A vaccine strategy against AIDS: An HIV gp41 peptide immunization prevents NKp44L expression and CD4$^+$ T cell depletion in SHIV-infected macaques

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We previously showed that a gp41 peptide (3S) induces expression of a natural killer (NK) ligand (NKp44L) on CD4$^+$ T cells during HIV-1 infection and that those cells are highly sensitive to NK lysis. In HIV-infected patients, anti-3S antibodies are associated with the maintenance of CD4$^+$ T cell counts close to their baseline values, and CD4$^+$ T cells decrease with the antibody titer. This study sought to determine whether anti-3S immunization could prevent NKp44L expression on these CD4$^+$ T cells *in vivo* and inhibits the subsequent decline in CD4$^+$ T cell counts by immunizing macaques with 3S and then infecting them with simian HIV$_{162P3}$. The results show that anti-3S antibodies inhibited NKp44L expression and NK activity and cytotoxicity. They also decreased the apoptosis rate of CD4$^+$ T cells in peripheral blood and lymph nodes. These data raise questions about the pathogenesis of HIV and present opportunities for both preventive and therapeutic HIV vaccine strategies.

Although CD4$^+$ T cell depletion appears to be the principal component of HIV disease, its underlying mechanisms remain controversial. Numerous reports offer a wide variety of tentative explanations for immune depression, including cell activation, the virus's direct cytopathic effects, and toxicity caused by its pathogenic determinants (1–4). One of the most intriguing phenomenons, however, is that many of the CD4$^+$ T cells that die during HIV infection are not infected (5). These cells must have died or been killed by a collateral effector mechanism not directly linked to viral replication. One plausible such mechanism is the expression of a natural killer (NK) ligand on CD4$^+$ T cells during HIV infection: we (6) demonstrated that NKp44L, the ligand of NKp44 NK receptor, is expressed on CD4$^+$ T cells of HIV-infected patients and showed that cells expressing NKp44L are highly sensitive to NK lysis. Ward *et al.* (7) have recently confirmed the specific expression of NKp44L on CD4$^+$ T cells after *in vitro* infection with HIV-1.

We also showed that a highly conserved motif of HIV gp41 envelope protein interacts with CD4$^+$ T cells to induce NKp44L (6). Humans at early stages of HIV infection produce antibodies against this peptide motif (called 3S) that can inhibit its *in vitro* expression, but this anti-3S antibody production decreases sharply thereafter. Although these antibodies did not neutralize the virus, they were associated with CD4$^+$ T cell counts and their rates of decrease. The antibody titer was also inversely associated with NKp44L expression (8). Together these results question the feasibility of immune intervention against gp41 to prevent the consequences of HIV infection.

Most studies, which have attempted to stimulate specific immune responses against gp41 HIV protein, have been applied to induce HIV neutralization. Many of those studies demonstrate that neutralizing Abs can protect against HIV-1 infection *in vitro* and in animal models, but *in vivo* proof of their activity in infected humans remains circumstantial (9, 10). Trkola *et al.* (11) showed that HIV-1 delay rebounded rapidly after cessation of antiretroviral therapy through passive transfer of the neutralizing Abs 2G12, 2F5, and 4E10 against gp41 epitopes. During the natural course of HIV infection, fully functional variants continuously emerge and compete for outgrowth in the presence of a rapidly evolving neutralizing Ab response, which exerts a high level of selective pressure. Non-neutralizing epitopes, which are usually very conserved, could also be the targets of immune intervention (10, 12). In that respect, the sharp conservation of the 3S-motif among all viral isolates (6) suggests that 3S-based peptides provide a major B-cell epitope that should be considered for use to limit virus pathogenicity, independently of pathogen replication.

The present study sought to determine whether anti-3S immunization could prevent NKp44L expression on these CD4$^+$ T cells *in vivo* and inhibit the subsequent decline in CD4$^+$ T cell counts by immunizing macaques with 3S-peptide and then infecting them with simian HIV (SHIV)$_{162P3}$.

Results and Discussion

Anti-3S Production After 3S-Keyhole Limpet Hemocyanin (KLH) Immunization in Macaques. Uninfected macaques were immunized with 3S peptide coupled to KLH carrier protein or KLH alone and subsequently infected by i.v. injection of SHIV$_{162P3}$, a CCR5-tropic virus. Profiles of viremia and CD4 count in SHIV162P3-infected *cynomolgus* macaques closely resembles naturally transmitted HIV strains in human patients (13, 14). All animals immunized with 3S-KLH, initially at monthly intervals, developed much stronger immune responses against 3S, whereas the animals immunized with KLH alone did not (Fig. 1*A*). Furthermore the macaque anti-3S antibodies had the functional capacity to prevent *in vitro* NKp44L expression on CD4$^+$ T cells, as previously reported for antibodies from HIV-infected patients (8). Sera from animals immunized against 3S (hereafter referred to as the immunized animals), but not from animals immunized by KLH alone (hereafter the controls), totally inhibited NKp44L expression on normal CD4$^+$ T cells incubated with 3S-peptide (Fig. 1*B*).

