In their recent report, Timothy W. Baba et al. state that a deletion mutant of simian immunodeficiency virus (SIVΔ3), which does not cause disease in adult macaques and has been successfully used as a vaccine against challenge with pathogenic virus (1), causes acquired immunodeficiency syndrome (AIDS) in newborn macaques. They ascribe the differential outcome of SIVΔ3 infection of neonatal and adult macaques to several possibilities including the amount of virus replication early after inoculation, the route of virus inoculation, and the developing neonatal immune system. However, their study does not allow separation of these important variables.

We found that high-dose intravenous inoculation of newborn rhesus macaques with molecularly cloned SIVmac239 (the parental virus from which SIVΔ3 was derived) resulted in persistently high amounts of virus in peripheral blood mononuclear cells (PBMC) and plasma (higher than those reported by Baba et al. for SIVΔ3). Rhesus newborns infected with SIVmac239 did not experience rapid CD4+ T lymphocyte depletion, and the time course before fatal immunodeficiency developed was consistent with that previously reported for SIVmac239-infected adult macaques (that is, 6 to 24 months) (2, 3). Thus, an age-related difference does not explain why rhesus infants inoculated with an attenuated triple-deletion mutant of SIVmac239 appear to experience a more rapid CD4+ T cell depletion and CD4+/CD8+ T cell ratio inversion than rhesus infants inoculated with the pathogenic parental virus, SIVmac239. We also found that absolute CD4+ T lymphocyte numbers were not a reliable marker of disease progression in infant rhesus macaques because of extreme variability of absolute lymphocyte counts in response to stress (for example, handling). Only absolute CD4+ T cell numbers that are persistently below 500 per microliter reliably suggested CD4+ T lymphocyte depletion in neonatal macaques (2-4).

Baba et al. hypothesized that the oral route of inoculation may be responsible for increased virulence of SIVΔ3 in newborns. Our observations with five orally and six intravenously inoculated newborn macaques did not demonstrate a more severe course of infection with uncloned pathogenic SIVmac239 for the oral route (2-4). With regard to the postulated age-dependence of SIV virulence, we have also compared the time course of infection of the nonpathogenic molecular clone, SIVmac111, and SIV/human immunodeficiency virus-1 (HIV-1) envelope chimeric viruses in macaques of different ages: We have no evidence that an SIV strain that is attenuated in older macaques becomes pathogenic when inoculated intravenously or orally into newborn macaques (2, 5). Instead, inoculation of fetal and newborn macaques with attenuated SIVmac111 proved to be a safe and effective vaccine against challenge with pathogenic uncloned SIVmac later in life (5). Finally, our studies indicate that the neonatal immune system was not overwhelmed by attenuated SIV isolates or by a pathogenic SIV clone (2, 3).

Caution must be used when assigning the underlying cause of death in SIV-infected macaques to immunodeficiency. For the one SIVΔ3-inoculated macaque that died in their study, the classical hallmarks of simian AIDS (such as the presence of opportunistic infections, encephalopathy, and so on) apparently were not demonstrated by Baba et al. Instead, this animal had severe anaemia and thrombocytopaenia, reportedly a result of peripheral autoimmune destruction of red blood cells and platelets. It is not clear whether this diagnosis of hemolytic anaemia was mainly based on a positive direct Coombs test. Many healthy macaques will react positively if human Coombs test reagents are used (6). Clinical hemolytic anaemia must be confirmed by additional evidence, such as hemoglobinuria, poikilocytosis, the presence of spherocytes, hemolytic or icteric plasma, and increased serum bilirubin and lactate dehydrogenase. The erythroid hyperplasia of the bone marrow, reported by Baba et al., is a finding that we do not see in anemic SIV-infected animals; rather, their bone marrow aspirates reveal a myeloid hyperplasia with the erythroid series being normal or only slightly increased (7). Findings in addition to an abundance of megakaryocytes in the bone marrow are needed to support the hypothesis of peripheral platelet destruction. SIV-infected animals often have a megakaryocyte hyperplasia of the bone marrow, but these megakaryocytes have increased cytoplasmic vacuolization, which suggests that the thrombocytopenia is a result of decreased platelet production rather than peripheral platelet destruction (6).

Extra care needs to be taken to exclude all other pathogens that can adversely affect the immune system and the health of macaques. Although the animals in the study by Baba et al. were polymerase chain reaction-negative (by an assay able to detect approximately one infected cell in 8000 PBMC) and seronegative for simian type D retroviruses, virus isolation is more reliable for diagnosis of this viral infection, but was not reported by Baba et al.

Until a more thorough analysis is completed and results of Baba et al. are confirmed, it would be premature to dismiss the potential of SIV nef-deletion mutants as live-attenuated vaccines.

