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Gag-specific immune enhancement of lentiviral infection after vaccination with an adenoviral vector in an animal model of AIDS

Bernard Klonjkowski[a], Dieter Klein[b], Sandra Galea[a], Françoise Gavard[a], Martine Monteil[a], Lidia Duarte[a], Annie Fournier[a], Sophie Sayon[a], Kamila Górna[a], Reinhard Ertl[b], Nathalie Cordonnier[a], Pierre Sonigo[c], Marc Eloit[a], Jennifer Richardson[a,*]

[a] UMR 1161 Virologie INRA, Afssa, ENVA, 7 avénue du Charles de Gaulle, 94704 Maisons-Alfort, France
[b] Department of Pathobiology, Institute of Virology, University of Veterinary Medicine, Vienna, Austria
[c] Bio-rad, Food science division, Life Science Group, 3 bd Raymond Poincaré, 92430 Marnes-la-coquette, France

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ABSTRACT

The evaluation of vaccine strategies in animal models is essential for the development of a vaccine against HIV. In efficacy trials conducted in non-human primate models of AIDS, vaccines based on adenoviruses compared favourably with other vaccine vectors. To determine whether this strategy could be transposed to another animal model, and by extension, to humans, we have evaluated the efficacy of adenoviral vectors in a natural model of AIDS, infection of the cat by the feline immunodeficiency virus (FIV). Recombinant canine adenoviruses expressing the envelope glycoproteins or the Gag protein of a primary strain of FIV were constructed. Three groups of six cats were immunised twice with vectors expressing FIV antigens or with a vector expressing an irrelevant antigen, green fluorescent protein, by intramuscular and subcutaneous routes. Humoral responses were elicited against the transgene product in 6/6, 3/6 and 0/6 cats after immunisation against green fluorescent protein, Gag or the envelope glycoproteins, respectively. Six weeks after the second administration, cats were challenged by the intraperitoneal route with the homologous strain, and viral burden in plasma was followed by quantitative RT-PCR. Immunisation with FIV antigens did not afford protection. Rather, viral RNA was detected at earlier time points in cats immunised against Gag than in cats immunised with a vector expressing an irrelevant antigen. Such immune-mediated enhancement did not appear to have a long-range impact on viral set point or inversion of the $CD4^+/CD8^+$ ratio. Thus, in the feline AIDS model pre-existing immunity against a viral antigen exacerbated acute phase infection.

1. Introduction

The evaluation of vaccine strategies in animal models is essential to instruct development of a vaccine against HIV. To this end, several animal species have been shown to be permissive to lentiviral infection, and some such infections lead to AIDS. This is notably the case for infection of certain non-human primate species and domestic cats by simian and feline immunodeficiency viruses (SIV and FIV), respectively. The primate models, and in particular infection of macaques with SIV or genetic chimeras of SIV and HIV (SHIV), have been most extensively employed. The genetic proximity of SIV and HIV, not to mention the genetic proximity of human and non-human primates, provides ample justification for this choice. Nevertheless, FIV infection more closely resembles HIV infection in one critical matter; that is, FIV causes AIDS in its natural host, while SIV does not [1,2]. The physiopathology of infections by FIV and HIV in their respective natural hosts presents striking analogies, and several intriguing differences. Both viruses preferentially infect $CD4^+$ T lymphocytes of the effector or memory phenotype, although the cell-surface receptors used for high-affinity attachment, CD4 and CD134 for HIV and FIV, respectively, differ [3,4]. Both viruses cause dysfunction of the $CD4^+$ lymphocyte subset early in infection, and progressive selective depletion of this subset, although FIV also infects the $CD8^+$ subset [5] and even B lymphocytes [6], and both ultimately cause immune system collapse.

Most vaccines that afford protection against viral infections elicit antibodies that neutralise viral infectivity [7]. As regards HIV vaccines, and despite intense international effort, the goal of eliciting antibodies capable of neutralising a broad spectrum of

* Corresponding author. Tel.: +33 1 43 96 73 71; fax: +33 1 43 96 73 96.
E-mail address: jrichardson@vet-alfort.fr (J. Richardson).

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primary HIV isolates has proven elusive (for review see [8]). A second strategy has thus emerged, that is, eliciting cell-mediated immunity, and more particularly, effector functions of CD8+ T lymphocytes. The scientific justification for this approach is based on abundant data attesting to the importance of $CD8^+$ T cells in controlling lentiviral infection, both in humans and non-human primates [9–14]. Since the immunological requirements for induction of B- and T-cell immunity diverge, the change in vaccinal paradigms has shifted interest towards vectored vaccines. Such vaccines, which deliver a gene ("transgene") encoding antigen rather than the antigen itself, give rise to endogenous antigen expression and thus efficient delivery of epitopes to the major histocompatibility class I pathway for antigen presentation to $CD8^+$ cells. Many such vectors have been derived from viruses, and notably from adenoviruses.