3S-KLH Immunization Decreases NKp44L Expression on CD4$^+$ T Cells of SHIV-Infected Macaques. Next, we compared viral load and NKp44L expression on CD4$^+$ T cell depletion in the immunized and control animals after they were infected by SHIV. The infection slightly enhanced anti-3S Ab responses of the immunized animals, which remained at high titers (Fig. 2*A*). In contrast, the antibody level in control animals was lower by a factor of ≈10. These antibodies appeared also later and decreased with time. As expected given that

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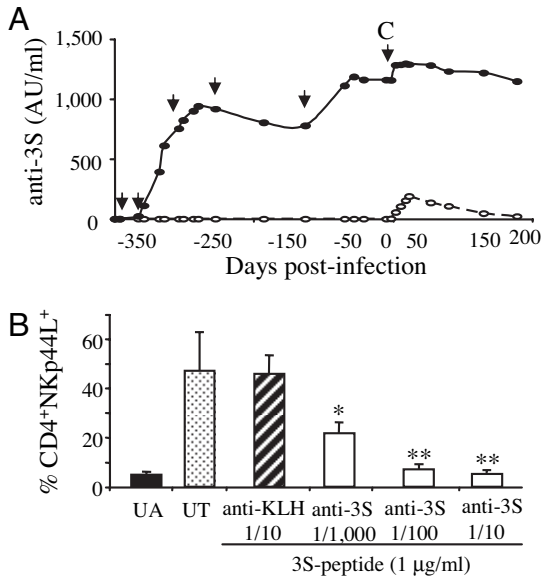

Fig. 1. Evaluation of anti-3S peptide immunization in assays of serum from SHIV-infected macaques. (*A*) Anti-3S antibodies (mean ± SD) are strongly detected in serum of macaques immunized with 3S/KLH (closed symbols, black line) and not in control KLH (open symbols, dotted line). Symbols I1 to I5 indicate the several immunizations, and C indicates the challenge with $SHIV_{162P3}$ on day 0. (*B*) NKp44L expression was strongly inhibited by serum (on day 0) from KLH/3S-immunized macaques in $CD4^+$ T cells. Peripheral blood mononuclear cells (PBMCs) from uninfected macaques were IL-2-activated and then not treated (UA; black bar) or treated with 3S peptide alone (UT; dotted bar) or in the presence of pooled sera from immunized animals on day 0 of SHIV infection. The IL-2-activated PBMCs were treated as described (8) with a 1/10 dilution of the pool from the six KLH-immunized animals (hatched bar) or with serial dilutions ranging from 1/1,000 to 1/10 of the pooled sera from the four KLH/3S-immunized animals (open bars). The data represent the mean ± SD of three uninfected animals. UT bars served as reference for the statistical analysis. **, $P < 0.01$; *, $P < 0.05$.

anti-3S antibodies had no neutralizing effect on viral infection *in vitro* (8), plasmatic viral load did not differ between the two groups of animals (Fig. 2*A*): all animals showed a similar peak followed by a drop in viral load. Similarly, viral load did not significantly differ in lymph nodes at day 21 in the immunized and control animals ($P = 0.20$; data not shown). Importantly, NKp44L expression on $CD4^+$ T cells differed significantly. In control animals, the percentage of cells expressing NKp44L increased over time, reaching 40% at day 42 and remained constant. In contrast, immunization before SHIV infection drastically inhibited NKp44L expression on CD4 T cells. However in one animal (no. 14599), NKp44L increased consistently after day 80 while remaining lower than controls. Therefore, although anti-3S antibodies did not affect viral replication, they suppressed *in vivo* NKp44L expression on $CD4^+$ T cells of SHIV-infected animals, as $CD4^+$ T cells incubated with anti-3S Abs did *in vitro* (Fig. 2*B*). The 3S-gp41 motif is localized between the N-terminal heptad repeat 1 (HR1) and the HR2 domains. Several models indicate that this region may have contact with the host cell membrane during the formation of the ectodomain core structure (15–17). Although anti-3S Abs had no neutralizing activities, our data also indicate that this 3S motif is transiently accessible during *in vivo* infection and is recognized by anti-3S Abs, which prevent its pathogenic effect on $CD4^+$ T cells.

