The Ability of Certain SIV Vaccines to Provoke Reactions Against Normal Cells

ALPHONSE J. LANGLOIS, KENT J. WEINHOLD, THOMAS J. MATTHEWS, MICHAEL L. GREENBERG, DANI P. BOLOGNESI*

CONTEMPORARY VACCINES AGAINST COMMON VIRAL DISEASES generally consist of either whole inactivated or live attenuated virus preparations (*1*). Their proven efficacy over the last century would appear to set the stage for developing vaccines for more complex viruses such as members of the herpes or retrovirus families. But concerns over safety, given the morbidity and mortality caused by such agents, and coupled with incidents in which whole virus vaccine preparations were still infectious or pathogenic, have stymied development efforts.

Whole virus vaccine preparations are usually grown in a cell type or biological system that favors high virus yields including permanent cell lines or embryonated eggs. There is no requirement that the host cells be human-derived, and often they are not. Such considerations have led to debate over the contribution of cellular materials to the immunogenicity of such vaccines. They have also prompted studies to determine the location and function of cellular antigens within virions, particularly for viruses that mature at the cell surface like myxoviruses and retroviruses. Relevant work in this area dates back to the 1930s. An insightful summary can be found in Jan Lindenmann's review (*2*), which includes his concept of potential synergy between viral and host antigens as a mechanism for provoking protective immune responses against experimental tumors.

The possibility that cellular material can influence vaccine immunogenicity was revived this year by investigators continuing the search for a vaccine for acquired immunodeficiency syndrome (AIDS) when they announced that sera from macaques receiving vaccines based on inactivated simian immunodeficiency virus (SIV) or SIV-infected cells contain reactivities to normal human cell components and that the respective titers correlate positively with protection (*3*). Their work was accompanied by the finding of protection induced by vaccination with uninfected human cells. We would like to add similar findings after study with sera obtained from various SIV vaccine trials in the United States.

We have also identified anticellular activity in sera from vaccinated macaques through collaborative studies with several groups investigating SIV vaccines (Table 1). Furthermore, with the assistance of Alan Schultz and Wayne Koff of the AIDS Vaccine Research and Development Branch, Division of AIDS, National Institute for Allergy and Infectious Diseases, we had the opportunity to examine approximately 200 additional serum samples in a blinded fashion for the presence of neutralizing antibodies as well as antibodies directed to cell surface components of human cells. The information presented here (Table 1) represents only those serum samples available to us for which protection data were known. Our results confirm and extend the reports of our British colleagues. We agree that sera from animals immunized with killed SIV invariably possess reactivities against uninfected human cells, which can almost certainly be attributed to derivation of the vaccines from established cell lines of human origin.

In our measurements of anticellular activities, we used two assays. One was based on the observation that sera from certain vaccinated animals produced a pronounced clumping of uninfected human cells. The other represented a direct measurement of antibodies binding to the surface of human cells by fluorescence-activated cell sorter (FACS) analysis (Fig. 1). The positive fluorescence values for sera from normal and SIV-infected macaques were within the range of the conjugate controls, whereas the values of sera from macaques immunized with whole killed SIV gave an increase in overall fluorescence intensity. Although the antihuman cell activity was observed most consistently when animals received inactivated whole virus vaccines, the magnitude of the reactions varied considerably depending on the preparation or vaccine protocol used. When examined by the clumping reaction, there was no strict correlation between titer (measured as the highest dilution of serum that produced a clumping reaction) and protection; high titers (greater than 1:1280) were, on the whole, predictive for subsequent protection. Exceptions included two animals that were challenged with high virus doses (*4*) and were not protected despite high levels of anticell activity. However, as established by others with whole inactivated SIV vaccines (*5*), there was no correlation between protection and the presence or absence of neutralizing antibodies. This situation markedly contradicts studies in chimpanzees with subunit human immunodeficiency virus (HIV) in which neutralizing antibodies induced by viral subunit vaccines emerged as the best correlate for protection (*6*).

As anticipated, none of the vaccines derived from recombinant vectors, viral subunits, or combinations thereof (Table 1) induced antibodies that reacted with uninfected human cells. This indicates that a cross reactivity between viral antigens and human cell antigens does not explain the aforementioned anti-cell activity. Among these recombinant vaccine approaches, one study resulted in protection of

Table 1. Reactivity with uninfected human cells. Clumping was noted on uninfected AA-5 human B lymphoblastoid cells and on a number of established T cell lines (H-9, HUT-78, and CEM). Titers ranged from 1:20 (low) to 1:2560 (high); values <1:20 were considered negative. FACS analysis was done with AA-5 cells and a 1:250 serum dilution. The minimum criteria for a positive result was threefold the background value. ND, not done; NA, not applicable.

Serum derived from animals immunized with	Cell clumping	FACS	Neutralizing (*18*) antibodies	Protection
Inactivated SIV*	+ (25/25)	+ (22/25)	+ (11/25)	+ (18/25)
Recombinant vectors†	− (0/2)	− (0/2)	+ (2/2)	− (0/2)
Recombinant subunits‡	− (0/4)	− (0/4)	+ (4/4)	− (0/4)
Recombinant vector + subunit§	− (0/4)	ND	+ (4/4)	+ (4/4)
Infected animals	− (0/3)	− (0/3)	+ (3/3)	NA
Uninfected animals	− (0/2)	− (0/2)	− (0/2)	NA

*Samples were obtained before challenge in studies described (*4, 19, 20*). †Samples were obtained before challenge after immunization with recombinant vaccinia bearing the complete SIV envelope either alone or together with the *gag* gene (*21*). ‡Samples were obtained before challenge after immunization with baculovirus derived gp140 and gp110 of SIV (*22*). §Samples were derived before challenge after immunization with vaccinia bearing the SIV envelope as primer followed by the entire envelope subunit produced in baculovirus as booster (*7*).

