4. Discussion

4.1 Induction and characterisation of neutralising antibodies against FeLV-A p15E in different species

Vaccines against FeLV represent the first example of successful vaccines preventing a retroviral disease. Three types of FeLV vaccines are currently available: inactivated whole virus preparations, inactivated mixed subunit preparations from FeLV-infected tissue culture filtrate and recombinant FeLV proteins. The commercially available vaccines containing inactivated FeLV subunit preparations are Fevaxyn, Leucocine and Leukocell2. Leucogen is an example of a recombinant vaccine, and comprises recombinant non-glycosylated surface envelope protein p45 (Tab.1).

None of the vaccines regularly induce virus neutralising antibodies following vaccination: such antibodies are usually detected only after recovery from challenge (Jarrett, 2001). However, a high proportion of cats are protected by vaccination, indicating that immune mechanisms other than neutralising antibodies may be involved. This conclusion is confirmed by results showing that a DNA vaccine engineered to promote the induction of FeLV-specific cytotoxic T cells provided protection against FeLV challenge without any detectable antibody responses (Hanlon et al., 2001). However, it should not be concluded from these findings, that neutralising antibodies are not important. The presence of neutralising antibodies in cats recovering from natural FeLV infection clearly correlates with resistance to infection and passive transfer of antibodies, either naturally through the colostrum (Hoover *et al.,* 1977) or experimentally by infusion (Haley et al*.,* 1985) protects cats against FeLV challenge. It is still possible that the methods used to detect neutralising antibodies are ineffective and that the level of such antibodies required to protect are lower than the detection threshold. Data presented here clearly show that the immunisation with Leucogen alone (group 56) was able to induce neutralising antibodies (group 56, Fig. 19, 20; Tab.4).

Numerous attempts have been undertaken to improve the efficacy of vaccination. For example, immunisation with the immunostimulating complexes (ISCOM) containing the gp70/gp85 precursor molecules not only induced virus neutralising antibodies, but also protection against infection (Osterhaus *et al.,* 1985) . The development of antibodies to gp70 and p15E were confirmed by Western blot (Osterhaus *et al.,* 1989). When compared with the commercial vaccine Leucocell, containing inactivated FeLV, the ISCOM preparation proved to be superior. Virus neutralising antibodies were also induced by synthetic peptides corresponding to a domain of gp70 of FeLV-A involved in infection (Weijer *et al.,* 1993). Since FeLV-B and FeLV-C might originate by recombination between FeLV and endogenous FeLV-related sequences (Bechtel *et al.,* 1998), neutralising antibodies against FeLV-A should also protect cats from natural infection with all subgroups.

Some commercial vaccines such as Leucogen, which comprise the 45 kDa unglycosylated surface envelope protein, do not contain p15E and for others containing p15E the role of this protein in the

induction of neutralising antibodies has not been addressed. For the first generation vaccines that do not fully protect animals from infection, addition of p15E protein as an additional component may be of great benefit.

4.1.1 Immunisation with FeLV-A p15E induces neutralising antibodies in different species including cats

As it had been shown for murine leukaemia virus (MuLV) that immunisation with gp70 plus p15E was more effective in inducing neutralising antibodies than was immunisation with gp70 alone (Schwarz et al., 1984), the ability of FeLV-A p15E to induce neutralising antibodies was studied in a goat, in rats and finally in cats. By immunisations of a goat and of rats with the ectodomain of FeLV-A p15E binding antibody titres up to $1x10^6$ were induced and neutralisation was observed from 51% to 99% (chapter 3.1, Tab.3). For antisera induced by p15E immunisations in rats neutralisation titres up to 1:16 were found (chapter 3.3, Fig. 19, C, F, Tab. 4), equivalent to an amount of 150-300µg/ml of p15E specific antibodies (chapter 3.1, Fig. 11). This observance is in accordance with previously published data showing the induction of neutralising antibodies by immunisation with the p15E protein of the gammaretrovirus PERV (Fiebig et al., 2003). Moreover it was shown that the efficiency of neutralising antibodies induced by immunisation with FeLV-A p15E is more than 50 fold increased in its natural host. Compared to the goat and rats, cats showed neutralisation titres up to 1:1024 (chapter 3.4 Fig. 24, Tab. 5) although titres of binding antibodies were similar to those observed in a goat and in rats (chapter 3.4, Tab.5).