Protective Effect of 3S-KLH Immunization on $CD4^+$ T Cells Decline. Hypothesizing that anti-3S antibodies would subsequently affect the decline in the $CD4^+$ T cells after SHIV infection, we compared the two groups to assess any effects on percentage and counts of $CD4^+$ T cells in peripheral blood and lymph nodes. The percentage of $CD4^+$ T cells observed over time after infection differed very significantly in the two groups (Fig. 2*C*). In control animals, the frequency of peripheral blood $CD4^+$ T cells decreased progressively; the difference compared with basal values increased with time ($P = 0.03$ at day 42 and $P = 0.05$ at day 188). In immunized animals, on the other hand, those percentages remained stable, never differing significantly from the values observed before infection ($P = 0.12$ at days 42 and 188). Similar differences were observed in lymph node $CD4^+$ T cells in the two groups (data not shown). Note that the peripheral blood $CD4^+$ T cell count reflected these differences. Counts fell significantly in control animals, whereas in immunized animals they never differed significantly from preinfection values (Fig. 2*D*). These results, which relate NKp44L expression to $CD4^+$ T cell depletion, were consistent with our earlier findings in HIV-infected patients and led us to hypothesize that NKp44L expression, by augmenting NK cell cytotoxicity, might be indirectly responsible for $CD4^+$ T cell depletion (6).

3S-KLH Immunization Protects $CD4^+$ T Cells from Apoptosis. Because clear differences were observed as early as 42 days after infection, we sought further proof by comparing apoptosis of $CD4^+$ T cells in immunized and control animals infected by SHIV during the period. Interestingly, anti-3S immunization induced a clear and significant reduction in $CD4^+$ T cell apoptosis, which persisted over time, together with the high level of anti-3S response (Fig. 3*A*). Similar significant differences were observed in lymph nodes (Fig. 3*A*). Furthermore, nearly all apoptotic cells expressed NKp44L, and this expression was clearly related to cell death. No significant cell apoptosis was detected in peripheral blood or lymph node cells that did not express this ligand (Fig. 3*B*). We note, however, that neither apoptosis alone nor 3S-associated $CD4^+$ T cell apoptosis induced NKp44L expression in $CD4^+$ T cells (data not shown).

Taken together, these results suggest that NKp44L is strongly associated *in vivo* with cell death during HIV infection and that the 3S motif is indirectly responsible for this cell death, because anti-3S antibodies control apoptosis. Similar correlation between NKp44L expression and apoptosis was also observed in $CD4^+$ T cells of HIV patients (unpublished data). However, although NKp44L expression appears closely related to apoptosis, not all $NKp44L^+$ T cells are apoptotic. Indeed, apoptotic cells accounted for <20% of $NKp44L^+$ cells on day 14 in control animals, a percentage that increased with time and reached 40% by the end of the study period (Fig. 3*C*). The same phenomenon was observed to a lesser degree in immunized animals, even though their rate of $CD4^+$ T cell apoptosis was significantly lower (Fig. 3*C*). These data suggest that a second event might be required to induce apoptosis.

3S-KLH Immunization Decreases NK Activation and Cytotoxicity. We next postulated that this second event necessary to induce the death of $CD4^+NKp44L^+$ cells was caused by the effect of NK activation and cytotoxicity directed at cells expressing NKp44L. To test this hypothesis, we compared these indicators in immunized and control animals. As Fig. 4 shows, even though the number of NK cells did not differ in the two groups (Fig. 4*A*), activation ($CD3^-CD8^+CD69^+$; Fig. 4*B*) and cytotoxicity (Fig. 4*C*) of NK cells were clearly lower in immunized than control animals. This was the case in both peripheral blood and lymph nodes (data not shown). In three of four animals, NK cytotoxicity in immunized animals did not differ from the level observed in both groups before SHIV infection. In animal 14599, which showed an increase of NKp44L after day 80, there was a parallel increase of NK cytotoxicity after such a period (Fig. 4*C*). NK cytotoxicity was highly correlated with both NK cell activation (Fig. 4*D*) and NKp44L expression (Fig. 4*E*) in both peripheral blood and lymph node cells (data not shown). These results considered together suggest that apoptosis induced by SHIV infection is the consequence of two sets of events, the first