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9. MOB-47 and MOB-55 are hamster mAbs raised against MuHF-N receptor β chain containing Fc fusion proteins that are specific for the murine receptor β chain and were generated according to a protocol outlined by K. Sheehan et al. J. Immunol. 142, 3884 (1989); 105, 1F1, and 2E2 are hamster mAbs specific for the MuHF-N receptor α chain. The MuHF-N receptor α and β chain epitopes recognized by 105 and MOB-55, respectively, are not blocked by ligand.
13. Nove CD4+ Mel14+ T cells from the ovalbumin (OVA)-reactive DO11.10 x BCR transgenic mouse (5) were purified by FACS as described (6) and differentiated in vitro into T1, T2 cells in the presence of 0.3 μM OVA peptide and irradiated BALB/c splenocytes by culture with MuL-12 (10 U/ml Hoffmann-La Roche, Nutley, NJ) and MuL-4 mAb (11811) (10 μg/ml DNA, Palo Alto, CA), or MuL-4 (200 U/ml, Genome, Cambridge, MA) and MuL-15 mAb (3 μg/ml TOSH), respectively. Seven days after primary stimulation, cells were restimulated with antigenic peptide in the absence of exogenous cytokine reagents and incubated for an additional 7 days.
15. We thank B. Unanue and P. Allison for helpful discussions. MuL-12 mAb was a gift from C. Tripp and B. Unanue, MuFh-α was a gift from S. Narula. Supported by grants from NIH and Genentech, Inc.
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The report by Baba et al. raises concern that attenuated HIV may not be safe as a vaccine to prevent syndrome AIDS. A major point of the report is that mucosal infection of newborn macaques with a triple gene-deleted preparation of attenuated SIV can result in an AIDS-like condition with a reduction in the CD4 cell count. This observation contrasts with the experience of Desrosiers and his colleagues who observed no ill effects in adult macaques that had been infected with a triple deletion SIV (1). Although the differences observed in the two studies might be accounted for by differences in the doses and routes of attenuated SIV administered, as noted by Baba et al., an important host factor that needs to be considered is the difference in the strength and maturation of the immune systems of neonates and adult animals.

The immune response potentials of adult and neonate macaques are likely to be different, such that antigen-presenting cell (APC) function for generating strong cellular immune responses could be deficient in the neonates, as they are in healthy human infants younger than 1 year of age (2). This type of deficiency could result in an inability or reduced ability of the cellular arm of the immune system to control the extent of replication of the attenuated virus. In contrast, the competent immune system of the adult animals would be expected to hold the attenuated SIV in check, and could result in protection against challenge with wild-type SIV. Such a defect in the APC function of neonates could permit viral replication and the generation of viral products that might be responsible for CD4+ T cell depletion. It has been reported that after priming in the presence of interleukin-12, human naive neonatal CD4+ T cells appear to develop a T helper cell zero (T(0) phenotype, whereas adult naive CD4+ T cells develop into T(1) cells (3). If this difference exists in macaques as well, it could contribute to differences in the immune potential between neonates and adults.

Concerning safety in the use of these attenuated viruses as vaccines, it might be argued that an attenuated HIV vaccine would be safe to use in adults whose immune systems are adequate to control a gene-deleted virus. However, the question remains as to whether the immune system of adults would continue to be sufficiently competent to hold the attenuated virus in check. Other infections, immune-suppressive drug therapy for other conditions, and aging could render the immune system inadequate to control the attenuated virus. Therefore, it is important to determine whether adult macaques that have been infected with attenuated SIV for an extended time will continue to maintain normal CD4 counts and remain without symptoms after immune suppression is induced.

Finally, central nervous system damage poses an additional safety issue for HIV vaccines that may be particularly relevant for infants. HIV-1 is frequently associated with neurologic and behavioral conditions in infants and children, in whom the virus may infect astrocytes as well as microglia, with the nef gene implicated in contributing to neuropathologic damage (4). Thus, it would be of value to have assessed neuro-pathologic and neurovirologic parameters at autopsy in the macaques receiving the nef, sfp, NRE-deleted live SIV vaccine.

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**REFERENCES**


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We have reported on the development of a second generation live nef attenuated vaccine strain (1) based on a gain-of-function approach that could address the safety issues raised by Baba et al. This concept is exemplified by the addition of a conditionally lethal suicide gene to a nef-deleted (loss-of-function) vaccine strain. Preliminary results suggest that the gain of a conditionally lethal function on top of a loss of a critical virus gene for growth further reduces virus load and perhaps affords greater safety. While we have worked on one prototypic effector gene [herpes simplex virus-1 (HSV-1) thymidine kinase], we realize that many other effectors are possible in attenuating a live vaccine through gain-of-function. Issues concerning a neutral or even a positive selective force needed to maintain a gain-of-function must be addressed before this approach is feasible. Genetic strategies for accomplishing this selection exist, and future refinements on a gain-of-function approach are likely.

Gain-of-function should be considered with loss-of-function in designing safe live attenuated HIV-1 vaccines.

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If SIVΔ3 is pathogenic in macaque infants (1, 2) then, by analogy, HIV with deleted genes might be pathogenic in human infants. Infants must not, therefore, be vaccinated or exposed to these potentially pathogenic HIV mutants. As there are no data thus far that nef-deleted SIV is pathogenic in adult macaques, the findings of Baba et al. only preclude the vaccination of women who might infect their infants or neonates with HIV having deleted genes. The solution may be to immunize immunocompetent men only. A strategy using an effective attenuated HIV vaccine in men could stop the spread of HIV. This strategy would be effective because men infect men, and men also infect women (3). Women do not infect women to any degree (3), if at all, and men do not infect infants or children under normal circumstances. Finally, women would not transmit HIV to immunized men. The cycle would be effectively broken. Eventually, all new infections except needle-transmitted HIV between women would be stopped by immunizing men only. It must still be determined if immunized men would shed enough virus to transmit the vaccine virus to women. Transmission of the vaccine strain between men would not be a problem, as any given two men would be immune in this protocol. Male macaques and SIVAΔ3 could be used to test this approach to vaccination.

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