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Repeated Low-Dose Mucosal Simian Immunodeficiency Virus SIVmac239 Challenge Results in the Same Viral and Immunological Kinetics as High-Dose Challenge: a Model for the Evaluation of Vaccine Efficacy in Nonhuman Primates

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Received 26 August 2003/Accepted 4 November 2003

Simian immunodeficiency virus (SIV) challenge of rhesus macaques provides a relevant model for the assessment of human immunodeficiency virus (HIV) vaccine strategies. To ensure that all macaques become infected, the vaccinees and controls are exposed to large doses of pathogenic SIV. These nonphysiological high-dose challenges may adversely affect vaccine evaluation by overwhelming potentially efficacious vaccine responses. To determine whether a more physiologically relevant low-dose challenge can initiate infection and cause disease in Indian rhesus macaques, we used a repeated low-dose challenge strategy designed to reduce the viral inoculum to more physiologically relevant doses. In an attempt to more closely mimic challenge with HIV, we administered repeated mucosal challenges with 30, 300, and 3,000 50% tissue culture infective doses (TCID50) of pathogenic SIVmac239 to six animals in three groups. Infection was assessed by sensitive quantitative reverse transcription-PCR and was achieved following a mean of 8, 5.5, and 1 challenge(s) in the 30, 300, and 3,000 TCID50 groups, respectively. Mortality, humoral immune responses, and peak plasma viral kinetics were similar in five of six animals, regardless of challenge dose. Interestingly, macaques challenged with lower doses of SIVmac239 developed broad T-cell immune responses as assessed by ELISPOT assay. This low-dose repeated challenge may be a valuable tool in the evaluation of potential vaccine regimes and offers a more physiologically relevant regimen for pathogenic SIVmac239 challenge experiments.

Worldwide, there are an estimated 42 million people who are currently living with human immunodeficiency virus (HIV). Heterosexual transmission is the predominant route of viral infection, particularly in Asia and sub-Saharan Africa where more than 35 million people are currently infected (29). The risk of HIV infection is affected by multiple factors that include transmission route, frequency of sexual contact, genetic predisposition, and immunocompetence of the individual (6, 9, 20). The frequency of HIV infection, particularly among women, has risen steadily, and there are twice as many young women (aged 15 to 24 years) as men that are currently infected with HIV in sub-Saharan Africa (29). According to the Joint United Nations Programme on HIV/AIDS, approximately 58% of HIV-infected individuals in sub-Saharan Africa are women and 9% are children (29). Both sexual and perinatal transmission of HIV are associated with a high plasma viral load (10, 14, 20, 23, 25, 27, 28).

Access to new and effective antiretroviral drugs is limited, and 5 million more people were infected during 2002 (29). Development of an effective vaccine strategy is therefore paramount. The majority of HIV vaccines in current clinical trials target cytotoxic T lymphocytes (CTL) because the generation of broadly neutralizing antibody response has been difficult to achieve (13, 22). Vaccines that specifically induce CTL have been tested in vaccinated macaques that were challenged with high doses of either simian-human immunodeficiency virus (SHIV) or simian immunodeficiency virus (SIV) (1, 4, 31). However, to evaluate such vaccines in the macaque model, a clinically relevant challenge is crucial to vaccine development. To date, even though amelioration of the disease course has been observed after challenge with the chimeric SHIV89.6P...
virus (3, 4, 8, 21, 24), few vaccination strategies have managed to significantly curtail the progression to simian AIDS (SAIDS) in animals challenged with highly pathogenic SIVs (239, 251, or E660) (5, 7). However, we and other groups have used SIVmac239 at doses of 10^3 to 10^5 50% tissue culture infective doses (TCID_{50}) when challenging animals for the evaluation of potential vaccines (2, 18). These high-dose challenges ensure that all control animals become infected after a single exposure. However, SIV challenge following administration of a potential vaccine should ideally reflect the rate of transmission with HIV. The actual dose of HIV transmitted via sexual contact has been investigated but has proved to be dependent upon the type of model used (6, 10, 26). A study in sub-Saharan Africa showed a correlation between plasma viral loads in excess of 35,000 copies/ml and transmission to HIV-negative partners. Conversely, individuals with fewer than 1,500 copies/ml were less likely to transmit the virus (10, 20). Therefore, it is likely that the rate of transmission depends upon the concentration of the virus in the inoculum. Unfortunately, the recovery and detection of virus in semen has proved difficult, and concentrations ranging from <10^3 to >10^5 HIV RNA copies/ml of seminal plasma have been reported previously (6, 30). The routine mucosal challenge inoculum used in nonhuman primate SIV challenge studies far exceeds the amount of HIV in semen and can be in excess of 8 x 10^7 SIV RNA copies/ml. Here we investigate whether a more relevant low-dose viral challenge can infect and cause disease in Indian rhesus macaques.