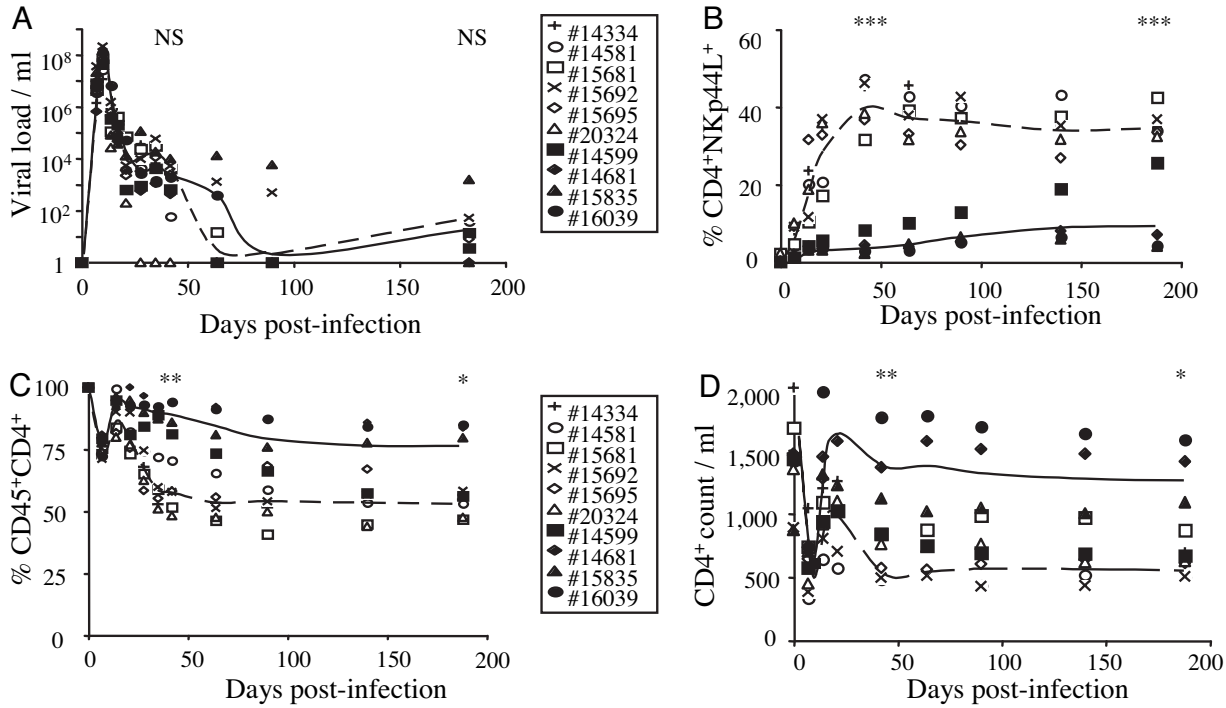

Fig. 2. Relative stability of the CD4 cells count after anti-3S immunization. (*A*) Similar viral load in macaques immunized with KLH/3S (closed symbols, black line) and KLH (open symbols, dotted line). Lines indicate the median values. (*B*) Very low level of NKp44L expression on $CD4^+$ T cells from macaques immunized with KLH/3S (closed symbols, black line) compared with control KLH (open symbols, dotted line). (*C* and *D*) Percentage (*C*) and absolute count (*D*) of $CD4^+$ T cells in peripheral blood from macaques immunized with KLH/3S (closed symbols; black line) and control KLH (open symbols, dotted line). Lines indicate the mean values. ***, $P < 0.005$; **, $P < 0.01$; *, $P < 0.05$; NS, not significant.

related to NKp44L expression, the second related to NK cell activation and cytotoxicity. Most important, they also demonstrate that anti-3S antibodies can prevent these related phenomena.

In summary, anti-3S immunization preceding SHIV infection of *cynomolgus* macaques prevented both NKp44L expression on $CD4^+$ T cells and the activation and cytotoxicity of NK cells. It

