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Vaccination with experimental feline immunodeficiency virus vaccines, based on autologous infected cells, elicits enhancement of homologous challenge infection

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Cats were vaccinated with fixed autologous feline immunodeficiency virus (FIV)-infected cells in order to present viral proteins to the immune system of individual cats in an MHC-matched fashion. Upon vaccination, a humoral response against Gag was induced. Furthermore, virus-neutralizing antibodies were detected in a Crandell feline kidney cell-based neutralization assay, but not in a neutralization assay based on primary peripheral blood mononuclear cells. Despite the induction of these FIV-specific responses, vaccinated cats were not protected. Instead, accelerated virus replication was found, an observation similar to what previous experiments using other vaccine candidates have shown. Here, the results of the present study are discussed in the light of enhancement of lentivirus infections as a complicating factor in lentivirus vaccine development.

Feline immunodeficiency virus (FIV) infection of the cat serves as a suitable model to study the pathogenesis of human immunodeficiency virus (HIV) infection and has been used to evaluate lentivirus vaccine development strategies (Willett *et al.*, 1997). Several of these have been based on the use of envelope glycoproteins of FIV. Envelope-based lentivirus vaccines, however, may induce enhanced rather than decreased susceptibility to challenge infection or disease (Hosie *et al.*, 1992; Lombardi *et al.*, 1994; Wang *et al.*, 1994; Siebelink *et al.*, 1995; Montelaro *et al.*, 1996; Richardson *et al.*, 1997). We have previously shown that cats vaccinated with recombinant envelope protein-based candidate vaccines exhibited an accelerated viraemia upon challenge infection, which was most likely mediated by virus-specific antibodies (Siebelink *et al.*, 1995). Antibody-mediated enhancement of HIV-1 *in vitro* proved to be related to the syncytium-inducing phenotype of the virus, which is directly related to the envelope glycoprotein structure (Allan *et al.*, 1990; Schutten *et al.*, 1995; Kostrikis *et al.*, 1996). In the present study, we have vaccinated cats with fixed autologous FIV-infected or uninfected peripheral blood mononuclear cells (PBMC) and challenged with the homologous molecular clone FIV-19k1. This approach was chosen to take advantage of the induction of cell-mediated immunity, since antigen presentation would take place in an MHC-matched fashion in each individual cat. We and others have recently shown that cellular immune responses against lentiviral proteins are of major importance in controlling lentivirus replication and disease (Flynn *et al.*, 1995, 1996; Hosie *et al.*, 1996; van Baalen *et al.*, 1997; Ogg *et al.*, 1998). Since we showed that paraformaldehyde is a relatively mild fixative for lentivirus membrane glycoproteins (Hulskotte *et al.*, 1997), we speculate that the envelope protein in this candidate vaccine was indeed presented in a way that closely resembles the native form, which may not always be the case with subunit vaccines. The HIV-1 envelope proteins of primary isolates are organized in oligomeric structures, which may be necessary for the induction of effective neutralizing antibodies (Poignard *et al.*, 1996). Also, when using this strategy we could exclude any

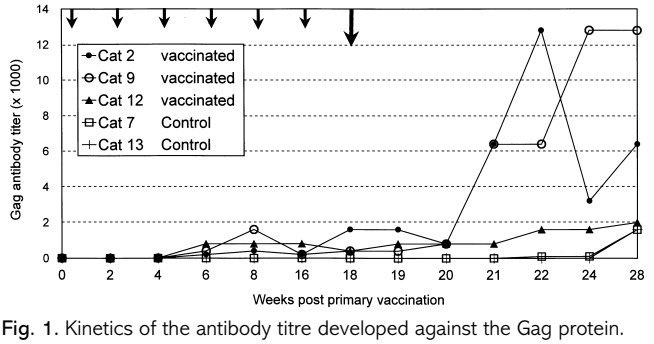

Fig. 1. Kinetics of the antibody titre developed against the Gag protein. Cats no. 2, 9 and 12 were vaccinated with fixed autologous FIV-19k1-infected PBMC. Cats no. 7 and 13 were vaccinated with fixed autologous uninfected PBMC. Small arrows, time-points of vaccination; large arrow, time-point of challenge.