To achieve a low-inoculum dose, we repeatedly challenged six animals intrarectally according to the strategy depicted in Fig. 1. Animals 1941 and 96107 received 30 TCID_{50}, AJ10 and AJ11 received 300 TCID_{50}, and 97009 and 98019 received 3,000 TCID_{50}. Challenge was repeated after 2 weeks and then at weekly intervals thereafter until infection was detected. The plasma virus concentration was tested prior to the next weekly dose; if positive, the challenges for that animal were terminated. From the time of the initial challenge, the animals were screened regularly for humoral and cellular immune responses by enzyme-linked immunosorbent assay and whole-proteome ELISPOT assay, respectively.

Repeated low-dose challenge results in SIVmac239 infection. Quantitative reverse transcription-PCR (RT-PCR) was used for early virus detection and determination of plasma viral concentration as described previously. Viral RNA was

![Graph showing number of challenges administered to each animal to establish a SIVmac239 infection with respect to dose.](http://jvi.asm.org/)

![Graph showing longitudinal SIVmac239 plasma viral concentration and antibody responses for each low-dose-challenge group. The plasma viral concentration and antibodies were detected as outlined in Materials and Methods. Animals were euthanized due to SAIDS-related illness at the times indicated. The major histocompatibility complex alleles were determined for each animal by using methods described previously (12). OD_{450}, optical density at 450 nm.](http://jvi.asm.org/)
extracted from 800 \mu\text{l} of plasma after the pelleted virus was digested with 1 mg of proteinase per ml, and viral RNA was precipitated. Plasma viral RNA concentrations were determined by quantitative RT-PCR using the Roche LightCycler (Roche Diagnostics Corporation, Columbus, Ind.). The forward primer was SIV-61F (5’-CCACCTACATTAGCCGA-3’), the reverse primer was SIV-143R (5’-CTGGCACTACTTCTGCTCCAAA-3’), and the probe was SIV-84T (FAM reporter, TAMRA quencher) [5’-CATTAAATGGCTGAGTGAATGAGGAG(A/G)AAGAA-3’]. Cycling conditions were as follows: 61°C for 15 min, 95°C for 30 s, 45 cycles at 95°C for 2 s, and 60°C for 12 s. Data were collected at the end of the extension phase only. As expected, SIVmac239 was detected in the plasma from animals 97009 and 98019 following a single challenge with 3,000 TCID\text{50} of SIVmac239 (Fig. 2). However, AJ10 and AJ11 were challenged eight and three times, respectively, (mean, 5.5 challenges) with 300 TCID\text{50} before they became infected. The lowest dose of 30 TCID\text{50} was administered 10 and 6 times (mean of eight challenges) to animals 1941 and 96107 to establish SIVmac239 infection (Fig. 2). Longitudinal analysis of the plasma viral concentration from all groups revealed similar kinetics regardless of inoculum or number of infections the animals received (Fig. 3). The plasma viral concentration peaks were in the expected range of 10\text{7} to 10\text{8} copies/ml, which subsequently reduced to approximately 10\text{6} copies/ml—a finding typical for this virus (Fig. 3) (19). The plasma viral concentration produced by low-dose challenge showed no difference when compared to that of 10 Mamu-B*17-negative animals exposed to a high-dose challenge (Fig. 4A). However, one animal, AJ11, was challenged three times with 300 TCID\text{50} of SIVmac239 and had a peak viral load of only 10\text{5} copies/ml, a plasma viral concentration 10-fold lower than that of the other animals that received low doses of SIVmac239. Indeed, following this peak plasma virus concentration, plasma virus in AJ11 became undetectable by our sensitive quantitative RT-PCR technique. Interestingly, this animal (AJ11) is Mamu-B*17 positive, an allele associated with the control of SIVmac239 (19). When compared to plasma viral concentrations in 13 other Mamu-B*17-positive macaques, the concentration of plasma virus was found not to differ in the acute phase, despite the high-dose challenge of SIVmac239 administered to these animals (Fig. 4B). Interestingly, comparison of the chronic-phase plasma virus concentration of AJ11 and the plasma virus concentration of the Mamu-A*01, B*17 double-positive long-term nonprogressors (shown in red in Fig. 5B) showed no difference. Animals 97009, 98019, AJ10, and 1941 were sacrificed due to SAIDS-related diseases at 26, 22, 26, and 10 weeks postinfection, respectively. We therefore conclude that low doses of SIVmac239 were pathogenic in these rhesus macaques regardless of dose or number of challenges received, and these findings fall within our expected range of life span with this virulent
virus (19). Two animals remain asymptomatic to date (at 35 weeks postinfection), 96107 and AJ11, with the former having a set point viral load of $>10^6$ copies/ml, and we are still unable to detect plasma virus in the latter Mamu-B*17-positive animal.

