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Immunological Significance of Recombinant Vaccinia Viruses Carrying the *HIV* Gene.

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ABSTRACT

We examined mouse immune response to 4 kinds of recombinant vaccinia viruses carrying the human immunodeficiency virus (HIV) Group-specific Antigen (gag) gene, including vac-gag/pol, which produces HIV-like particles with processed gag proteins; vac-gag, which also produces HIV-like particles but with unprocessed gag protein; and vac-gag-pol-fuse and vac-es-gag/pol, neither of which produces such particles but releases reverse transcriptase (RT) and gag protein, respectively, from infected cells. Although infection of mice with recombinant vaccinia viruses induced production of the anti-p24 antibody in all mice, vac-gag/pol and vac-es-pol induced higher production than the other two recombinants. Increase in [3H]thymidine uptake by splenic lymphocytes following p24 antigen stimulation was most evident in mice infected with vac-gag/pol. Thus, the highest immune reaction, both humoral and cellular, was elicited by vac-gag/pol, indicating that among those tested, this recombinant vaccinia virus is the best candidate for a vaccine that induces anti-HIV gag immunity.

KEYWORDS : Lightning conductor, protection angle, lightning area

Introduction

The envelope antigen (env) has been a primary target for the development of vaccines against human immunodeficiency virus (HIV), the causative agent of acquired immune deficiency syndrome (AIDS). Previous studies showed that recombinant vaccinia viruses constructed as vaccine candidates evoked humoral and cell-mediated immune responses against various pathogens (Bennick JR. et.al. 1984, Panicali D. et.al. 1983, Paoletti E. et.al. 1984, Smith GL. et.al. 1984). There are reports that recombinant vaccinia viruses carrying the env region of HIV are capable of inducing antibodies against the env protein in mice and chimpanzees (Flu S-L. et.al. 1986, Flu S-L. et.al. 1987). Furthermore, Zarling et al confirmed the presence of T-cell responses to the env antigen in macaques immunized with a recombinant virus carrying the env gene (Zarling JM. et.al. 1986). However, it is known that the mutation rate of the env gene is high, and it has also been reported that immunity against gp120 of the env protein elicited by HIV infection exerted deleterious effects on the immune system (Lanzavecchia A. et.al. 1988, Siliciano RF. et.al. 1988, Weinhold KJ. et.al. 1988). These findings suggest problems to be overcome before using the env protein as the target for vaccines.

Recently, attention has been paid to the Group-specific Antigen (gag) including viral core antigen because of the low rate of mutation in the gag region and specific decreases in anti-gag antibodies before the development of AIDS in HIV-infected patients (Shiipbach J. et.al. 1985, Weber JN. et.al. 1987). Moreover, cellular immunity against viral core antigens is known to play a definitive role in recovery from viral infections (Yewdell JW. et.al. 1985), and several reports have indicated that cellular immunity against gag proteins was detectable in HIV infection (Nixon DF. et.al. 1988, Plata F. et.al. 1987, Walker BD. et.al. 1987). The primary product of the *gag* gene is the p55 gag precursor, which is processed into p17, p24 and p15 mature gag proteins by the protease encoded by the pol gene (Nixon DF. et.al. 1988). Several workers have already succeeded in expressing HIV gag proteins in mammalian cells using recombinant vaccinia vectors (Karacostas V. et.al. 1989, Shioda T. et.al. 1990, Hayashi T. et.al. 1992, Hayashi T. et.al. 1993) and recent reports showed that recombinant vaccinia viruses carrying the *gag* gene can produce HIV-like particles in mammalian cells (Karacostas V. et.al. 1989, Shioda T. et.al. 1990, Hayashi T. et.al. 1992, Hayashi T. et.al. 1993). However, only a little is known about the immunological potency of these recombinant vaccinia viruses. In the present study we addressed this matter using mice of 3 inbred strains as the host animal and 4 different kinds of recombinant vaccinia viruses carrying the *gag* gene as the immunogen. The recombinants included 3 previously described and one newly constructed (Shiipbach J. et.al. 1985).

Although several studies on the expression of the *HIV gag* gene in recombinant vaccinia viruses have been reported, only a report described the immunological potency of the recombinants (Karacostas V. et.al. 1989, Koup RA. et.al. 1989, Shioda T. et.al. 1990, Hayashi T. et.al. 1992, Hayashi T. et.al. 1993, Mak TK. et.al. 2008). They constructed 5 recombinants, in which v-gag 1 and v-gag 4 were similar to our vac-gag/pol and vac-gag, respectively. C57/B6 mice immunized with these recombinants at a single dose of 10^7 PFU per mouse by footpad inoculation mainly generated antibodies directed to p24 but occasionally those against p17 as well. Cellular immunity against the gag proteins was not examined in the mice. However, these authors found lymphoproliferative responses to psoralen-inactivated HIV-1 in chimpanzees immunized twice with a recombinant carrying the 3′-truncated *gag* gene (v-gag 5), each at a viral dose of 2 x 10^8 PFU, by skin scarification. In the present study we examined the immunogenic properties of our 4 recombinants carrying the HIV *gag* gene. All the recombinants could effectively elicit anti-p24 antibody in mice of 3 inbred strains. Vac-gag/pol and vac-es-gag/pol seemed to be more effective than vac-gag and vac-gag-pol-fuse. Interestingly, there was no obvious correlation between the neutralizing antibody titer against vaccinia virus and that of anti-p24 antibody. In contrast to the production of anti-p24 antibody, splenic lymphocyte response to the p24 antigen, which was estimated as an indicator of cellular immunity, was most evident in the mouse group immunized with vac-gag/pol, irrespective of mouse strain, although the response index greatly varied from mouse to mouse, and a high viral dose of 10^8 PFU per mouse was necessary to induce the response. Vac-es-gag/pol also seemed to be effective in evoking the response, but statistically there was no significant difference between vac-es-gag/pol and vac-gag in all mouse strains. All the findings in the present study strongly suggest that vac-gag/pol is superior to the other recombinants carrying the gag gene in inducing immune responses, both humoral and cellular, against the gag protein. The most characteristic feature of vac-gag/pol is the capability to produce HIV-like particles composed of the processed gag proteins, which may well be correlated to the high immunological potency (Hayashi T. et.al. 1992, Hayashi T. et.al. 1993, Klein F. et.al. 2013). Vac-gag is also able to produce HIV-like particles, but its gag protein is the unprocessed precursor (Hayashi T. et.al. 1992, Hayashi T. et.al. 1993, Hayden EC. 2015). The newly constructed recombinant, vac-es/gag/pol, induced a fairly high production of anti-p24 antibody as well as considerable splenic lymphocyte response, which might correspond well to the efficient release of a modified gag protein from infected cells. However, this recombinant could not exceed vac-gag/pol in its immunological potency. We had expected that the sera from mice infected with these recombinants possessed neutralizing activity against HIV, since two mouse monoclonal antibodies against the p17 gag protein were reported to have the ability to neutralize HIV (Papsidero LD. et.al. 1989, Thippeshappa R. et.al. 2015). However, our mouse sera failed to show this ability, and it remains to be examined whether these sera have antibody against the p17 gag protein. Although we measured here the splenic lymphocyte response to the p24 antigen as an indicator for evaluating cellular immunity, it is important to further clarify whether our recombinants, especially vac-gag/pol, are able to induce cytotoxic T cells against the cells expressing the gag antigen. If they have such an ability, we could expect that they interfere with the onset of AIDS from asymptomatic HIV carrier (Kannagi NI. et.al. 1990).

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