4.1.2 The humoral immune response against the transmembrane proteins of HIV, PERV and FeLV-A shows the detection of similar located epitopes

For antisera obtained from immunisation with p15E in a goat, in rats and in cats two linear epitope regions were identified in the ectodomain of FeLV-A p15E termed E1 and E2 each subdivided into two epitopes E1a, b and E2 a, b (Fig. 42 C). In a previous study, several regions of gp70 and of p15E able to elicit FeLV-neutralising antibodies were identified by immunisation with synthetic peptides coupled to a carrier protein (Elder et al., 1987). In this case, the peptides corresponded to the Gardner-Arnstein virus and the antisera were induced in rabbits. Interestingly, one of the peptides inducing neutralising antibodies, I-7 (corresponding to the C-terminal end of p15E) contained the epitope E2b that was detected here and after immunisation with PERV p15E (Fiebig et al., 2003). In addition, the peptides C-18 and I-6 also induced neutralising antibodies and contained the epitopes E1b and E2a, respectively (Elder et al., 1987). However, no detailed epitope mapping of the neutralising rabbit sera had been performed.

Of greatest interest is the similar location and partial sequence homology of the epitopes described for

p15E of PERV (Fiebig et al., 2003) and FeLV (Fig. 25) with epitopes described for HIV. Monoclonal antibodies that neutralise a broad spectrum of HIV subtypes have been generated using antibody-producing cells from HIV-infected patients. The first such antibody, 2F5 (Muster et al., 1993), recognises a linear epitope ELDKWA localised at the N-terminal end of gp41 and the second, 4E10, recognises the epitope NWFNIT (chapter 3.3, Fig. 25). This epitope has partial homology to the E2 epitope of PERV (FEGWFN) (Fiebig et al., 2003) and the E2b epitope described here for FeLV (WFEGWFN). Although this epitope was not recognised by the FeLV p15E-specific goat serum, it was detected as the major C-terminal epitope by the sera from the immunised rats. In gp41, the 4E10 and the 2F5 epitopes are located in close proximity (LL**ELDKWA**SLW**NWFNIT**) (Fig. 40A). A second epitope, N-terminal to FEGWFN, was also detected by the FeLV p15E-specific goat and rat sera (Fig. 40C). This second epitope, designated E2a, contained (in the case of the goat serum) the core sequence KQRQQLF. Although the distance between these two epitopes (RL**KQRQQLF**DSQQGW**FEGWFN**K) is much greater than between the 2F5 and the 4E10 epitopes, their general proximity and immunogenicity suggests that the E2a and E2b epitopes may represent the functional equivalents of the 2F5 and 4E10 epitopes, respectively.
It is noteworthy that the recognition of the epitopes is dependent on the different species. The neutralising goat serum specific for PERV p15E recognised the 4E10 equivalent in the C-terminal end and the epitope E1a in the N-terminal end (Fiebig et al., 2003) (Fig.40B). The FeLV p15E specific goat serum recognised the 2F5 equivalent in the C-terminal end and E1a in the N-terminal end (Fig. 25, 40C). In contrast, sera from rats immunised with PERV p15E recognised a 4E10 equivalent and/or a 2F5 equivalent in the C-terminal end and an E1a epitope in the N-terminal end (Fiebig et al., in preparation) (Fig. 40B). Sera from rats immunised with FeLV p15E recognised a 4E10 equivalent and/or a 2F5 equivalent in the C-terminal end and E1a and/or E1b in the N-terminal end (Fig. 40C).

Immunisation of cats with p15E of FeLV-A induced neutralising antibodies, which recognised epitopes similar to those described for sera after immunisation of rats and goat with FeLV-A and PERV p15E (Fig. 25). Interestingly, cats infected with FeLV also develop antibodies against p15E, although the reactions by immunoblot are weak and ELISA titres are low (Tab.5, Fig.23). Epitope mapping revealed a variety of epitopes recognised by sera from FeLV-infected animals, including epitopes detected by sera from p15E-immunised cats, albeit comparatively weakly (chapter 3.3, Fig. 25). This suggests that natural FeLV infection results in a weak induction of antibodies specific for the viral transmembrane protein p15E and a low induction of neutralising antibodies.