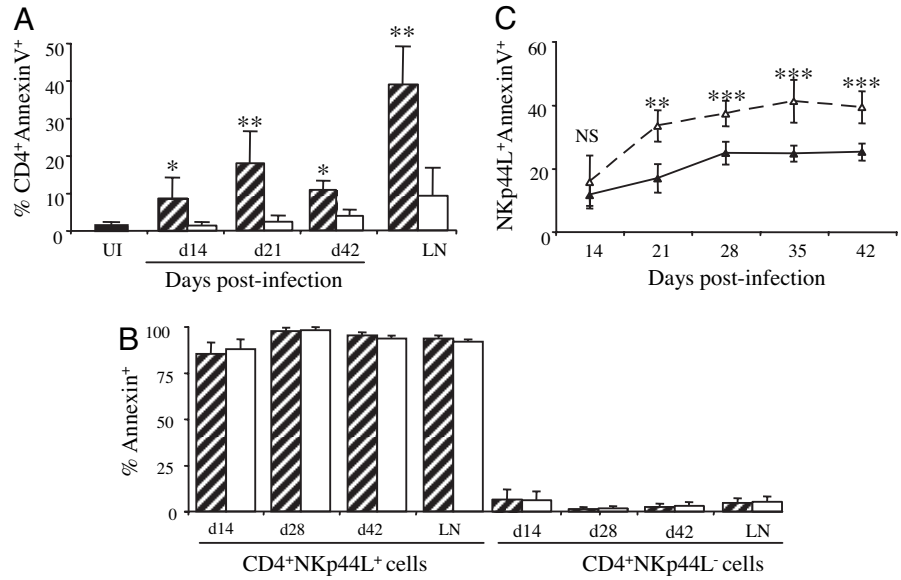

Fig. 3. Protection against $CD4^+$ T cell apoptosis by anti-3S peptide immunization. (*A*) Lower expression of annexin V on $CD4^+$ T cells from macaques immunized with KLH/3S (open bars) than with control KLH (hatched bars) at 14, 21, and 42 days after SHIV infection. A similar experiment was performed on lymph nodes (LN) collected at 37 days postinfection. As a control, annexin V frequency was determined in three uninfected animals (closed bar). (*B*) Inside the apoptosis (annexin V-positive) subpopulation, almost all cells expressed NKp44L in both groups: animals immunized by KLH/3S (open bars) or by control KLH (hatched bars) at 14, 21, and 42 days postinfection and in lymph nodes (LN). $CD4^+NKp44L^+$ (*Left*) and $CD4^+NKp44L^-$ (*Right*) cells that do and do not express NKp44L, respectively, are shown. (*C*) Lower expression of annexin-V on $CD4^+$ T cells that expressed NKp44L from macaques immunized with KLH/3S (black line) compared with control KLH (dotted line). Lines indicate the means; ***, $P < 0.005$; **, $P < 0.01$; *, $P < 0.05$.