**Humoral and cellular immune responses.** To investigate whether low-dose repeated challenge may result in antibody responses that differ from those seen in macaques exposed to a high-dose viral challenge, we monitored SIV-specific plasma antibody responses by using the commercially available HIV type 2-specific enzyme-linked immunosorbent assay kit from the Genetic Systems Corporation/Sano-Pasteur Diagnostics (Redmond, Wash.). The detection of antibody from the low-dose, repeatedly challenged animals produced the same kinetics as that seen in high-dose-challenge animals.

It is possible that infection with a low-dose viral inoculum might allow for the development of broad and vigorous SIV-specific cellular responses. In order to monitor the development and breadth of cellular responses before and after each virus challenge, we screened the animals regularly according to our animal protocols. Therefore, to assess SIV-specific T-cell responses, we used a gamma interferon ELISPOT assay (U-Cytech BV, Utrecht, The Netherlands) at regular intervals (Fig. 5) during the repeated intrarectal challenges using freshly isolated Ficoll-separated peripheral blood mononuclear cells ($10^5$/well). Peptide pools made up of 10 15-mers overlapping by 11 amino acids (final concentration, 5 $\mu$g/ml), which spanned the entire SIVmac239 proteome, were used to detect T-cell immune responses as described previously (11, 15–17). The peripheral blood mononuclear cells and peptides were coincubated for 16 to 18 h, and the spots were visualized directly by using an AID reader system (Cell Technologies, Inc., Columbia, Md.). Responses were considered positive when the frequency of gamma interferon-secreting T cells exceeded the mean spot-forming cell count of the negative controls plus twice the standard deviation. In all of the study animals, we detected T-cell responses across the SIVmac239 proteome from the earliest time point tested after successful intrarectal challenge until the time of sacrifice. At more than 27 weeks postinfection, the two animals that are still alive, AJ11 and 97106, had broad T-cell responses to 27 and 24% of the SIVmac239 pools tested, respectively (Fig. 4). Analysis of the average number of pools recognized by animals in the different groups shows that, at time points before 11 weeks, 11.5 (14%), 19.5 (23%), and 46.5 (56%) SIVmac239 pools per animal were recognized in the 3,000, 300, and 30 TCID$_{50}$ groups, respectively. This would suggest that more immune responses are recognized in the lower-dose-challenge inoculum groups than in the 3,000-TCID$_{50}$ dose of SIVmac239 early in infection. However, given the small number of animals and the inherent genetic variation in outbred rhesus macaques, this trend may not be significant.

Our low-dose-challenge experiments clearly show that infection can be achieved by repeated exposure to low virus doses across mucosal surfaces. Infection with these low virus doses results in typical plasma virus concentrations and the normal
development of humoral immune responses. Furthermore, broad, multiple-epitope-specific cellular immune responses develop in animals that are infected with repeated exposure to low doses of SIVmac239. Since these repeated low-dose exposures more closely resemble exposure to HIV, it will be interesting to determine whether vaccine-induced CTL in the mucosa can ameliorate the disease course after challenge with a low dose of this highly pathogenic SIVmac239 clone.

We thank Bill Rehrauer and Tim Jacoby for major histocompatibility complex complex topics. We also thank Eva Rakasz for assistance with the antibody titers and for helpful discussions.

This work is supported by NIH grants AI46366, AI49120, RR15371, and RR00167. David Watkins is an Elizabeth Glaser Scientist.

REFERENCES