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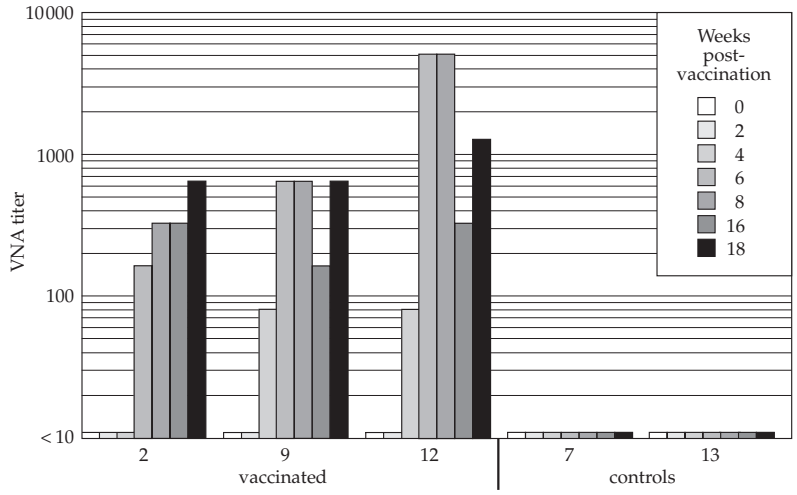

Fig. 2. Virus-neutralizing antibody titres of the individual cats at different weeks post-vaccination as measured in the CrFK virus-neutralization assay. Cats no. 2, 9 and 12 were vaccinated with fixed autologous FIV-19k1-infected PBMC. Cats no. 7 and 13 were vaccinated with fixed autologous uninfected PBMC.

beneficial role of antibodies against cellular proteins in protection against infection as has been described in simian immunodeficiency virus vaccine studies (Stott, 1991; Osterhaus *et al*., 1992).