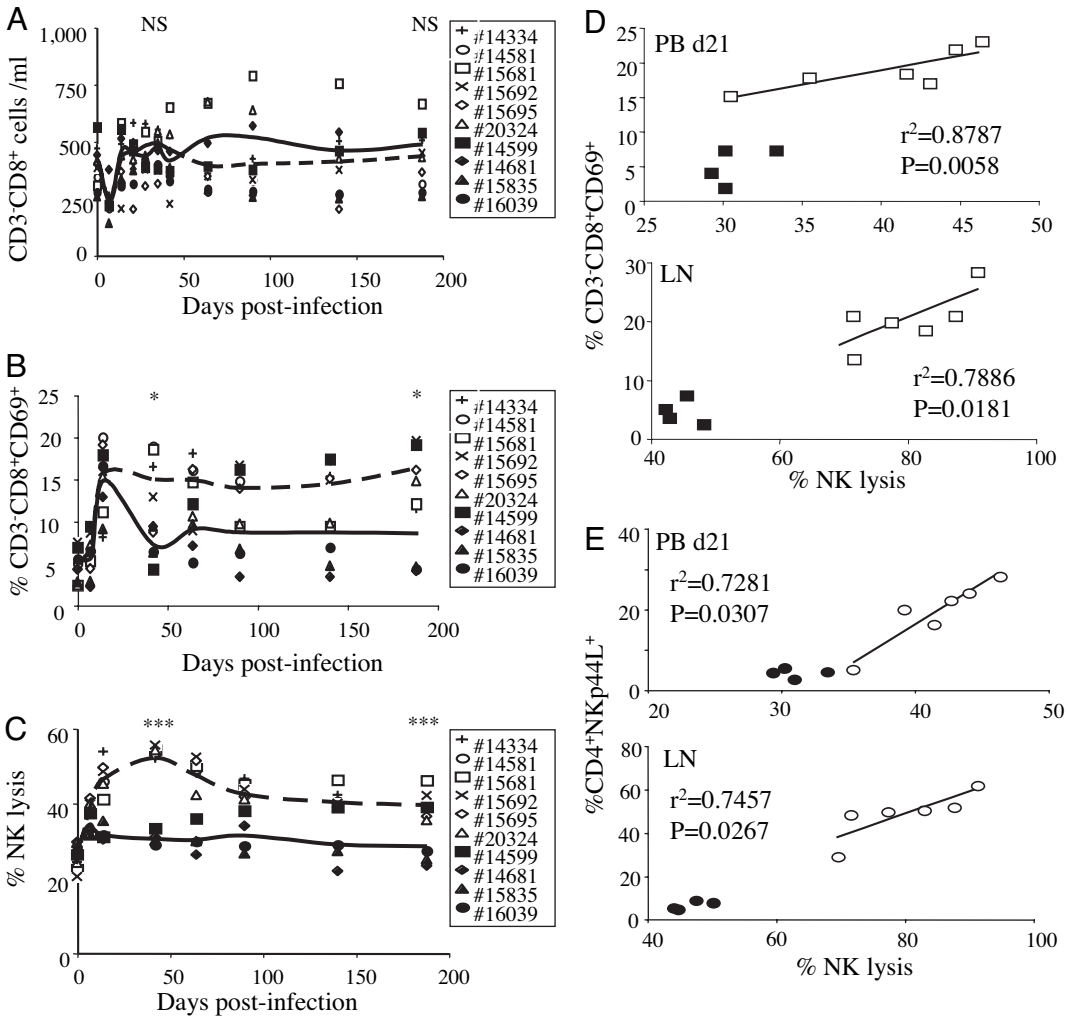

Fig. 4. Critical role of anti-3S immunization in the inhibition of NK activities. (*A*) Similar NK cell counts ($CD3^-CD8^+$) in peripheral blood from macaques immunized with KLH/3S (closed symbols; black line) and control KLH (open symbols, dotted line). Lines indicate the mean values. (*B*) Lower expression of CD69-activated marker on NK cells from animals immunized with KLH/3S (closed symbols; black line) compared with control KLH (open symbols, dotted line). A similar experiment was performed on lymph nodes (LN) collected at 37 days postinfection (data not shown). Lines indicate the mean values. (*C*) Lower NK lysis in macaques immunized with KLH/3S (closed symbols; black line) than control KLH (open symbols, dotted line). A similar experiment was performed on lymph nodes (LN) collected at 37 days postinfection (data not shown); Lines indicate the mean values. ***, $P < 0.005$; **, $P < 0.01$; *, $P < 0.05$; NS, not significant. (*D*) Correlation between percentage of activated NK cells ($CD3^-CD8^+CD69^+$) and NK lysis in peripheral blood at 21 days postinfection (*Upper*) and in lymph node at 37 days postinfection (*Lower*). (*E*) Correlation between NK lysis and percentage of $NKp44L^+$ $CD4^+$ T cells, in peripheral blood at 21 days postinfection (*Upper*) and in lymph nodes at 37 days postinfection (*Lower*). A black line reports the regression linear curve for the six control KLH-immunized animals.

therefore prevented a decline in $CD4^+$ T cells but had no effect on viral load. This effect was long lasting for three of four animals. In one animal, however, there was an escape phenomenon after day 80: a late increase of NKp44L was accompanied by CD4 cells drop and increase of NK cytotoxicity, emphasizing the relationship between these parameters. Taken globally, these results confirm in a macaque model of AIDS what we previously showed in humans, that the 3S-gp41 epitope can induce NKp44L expression (6). They also show clearly that *in vivo* ligand expression on $CD4^+$ T cells is related to this 3S interaction. The finding that all apoptotic cells expressed NKp44L demonstrates that expression of this ligand by target cells is a prerequisite for their *in vivo* apoptosis during SHIV infection. Because the 3S peptide by itself did not induce apoptosis, nor did apoptosis induce NKp44L expression, our results also indicate that NKp44L may increase the susceptibility of a population of $CD4^+$ T cells to an external death signal.

These findings, which show the strong relation of NKp44L expression and apoptosis to NK activation and cytotoxicity, provide support for the hypothesis that this second external event is caused by NK cytotoxicity. Numerous reports show that NK cells exhibit a variety of different behaviours during HIV-1 infection (18). Interestingly, the increased NK activity in some HIV-exposed patients suggests that NK cells may help protect against infection (19). Conversely, several studies show alterations in the number and function of NK cells during HIV infection and progression to AIDS (20, 21). On the other hand, various distinct allelic combinations of the NK inhibitory receptor KIR3DL1 and HLA-B loci significantly and strongly influence both AIDS progression and plasma HIV RNA abundance (22). Yet, none of the above studies referred to a correlation between the level of NK activity and the NK ligand expression. It is clear that further experiments are needed to conclusively demonstrate the role that NK cells may have *in vivo*. However, our previous *in vitro* data, together with the results reported herein, support the hypothesis of NK cells' deleterious effect and the importance of NKp44L in AIDS pathogenesis.

Our results also emphasize the fact that the 3S motif is a pathogenic viral determinant, which strongly affects disease progression, most especially the $CD4^+$ T cell depletion. These results demonstrate that a key epitope induces such pathogenic phenomena *in vivo*. Although these data do not totally rule out the possibility that other factors contribute to $CD4^+$ T cell depletion, they strongly support the hypothesis that the 3S viral peptide plays a major role in the immune depression of HIV and SHIV infection. They may provide insight to improve our understanding of the lack of pathogenicity of natural SIV lentivirus infection in African green monkeys, a pathogenic lentivirus to the natural host, and the different factors that might control viral burden and pathogenicity (23, 24).