Adenoviruses (Ad) have been isolated from humans and many animals, including bovine, ovine, porcine, canine and avian species. Most adenoviral-based vectors have been derived from human Ad, and in particular from serotype 5, and most such vectors have been rendered replication-incompetent by the deletion of a genetic region, E1, required for progression of the viral life cycle. Such non-replicative Ad5-based vectors present many attractive features as candidate vaccines [15–17], not least because they elicit potent humoral and cell-mediated immune responses in mammalian hosts against the antigens encoded by the inserted foreign genes, and have afforded protection in many infectious settings [18–20]. When different vectors expressing the same heterologous antigen have been compared, Ad5-based vectors have proven particularly immunogenic, and notably as regards induction of antigen-specific $CD8^+$ T cells [21–23]. The qualities of Ad5 that underpin such immunogenicity are not as yet fully elucidated, but include its capacity to elicit strong innate immunity. Parenteral administration of Ad5 in mice induces an intense inflammatory response, characterised by the secretion of high levels of proinflammatory cytokines and the induction of a maturation process in immature dendritic cells [24,25], presumably creating conditions conducive to the induction of antigen-specific adaptive immunity. Nevertheless, in humans natural exposure to Ad5 is common [26,27], and pre-existing immunity against Ad5 markedly diminishes immune responses elicited against the transgene product of Ad5-based vaccines [21,28–31]. Several strategies of circumventing pre-existing immunity to Ad5 have been envisaged [32], and notably the development of vectors derived from animal adenoviruses against which humans are not naturally immune (for reviews see [33,34]).

In the course of a comparative study of several vaccine vectors conducted in a non-human primate model of AIDS, the best level of protection was afforded by an adenoviral vector [23]. In view of the difficulty in translating results obtained in animal models to humans, it appears essential to evaluate the most promising strategies in several animal models of AIDS, since a strategy that can be transposed between animal models is more likely to be extrapolated successfully to humans. We have therefore evaluated the efficacy of adenoviral vectors in a non-primate model of AIDS, infection of the domestic cat by FIV. Recombinant canine Ad (Cav) expressing the envelope glycoproteins or the Gag protein of a primary strain of FIV were generated. Following immunisation, cats were challenged with the homologous virus. Immunisation with FIV antigens did not afford protection. Rather, viral RNA was detected at earlier time points in cats immunised against Gag than in cats immunised with a Cav vector expressing an irrelevant antigen. Thus, in the feline AIDS model pre-existing immunity against a viral antigen exacerbated acute phase infection.

2. Materials and methods

2.1. Cats and mice

Six-week-old female BALB/cAnNCrl mice were purchased from Charles River Laboratories and housed in a level A2 biosecurity facility. Two-month-old male specific-pathogen free (SPF) cats were purchased from Harlan (USA) and housed in a level A2 biosecurity facility. Animal experimentation was conducted in compliance with European guidelines, according to protocols approved by the local ethics committee.

2.2. Tissue culture

The canine kidney cell line DK-E1, stably transfected with the E1 region of Cav-2, was cultivated in Dulbecco's modified Eagle's medium supplemented with 10% heat-inactivated fetal calf serum (FCS), 100 IU of penicillin per ml, and 100 μg of streptomycin per ml. The human embryonic kidney cell line HEK-293 [35] was cultivated as described for DK-E1 cells.

Feline peripheral blood mononuclear cells (PBMC) were isolated from the blood of cats by density gradient centrifugation and cultivated for 3 days in RPMI 1640 medium containing 50 μM 2-mercaptoethanol, 10 mM HEPES, and 5 μg per ml of the mitogen concanavalin A (Sigma), as well as FCS and antibiotics as described for the DK-E1 cell line. For subsequent culture, medium was supplemented with 100 units per ml of recombinant human interleukin-2 (Eurobio). For infection with FIV, concanavalin A was omitted from the culture medium.

2.3. Challenge FIV virus

A stock of the Wo strain of FIV (FIV_{Wo}) [36] was prepared from the supernatant of acutely infected feline PBMC. In brief, a culture of mitogen-activated PBMC was inoculated with FIV_{Wo} and viral replication was monitored for detection of the p24 subunit of the FIV Gag protein in cell supernatant using commercially available reagents (PetChek® FIV (Ag), IDEXX). Once p24 was detected, fresh mitogen-activated PBMC were added to the culture, and viral replication was monitored as described. Once high levels of p24 were detected, the medium was replaced. After overnight incubation, the supernatant was recovered, filtered (0.45-μm pore-size Spin-X filters, Costar # 8162), aliquoted and frozen in liquid nitrogen. The stock was titered by endpoint dilution in mitogen-activated feline PBMC. An *in vivo* titer was determined by infecting groups of six 2-month-old male SPF cats with serial dilutions of the stock and by monitoring for seroconversion by using commercially available materials (Feline leukemia virus antigen/Feline immunodeficiency virus antibody test kit, IDEXX).

2.4. Construction of recombinant Cav-2 genomes

2.4.1. Cav-env

Following isolation of DNA from a lymph node of a cat experimentally infected with FIV_{Wo}, the *env* gene (Genbank accession number AF298778) was amplified by PCR and inserted into the cloning vector pPCR-Script Amp as previously described [37]. The open reading frame (ORF) of *env* was subsequently sub-cloned into a Cav-2 shuttle vector, a full description of which being available upon request, within an expression cassette comprising the enhancer/promoter region from the intermediate/early (IE) gene of human cytomegalovirus (CMV) and the simian virus 40 (SV40) late polyadenylation signal, as upstream and downstream regulatory elements, respectively. By means of homologous recombination in the BJ5183 strain of *E. coli* [38], the expression