PBMC of five 6-month-old cats (nos 2, 7, 9, 12, 13) were obtained and cultured as described previously (Siebelink *et al*., 1995) and were infected after 7 days with 1000 CID_{50} of molecular clone FIV-19k1 (Siebelink *et al*., 1992). In parallel, cultures with uninfected cells from each cat were maintained. Virus production was measured in an antigen ELISA (Siebelink *et al*., 1990). As soon as FIV antigen was detected in the culture supernatant, the percentage of infected cells was determined by an immunofluorescence assay, using serum of an experimentally FIV-infected specific-pathogen-free (SPF) cat. The number of infected PBMC for cats no. 2, 9 and 12 was approximately 5%, whereas this was found to be approximately 1% for cats no. 7 and 13. This variability in the *in vitro* rate is common in our experience when infection is allowed to progress for a limited period of time. This observation is probably due to minor differences in culture conditions. Subsequently, infected as well as uninfected cells from all cats were inactivated with 1·25% paraformaldehyde as previously described (Yamamoto *et al*., 1991). Complete inactivation of the vaccines was confirmed *in vitro* by co-cultivation of the vaccines with autologous cells and *in vivo* by attempting to reisolate FIV from the PBMC of cats following two inoculations with fixed autologous FIV-infected cells. Neither method led to antigen production as measured in an antigen ELISA (Siebelink *et al*., 1990). Three cats (nos 2, 9 and 12) were vaccinated with fixed autologous FIV-19k1-infected cells. The two other cats (nos 7 and 13) served as controls and received fixed autologous non-infected cells. The cats were vaccinated six times at weeks 0, 2, 4, 6, 8 and 16 intravenously and subcutaneously, each time with 5×10^6 cells. Challenge infection was performed intramuscularly with 10 CID_{50} of molecular clone FIV-19k1 at week 18. Blood samples were collected at weekly intervals during the first 4 weeks and at week 6 and 10 post-challenge. We determined the development of Gag-specific antibodies in plasma by ELISA as described before (Siebelink *et al*., 1995). All cats vaccinated with fixed autologous FIV-infected cells developed a Gag-specific antibody response after three vaccinations (Fig. 1). On the day of challenge the titres of the three vaccinated cats varied from 400 to 1600, while the two controls remained sero-negative. Antibody responses measured against two Env peptides (SU and TM) were also determined as previously described (Siebelink *et al*., 1995) and were negative for all cats except at one time-point for cat no. 12, which showed an ELISA titre of 200 after three vaccinations (data not shown). Virus-neutralizing antibody titres were determined using two systems: the first is based on inhibition of infection of Crandell feline kidney (CrFK) cells with CrFK cell-adapted virus FIV-AM6c (Siebelink *et al*., 1995), and the second is based on inhibition of infection of primary PBMC with molecular clone FIV-19k1 (Siebelink *et al*., 1993). In the assay based on CrFK cells, virus-neutralizing antibodies could be detected in sera of the cats vaccinated with fixed autologous FIV-infected cells from the second vaccination onward, reaching titres of 640–1280 on the day of challenge (Fig. 2). FIV-neutralizing antibodies could not be demonstrated in the two control cats before challenge. No virus-neutralizing activity could be detected in the serum of any cat on the day of challenge in the assay based on primary PBMC. Gag-specific antibodies became detectable in the control cats at 10 weeks post-challenge, reaching a titre of 1600 in both cats. In the vaccinated cats, an anamnestic response against Gag was evident from 2 weeks post-challenge onward (Fig. 1). These titres increased substantially in the following weeks, ranging from 800 to 13 000, except for cat no. 12, which showed a lower titre rise upon challenge. Cell-associated virus loads were determined with an infectious centre test as previously described (Siebelink *et al*., 1995). In the cats vaccinated with fixed autologous FIV-infected cells, 10–36 and 30–80 FIV-infected cells per 10^6 PBMC were demonstrated 2 and 3 weeks post-challenge