More importantly, anti-3S immunization appears to prevent $CD4^+$ T cell depletion in pathogenic infection. Additional experiments should demonstrate whether anti-3S vaccines or monoclonal antibodies or both have a therapeutic effect in infected individuals, by limiting $CD4^+$ T cell depletion or promoting immune restoration during continued viral replication. Further studies are also needed to understand mechanisms of escape observed in one animal after day 80. The results reported herein open the way for additional strategies of immune intervention aimed at controlling disease development. Yet rather than choosing between a vaccine strategy against pathogenicity proved effective against tetanus, diphtheria and cholera bacteria, that is, inoculation against toxins, and a different strategy aiming at neutralizing the virus (25–27), we submit that these both types of preventive vaccinations should be envisioned as complements.

In conclusion, our results raise questions about our understanding of HIV pathogenesis and present opportunities for prevention and treatment of the CD4 immune depression induced by HIV-1.

Materials and Methods

Vaccination Protocol and Virus Challenge. Ten adult *cynomolgus* macaques (*Macaca fascicularis*), each weighing 4–6 kg, were imported from Mauritania. They were housed in individual cages in level 3 biosafety facilities. All experimental procedures were conducted in compliance with European Community legislation for animal care. Four animals were immunized with 200 μg of 3S-peptide coupled with KHL in incomplete Freund's adjuvant (IFA) and boosted five times at -55, -50, -46, -42, and -16 weeks before SHIV infection. Two other animals were immunized with 200 μg of KLH alone in IFA following the same immunization schedule, and four more control macaques received only the last KLH injection.

Sixteen weeks after the last immunization, all animals were challenged i.v. with 1 ml of pooled plasma of *cynomolgus* macaques collected at peak of viremia during primary infection (days 12–17 postinfection) after intrarectal inoculation of pathogenic $SHIV_{162P3}$, as described (13), obtained from the National Institutes of Health AIDS Research and Reference Reagent Program (catalog no. 6526). This pool of plasma contained 46.6×10^8 copies of viral RNA per milliliter. Viral RNA copies in plasma of challenged macaques was quantified by RT-PCR by using primer pairs for gag as described (28). Detection limit is 60 viral RNA copies per milliliter.

Flow Cytometric Analysis. Four-color FACS analysis was performed on freshly harvested blood cells. Isotype-matched Ig served as the negative control (BD Biosciences PharMingen). Briefly, 100 μl of peripheral blood was stained for 30 min, at room temperature under gentle agitation, with an appropriate antibody mixture provided by BD Biosciences Pharmingen including anti-CD45 (TÜ116), anti-CD3 (SP34), anti-CD4 (L200), and anti-CD8 (RPA-T8). NKp44L expression was determined by using anti-NKp44L mAb (no. 7.1, IgM), as described (5). After staining, the cells were washed on PBS and then the erythrocytes were gently lysed with 1 ml of the FACS lysing solution kit (BD Biosciences Pharmingen), for 10 min under slow agitation (250 rpm at room temperature). After extensive washing in PBS, and resuspension with 300 μl of PBS, at least 30,000 events were analyzed on a FACScalibur (BD Biosciences Pharmingen). Results were analyzed with CellQuest Pro software (BD Biosciences Pharmingen) and expressed as the percentage of all mAb-positive $CD45^+$ cells, without discriminate on the FCS/SSC profile.

NK Cytotoxicity Assay and ELISA. Cytotoxicity of NK cells from peripheral blood samples was evaluated in a 4-h ^{51}Cr release assay, as described (6), against the MHC class I-deficient erythroleukemia K562 cell line at several effector-to-target cell ratios. Quantification of anti-3S antibodies was performed by ELISA, as described (8). Anti-3S antibody quantities were expressed in arbitrary units (AU). This test has a detection limit of 10 AU/ml.

Peptide. The synthetic 15-mer peptide NH2-PWNASWSNKSLDDIW-COOH chemically coupled to the KLH was purchased from Covalabs. HPLC profile show that peptide was >90% pure.

Statistical Analysis. Statistical analysis used the Mann–Whitney or Wilcoxon tests, appropriate for small sample sizes, with Graphpad Prism 4 software.

