunization with inactivated whole virus vaccines [5]. However, the correlates of protection have not been identified. With the observation that uninfected cells could also immunize against SIV infection [7], the question remains whether immune responses to any viral antigens would be necessary or sufficient to confer protection. Because only recombinant viral antigens were used in the experiment reporter here, the possibility of anticellular immunity being responsible for the protection observed was remote. Indeed, we [18] and Langlois et al. [23] have shown that sera from these animals did not contain evidence of anticellular antibodies. Results presented here therefore demonstrate that SIV envelope antigens alone are sufficient to elicit protective immunity in macaques against a low-dose intravenous challenge by the homologous virus. Similarly, envelope glycoproteins of HIV-1 have also been shown to elicit protective immunity in the chimpanzee model [24].

A successful vaccine must be able to protect against multiple viral isolates. The immunized macaques described here generated antibodies that neutralized not only the homologous strain of SIV but also an uncloned stock of SIVmac251, which is approximately 9% divergent from SIVmne in the env region [25]. It remains to be shown whether recombinant subunit vaccines could protect against heterologous virus challenge, as has been demonstrated for whole inactivated SIV vaccines [26].

A combination immunization regimen similar to this model is now under evaluation in humans as candidate AIDS vaccines [27]. Although protection in the SIV system does not necessarily predict efficacy in humans against HIV-1 infection, our findings do argue for further testings of this combination immunization approach to define the limits and the correlates for protection in animal models and, ultimately, to determine efficacy in humans.

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[20] The in vitro titer of the challenge stock of SIVmne E11S was 1 X [10.sup.6] to 9 X [10.sup.6] tissue culture infectious dose (TCID) per milliliter on AA2 CL 1 cells. Rhesus macaques (three or four animals per group) inoculated intravenously with 1 ml of this stock virus diluted [10.sup.3]- or [10.sup.4]-fold were 100% infected, whereas one of four animals inoculated with [10.sup.5] dilution was infected and none at [10.sup.6] or higher (R. E. Benveniste and G. Eddy, in preparation). Of three rhesus macaques inoculated with E11S at [10.sup.3] dilution (10), all developed CD4 cell depletion and one died at 116 weeks after infection. E11S (at [10.sup.1] to [10.sup.3] dilution) has also been inoculated intravenously into six Macaca nemestrina at the Washington Primate Center. All animals died between 19 and 153 weeks after inoculation; four had CD4 cell depletion and the other two died with thrombocytopenia before significant CD4 cell depletion was evident (L. Kuller, W. M. Morton, and R. E. Benveniste, in preparation).

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