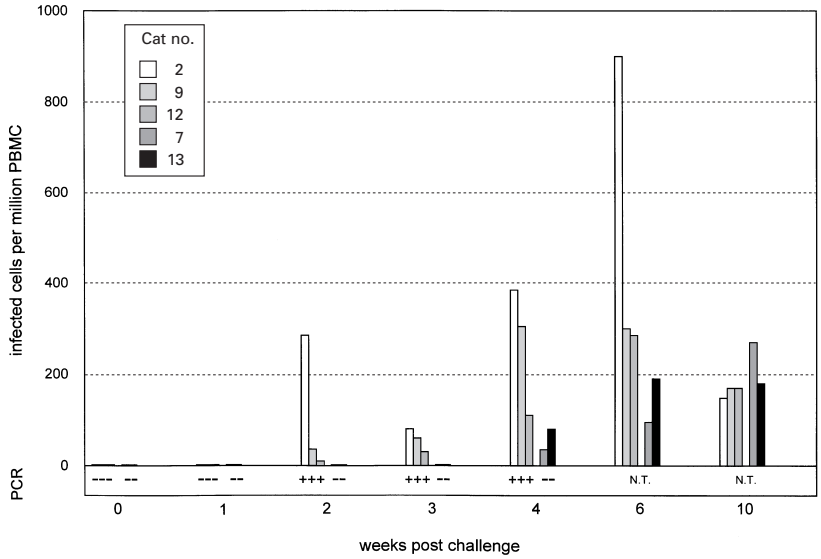

Fig. 3. The upper part of the figure shows the numbers of FIV-infected cells per 10^6 PBMC at different time-points post-challenge. Serially diluted PBMC samples (10^3, 3×10^3 and 10^4 cells) from the vaccinated and challenged cats were co-cultivated with 10^5 ConA- and IL-2-stimulated PBMC from an SPF cat in ten duplicate wells. After 3 weeks the culture supernatants were tested for the presence of FIV antigen by ELISA. The numbers of infected cells in the PBMC were calculated by assuming that one infected cell would give rise to antigen production. The lower part represents the presence of proviral DNA in PBMC of the cats at different time-points post-challenge, as determined with a nested *gag* PCR. Primer pair FIVDP1 (5′ GGCCCTCCACAGGCATATCC) and FIVDP2 (5′ GCATTTTATATCCTGGTGAGCC) was used for the first reaction and primer pair FIVDP3 (5′ GGCAAGAGAAGGACTAGGAGG) and FIVDP4 (5′ GCACAGCTCGAGGAGACTTAGC) was used for the nested reaction. −, Negative in PCR; +, positive in PCR; NT, not tested. Cats no. 2, 9 and 12 were vaccinated with fixed autologous FIV-19k1-infected PBMC. Cats no. 7 and 13 were vaccinated with fixed autologous uninfected PBMC.

respectively, whereas in the control cats no FIV-infected cells could be detected (Fig. 3). From 4 weeks post-challenge onward FIV-infected cells could also be determined in the two control cats. From 6 weeks post-challenge onward no differences in virus load between the two groups could be demonstrated. These observations were confirmed using a nested PCR based on the *gag* gene of FIV (Fig. 3). The cats vaccinated with fixed autologous FIV-19k1-infected PBMC contained proviral DNA in PBMC from 2 weeks post-challenge onward, whereas proviral DNA in the control cats could be shown from 4 weeks post-challenge onward. To determine whether antibodies were the cause of the observed enhancement, we purified immunoglobulins from plasma of both groups of cats, collected on the day of challenge, by means of protein A affinity chromatography. Naïve kittens were inoculated with 10 ml of whole plasma or an equivalent amount of purified immunoglobulins and subsequently challenged intramuscularly with 10 CID_{50} of molecular clone FIV-19k1. Blood samples were taken at the same time-points as described for the vaccinated cats. The virus loads of the cats were monitored using the infectious centre test and PCR. No differences in the kinetics of FIV infection and virus loads were observed between the four groups of cats.

Although we did not show the induction of virus-specific MHC class I-restricted CTL, we may expect that in these experiments, presentation of the envelope protein of FIV in its natural form and presentation of antigenic peptides derived thereof by matched MHC molecules did indeed result in the induction of these CTL. Consequently, if we assume that such CTL contribute to protective immunity we would have expected that this type of immunization would have led to protective immunity against a homologous challenge infection with molecularly cloned FIV. Instead, an accelerated viraemia was observed after challenge infection. Enhancement of challenge infection was observed in a previous study, after vaccination of cats with FIV subunit vaccines (Siebelink *et al.*, 1995) based on recombinant envelope protein incorporated into immune-stimulating complexes (iscom) (Rimmelzwaan *et al.*, 1994). Vaccine-induced envelope protein-specific antibody titres, as measured in the virus-neutralization assay based on CrFK cells, were similar in both studies. However, antibody titres directed against two peptides derived from the envelope protein were much lower in the cats vaccinated with fixed autologous FIV-infected cells than in the cats vaccinated with FIV Env iscom, which may explain the failure to transfer the phenomenon of accelerated viraemia to naïve kittens by means of plasma and purified immunoglobulins from this plasma in the present study. Besides the role of complement- and Fc-receptors in antibody-mediated enhancement, it has recently been described that the virus phenotype may also be involved (Schutten *et al.*, 1995). This was shown with monoclonal antibodies and virtually identical virus isolates derived from one patient. Some monoclonal antibodies neutralized syncytium-inducing viruses, whereas the replication of a non-