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1. Grossman Z, Meier-Schellersheim M, Sousa AE, Victorino RM, Paul WE (2002) $CD4^+$ T cell depletion in HIV infection: Are we closer to understanding the cause? *Nat Med* 8:319–323.
2. Gougeon ML (2003) Apoptosis as an HIV strategy to escape immune attack. *Nat Rev Immunol* 3:392–404.
3. Derdeyn CA, Silvestri G (2005) Viral and host factors in the pathogenesis of HIV infection. *Curr Opin Immunol* 17:366–373.
4. Grossman Z, Meier-Schellersheim M, Paul WE, Picker LJ (2006) Pathogenesis of HIV infection: What the virus spares is as important as what it destroys. *Nat Med* 12:289–295.
5. Alimonti JB, Ball TB, Fowke KR (2003) Mechanisms of $CD4^+$ T lymphocyte cell death in human immunodeficiency virus infection and AIDS. *J Gen Virol* 84:1649–1661.
6. Vieillard V, Strominger JL, Debré P (2005) NK cytotoxicity against $CD4^+$ T cells during HIV-1 infection: A gp41 peptide induces the expression of an NKp44 ligand. *Proc Natl Acad Sci USA* 102:10981–10986.
7. Ward J, *et al.* (2007) HIV modulates the expression of ligands important in triggering natural killer cell cytotoxic responses on infected primary T cell blasts. *Blood* 110:1207–1214.
8. Vieillard V, Costagliola D, Simon A, Debré P (2006) French Asymptomatiques à Long Term (ALT) Study Group specific adaptive humoral response against a gp41 motif inhibits CD4 T cell sensitivity to NK lysis during HIV-1 infection. *AIDS* 20:1795–1804.
9. Burton DR, *et al.* (2004) HIV vaccine design and the neutralizing antibody problem. *Nat Immunol* 5:233–236.
10. Zolla-Pazner S (2004) Identifying epitopes of HIV-1 that induce protective antibodies. *Nat Rev Immunol* 4:199–210.
11. Trkola A, *et al*. (2005) Delay of HIV-1 rebound after cessation of antiretroviral therapy through passive transfer of human neutralizing antibodies. *Nat Med* 11:915–922.
12. Nakowitsch S, et al. (2005) HIV-1 mutants escaping neutralization by the human antibodies 2F5, 2G12, and 4E10: *In vitro* experiments versus clinical studies. *AIDS* 19:1957–1966.
13. Harouse JM, Gettie A, Tan RC, Blanchard J, Cheng-Mayer C (1999) Distinct pathogenic sequela in rhesus macaques infected with CCR5 or CXCR4 utilizing SHIVs. *Science* 284:816–819.
14. Harouse JM, *et al*. (2001) Mucosal transmission and induction of simian AIDS by CCR5-specific simian/human immunodeficiency virus SHIV(SF162P3). *J Virol* 75:1990–1995.
15. Chan DC, Fass D, Berger JM, Kim PS (1997) Core structure of gp41 from the HIV envelope glycoprotein. *Cell* 89:263–273.
16. Weissenhorn W, Dessen A, Harrison SC, Skehel JJ, Wiley DC (1997) Atomic structure of the ectodomain from HIV-1 gp41. *Nature* 387:426–430.
17. Wang S, *et al*. (2002) Interhelical interactions in the gp41 core: Implications for activation of HIV-1 membrane fusion. *Biochemistry* 41:7283–7292.
18. Fauci AS, Mavilio D, Kottilil S (2005) NK cells in HIV infection: Paradigm for protection or targets for ambush. *Nat Rev Immunol* 5:835–843.
19. Scott-Algara D, *et al.* (2003) Cutting edge: Increased NK cell activity in HIV-1-exposed but uninfected Vietnamese intravascular drug users. *J Immunol* 171:5663–5667.
20. Scott-Algara D, Paul P (2002) NK cells and HIV infection: Lessons from other viruses. *Curr Mol Med* 2:757–768.
21. Alter G, Altfeld M (2006) NK cell function in HIV-1 infection. *Curr Mol Med* 6:621–629.
22. Martin MP, *et al.* (2007) Innate partnership of HLA-B and KIR3DL1 subtypes against HIV-1. *Nat Gen* 39:733–740.
23. Diop OM, *et al.* (2000) High levels of viral replication during primary simian immunodeficiency virus SIVagm infection are rapidly and strongly controlled in African green monkeys. *J Virol* 74:7538–7547.
24. Hirsch VM (2004) What can natural infection of African monkeys with simian immunodeficiency virus tell us about the pathogenesis of AIDS? *AIDS Rev* 6:40–53.
25. Andre FE (2003) Vaccinology: Past achievements, present roadblocks, and future promises. *Vaccine* 21:593–595.
26. Plotkin SA (2005) Vaccines: Past, present, and future. *Nat Med* 11:5–11.
27. Kabir S (2007) Cholera vaccines. *Lancet Infect Dis* 7:176–178.
28. Dioszeghy V, *et al.* (2006) *J Virol* 80:236–245.