The authors are at the Center for AIDS Research, Department of Surgery, Duke University Medical Center, Durham, NC 27710.

*To whom correspondence should be addressed.

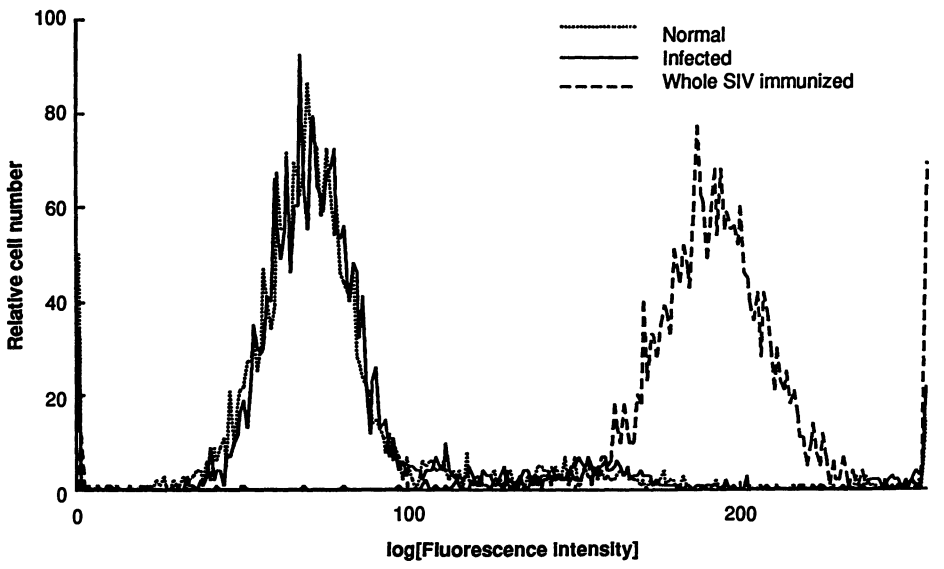

Fig. 1. FACS analysis of antihuman cell reactivity by individual sera from normal, infected, and whole virus–immunized macaques. See legend, Table 1. Note that the cytofluorographs for normal and infected sera are nearly superimposable. The positive fluorescence values for the three sera are 6.0% (normal), 7.2% (infected), and 97.5% (immunized) relative to the conjugate control.

all four vaccinated animals (7). It is not apparent why this approach was successful while the other protocols illustrated in Table 1 or even comparable priming and boost protocols conducted by other investigators (*5*) were unable to induce protection. The explanation does not concur with the presence or concentrations of neutralizing antibodies before challenge. However, until such assays are standardized in relation to the challenge viruses and other nuances particular to neutralization of SIV, the role of neutralizing antibodies in protection should remain open. This study does indicate, however, that protection can be achieved through immunization with virion antigens alone, as in the case with HIV (*6*).

These revelations raise three questions that are vital for vaccine development against this family of viruses. (i) What is the identity of the antigenic target or targets responsible for protection by inactivated whole virus vaccines (and the successful recombinant protocol). (ii) What are the immunological mechanisms and their targets on the virion or infected cell by which protection is achieved; it should be underscored that the anticellular antibodies may be but an indicator of a more comprehensive anticell immune response. And (iii) What is the importance of the relation between the host cell origin of the viruses used for immunization and for challenge.

Both viral and cellular antigens must now be considered as possible targets involved in protection because the virus used for challenge inocula (Table 1) was also grown in human cells. Molecules encoded by cellular genes can be incorporated into the budding virions along with virally encoded molecules as integral components or associated with the virus preparations as contaminants difficult to remove by purification. Indeed, there are ample precedents for incorporation into retroviruses of cellular materials including nucleic acids, enzymes, and a variety of other components (*8*) such as major histocompatibility (MHC) products (*9*). MHC products, which appear to be selectively incorporated into virions (*10*), could explain the strong protection achieved by inactivated virus vaccines given their unparalleled potency as inducers of the transplantation rejection reaction and their association with purified HIV preparations (*11*). Among other candidates are antibodies to CD4 (*12*), to complexes of envelope and CD4 (*13*), or to LFA-1 (*14*), all of which can block infection in vitro.

In conclusion, use of SIV derived from human cells as both a vaccine source and as a challenge virus has made pinpointing reactivities responsible for protection difficult. However, at this time these results with killed virus vaccines do not necessarily represent a setback in vaccine research against SIV and HIV (*15–17*). On the contrary, whole, inactivated SIV preparations induce the strongest and most consistent protection thus far experienced in experimental animal studies. Understanding how such protection is achieved is likely to provide lessons for vaccine development, particularly if the phenomenon can be extended to viruses, which have been grown in and have incorporated antigens from cells of the test species of the vaccine.

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