syncytium-inducing variant proved to be enhanced. A similar mechanism may play a role in the vaccination studies performed in the equine infectious anaemia virus model, where protection was found against homologous challenge after vaccination with recombinant envelope protein. In contrast, enhancement of infection was found in animals challenged with a heterologous virus (Montelaro *et al.*, 1996). This principle may also have played a role in our previous study, in which cats were vaccinated with recombinant envelope protein incorporated into iscom and where a biological FIV isolate was used for the challenge infection. This isolate contains a swarm of viruses, including the highly homologous molecular clones FIV-19k1 and FIV-19k32 (Siebelink *et al.*, 1992). It is of interest to note that FIV-19k1 is neutralized by its homologous serum, while FIV-19k32 was enhanced by the same antiserum (K. Siebelink, personal communication), indicating that there is a delicate balance between enhancement and neutralization. In the present study, however, it is unlikely that this mechanism of heterotypic enhancement of infection played a role, since a homologous molecular clone was used for challenge. Recently immune activation has been suggested as a possible mechanism for enhanced replication of FIV in a vaccination study (Richardson *et al.*, 1997). Furthermore, immune activation, including the activation of T-cells, seems to play a role in HIV-1-infected individuals who are vaccinated against opportunistic pathogens and who show a rise in HIV-1 virus load in plasma as a consequence of this (Ho, 1992; Staprans *et al.*, 1995; Brichacek *et al.*, 1996; Goletti *et al.*, 1996). It may be speculated that in the present study, in which a challenge infection was carried out only 2 weeks after the last booster vaccination, immune activation may also have played a role. To evaluate this possibility, we determined the induction of IFN-γ and IL-4 in PBMC after vaccination and challenge in both groups of cats. No differences in cytokine expression were observed, suggesting that immune activation did not play a role in the enhanced virus replication. However, cytokines other than IFN-γ and IL-4 may have played a role in the observed enhancement. Recently, it has been described that IL-10 upregulates expression of CCR-5 (Sozzani *et al.*, 1998), the chemokine receptor involved in infection of macrophage-tropic variants of HIV-1 (Choe *et al.*, 1996). This resulted in an enhanced *in vitro* infection of peripheral blood lymphocytes with HIV-1. Similar mechanisms, affecting primary or secondary receptors used by FIV, may be involved in the outcome of the study described here. The results obtained in the present experiments and in other studies referred to above show that enhancement of lentivirus infections can be a major problem in vaccine development. Several mechanisms can form the basis of this and it is important that the contribution of these mechanisms are addressed.

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References

Allan, J. S., Strauss, J. & Buck, D. W. (1990). Enhancement of SIV infection with soluble receptor molecules. *Science* **247**, 1084–1088.

Brichacek, B., Swindells, S., Janoff, E. N., Pirruccello, S. & Stevenson, M. (1996). Increased plasma human immunodeficiency virus type 1 burden following antigenic challenge with pneumococcal vaccine. *Journal of Infectious Diseases* **174**, 1191–1199.

Choe, H., Farzan, M., Sun, Y., Sullivan, N., Rollins, B., Ponath, P. D., Wu, L., Mackay, C. R., LaRosa, G., Newman, W., Gerard, N., Gerard, C. & Sodroski, J. (1996). The beta-chemokine receptors CCR3 and CCR5 facilitate infection by primary HIV-1 isolates. *Cell* **85**, 1135–1148.

Flynn, J. N., Beatty, J. A., Cannon, C. A., Stephens, E. B., Hosie, M. J., Neil, J. C. & Jarrett, O. (1995). Involvement of *gag*- and *env*-specific cytotoxic T lymphocytes in protective immunity to feline immunodeficiency virus. *AIDS Research and Human Retroviruses* **11**, 1107–1111.

Flynn, J. N., Keating, P., Hosie, M. J., Mackett, M., Stephens, E. B., Beatty, J. A., Neil, J. C. & Jarrett, O. (1996). Env-specific CTL predominate in cats protected from feline immunodeficiency virus infection by vaccination. *Journal of Immunology* **157**, 3658–3665.

Goletti, D., Weissman, D., Jackson, R. W., Graham, N. M., Vlahov, D., Klein, R. S., Munsiff, S. S., Ortona, L., Cauda, R. & Fauci, A. S. (1996). Effect of *Mycobacterium tuberculosis* on HIV replication. Role of immune activation. *Journal of Immunology* **157**, 1271–1278.

Ho, D. D. (1992). HIV-1 viraemia and influenza. *Lancet* **339**, 1549.

Hosie, M. J. & Flynn, J. N. (1996). Feline immunodeficiency virus vaccination: characterization of the immune correlates of protection. *Journal of Virology* **70**, 7561–7568.

Hosie, M. J., Osborne, R., Reid, G., Neil, J. C. & Jarrett, O. (1992). Enhancement after feline immunodeficiency virus vaccination. *Veterinary Immunology and Immunopathology* **35**, 191–197.

Hulskotte, E. G. J., Dings, M. E. M., Norley, S. G. & Osterhaus, A. D. M. E. (1997). Chemical inactivation of recombinant vaccinia viruses and the effect on antigenicity and immunogenicity of recombinant simian immunodeficiency virus envelope glycoproteins. *Vaccine* **15**, 1839–1845.

Kostrikis, L. G., Cao, Y., Ngai, H., Moore, J. P. & Ho, D. D. (1996). Quantitative analysis of serum neutralisation of human immunodeficiency virus type 1 from subtypes A, B, C, D, E, F, and I: lack of direct correlation between neutralisation serotypes and genetic subtypes and evidence for prevalent serum-dependent infectivity enhancement. *Journal of Virology* **70**, 445–458.

Lombardi, S., Garzelli, C., Pistello, M., Massi, C., Matteucci, D., Baldinotti, F., Cammarota, G., da Prato, L., Bandecchi, P., Tozzini, F. & Bendinelli, M. (1994). A neutralising antibody-inducing peptide of the V3 domain of feline immunodeficiency virus envelope glycoprotein does not induce protective immunity. *Journal of Virology* **68**, 8374–8379.

Montelaro, R. C., Grund, C., Raabe, M., Woodson, B., Cook, R. F., Cook, S. & Issel, C. J. (1996). Characterization of protective and enhancing immune responses to equine infectious anemia virus resulting from experimental vaccines. *AIDS Research and Human Retroviruses* **12**, 413–415.

Ogg, G. S., Jin, X., Bonhoeffer, S., Dunbar, P. R., Nowak, M. A., Monard, S., Segal, J. P., Cao, Y., Rowland-Jones, S. L., Cerundolo, V., Hurley, A., Markowitz, M., Ho, D. D., Nixon, D. F. & McMichael, A. J. (1998). Quantitation of HIV-1-specific cytotoxic T lymphocytes and plasma load of viral RNA. *Science* **279**, 2103–2106.

Osterhaus, A., de Vries, P. & Heeney, J. (1992). AIDS vaccine developments. *Nature* **355**, 684–685.

Poignard, P., Klasse, P. J. & Sattentau, Q. J. (1996). Antibody neutralization of HIV-1. *Immunology Today* **17**, 239–246.

Richardson, J., Moraillon, A., Baud, S., Cuisinier, A. M., Sonigo, P. & Pancino, G. (1997). Enhancement of feline immunodeficiency virus (FIV) infection after DNA vaccination with the FIV envelope. *Journal of Virology* **71**, 9640–9649.

Rimmelzwaan, G. F., Siebelink, K. H. J., Huisman, R. C., Moss, B., Francis, M. J. & Osterhaus, A. D. M. E. (1994). Removal of the cleavage site of recombinant feline immunodeficiency virus envelope protein facilitates incorporation of the surface glycoprotein in immune-stimulating complexes. *Journal of General Virology* **75**, 2097–2102.

Schutten, M., Andeweg, A. C., Bosch, M. L. & Osterhaus, A. D. (1995). Enhancement of infectivity of a non-syncytium inducing HIV-1 by sCD4 and by human antibodies that neutralize syncytium inducing HIV-1. *Scandinavian Journal of Immunology* **41**, 18–22.

Siebelink, C. H., Windrich, R. W., Chu, I., Groen, J., Weijer, K., UytdeHaag, F. G. & Osterhaus, A. D. (1990). An enzyme linked immunosorbent assay (ELISA) for the detection of feline immunodeficiency virus (FIV) antigen in cell culture and FIV specific antibodies in feline serum. *Development in Biological Standardization* **72**, 189–196.

Siebelink, K. H., Chu, I. H., Rimmelzwaan, G. F., Weijer, K., Osterhaus, A. D. & Bosch, M. L. (1992). Isolation and partial characterization of infectious molecular clones of feline immunodeficiency virus obtained directly from bone marrow DNA of a naturally infected cat. *Journal of Virology* **66**, 1091–1097.

Siebelink, K. H., Rimmelzwaan, G. F., Bosch, M. L., Meloen, R. H. & Osterhaus, A. D. (1993). A single amino acid substitution in hypervariable region 5 of the envelope protein of feline immunodeficiency virus allows escape from virus neutralization. *Journal of Virology* **67**, 2202–2208.

Siebelink, K. H., Tijhaar, E., Huisman, R. C., Huisman, W., de Ronde, A., Darby, I. H., Francis, M. J., Rimmelzwaan, G. F. & Osterhaus, A. D. (1995). Enhancement of feline immunodeficiency virus infection after immunization with envelope glycoprotein subunit vaccines. *Journal of Virology* **69**, 3704–3711.

Sozzani, S., Ghezzi, S., Iannolo, G., Luini, W., Borsatti, A., Polentarutti, N., Sica, A., Locati, M., Mackay, C., Wells, T. N., Biswas, P., Vicenzi, E., Poli, G. & Mantovani, A. (1998). Interleukin 10 increases CCR5 expression and HIV infection in human monocytes. *Journal of Experimental Medicine* **187**, 439–444.

Staprans, S. I., Hamilton, B. L., Follansbee, S. E., Elbeik, T., Barbosa, P., Grant, R. M. & Feinberg, M. B. (1995). Activation of virus replication after vaccination of HIV-1-infected individuals. *Journal of Experimental Medicine* **182**, 1727–1737.

Stott, E. J. (1991). Anti-cell antibody in macaques. *Nature* **353**, 393.

van Baalen, C. A., Pontesilli, O., Huisman, R. C., Geretti, A. M., Klein, M. R., de Wolf, F., Miedema, F., Gruters, R. A. & Osterhaus, A. D. M. E. (1997). Human immunodeficiency virus type 1 Rev- and Tat-specific cytotoxic T lymphocyte frequencies inversely correlate with rapid progression to AIDS. *Journal of General Virology* **78**, 1913–1918.

Wang, S. Z., Rushlow, K. E., Issel, C. J., Cook, R. F., Cook, S. J., Raabe, M. L., Chong, Y. H., Costa, L. & Montelaro, R. C. (1994). Enhancement of EIAV replication and disease by immunization with a baculovirus-expressed recombinant envelope surface glycoprotein. *Virology* **199**, 247–251.

Willett, B. J., Flynn, J. N. & Hosie, M. J. (1997). FIV infection of the domestic cat : an animal model for AIDS. *Immunology Today* **18**, 182–189.

Yamamoto, J. K., Okuda, T., Ackley, C. D., Louie, H., Pembroke, E., Zochlinski, H., Munn, R. J. & Gardner, M. B. (1991). Experimental vaccine protection against feline immunodeficiency virus. *AIDS Research and Human Retroviruses* **7**, 911–922.

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