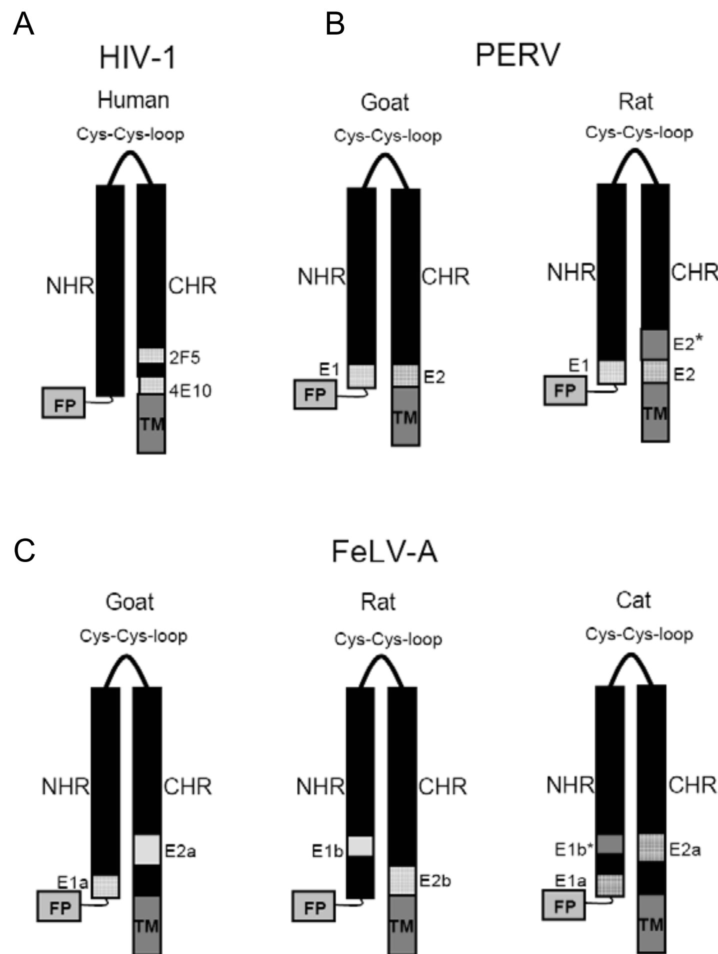

Figure 40 Schematic presentation of epitopes recognized in the ectodomains of transmembrane proteins of HIV-1, PERV and FeLV-A. Indicated are the cystein-loop (Cys-Cys-loop), the N- and C-terminal helix regions (NHR, CHR), the fusion peptide (FP) and the transmembrane domain (TM). (**A**) Localization of the 2F5 and the 4E10 epitopes in HIV gp41. (**B**) Detection of epitopes E1 and E2 induced by immunisation of PERV p15E in a goat and in rats. The E2* epitope is sporadically detected in rats. (**C**) Detection of epitopes E1a, b and E2a, b induced by immunisation of FeLV-A p15E in a goat, in rats and in cats. The E1b epitope is sporadically detected in immunised cats and epitopes E1a, b and E2a also could be detected in FeLV-A infected cats.

Synthetic peptides corresponding to the PERV E1 and E2 epitopes inhibit the neutralising activity of the goat serum more effectively when added together and it was therefore proposed they represent a conformational epitope (Fiebig et al., 2003). Although there is still no clear evidence for this, recent studies have shown that conformational changes take place in the transmembrane envelope protein of all retroviruses during the infection process that bring the two epitopes into close proximity (Gallo et al., 2003). After binding of the surface envelope protein (gp70 for FeLV and PERV and gp120 for HIV-1) to their corresponding receptors, the N-terminal helix and the C-terminal helix interact (E1 and

E2 are located at the N-terminal and C-terminal ends, respectively). The neutralising antibodies may inhibit either the interaction of both helices or later events in the infection process.

The mechanism of action of the human mAb2F5 is still poorly understood, although it is known that the antibody binds to the virion before attachment of the virus to the cell (Sattentau et al., 1993; Schmolke et al., in preparation). It was also shown that 2F5 does not influence the interaction between the helices (Golding et al., 2002) but rather inhibited later steps during infection (de Rosny et al., 2004). All attempts to induce neutralising antibodies using recombinant proteins containing the E2 epitope of HIV-1 gp41 (ELDKWA), have so far failed (Tian et al., 2001; Liao et al, 2000; Lu et al., 2000). Although final proof has to be presented, e.g., by immunoprecipitation of purified virus, binding of the HIV neutralising antibody 2F5 to the virions before attachment to the cells was shown (Sattentau et al., 1993). Also p15-specific antibodies induced in rats (Fig. 14) and cats (Fig. 26) were able to bind to the cell surface of infected cells as shown in immunofluorescence studies, suggesting the availability of the epitopes on the virion itself despite the fact that most of the transmembrane envelope protein is occluded by the surface envelope protein gp70.

These data obtained from the epitope characterisation show a greater similarity than previously suspected between gammaretroviruses such as PERV and FeLV and lentiviruses such as HIV-1 with regard to the localisation of target epitopes for broadly neutralising antibodies in the transmembrane envelope proteins. This similarity may be based on a common requirement for conformational changes during retroviral infection that can be inhibited by antibodies and hence prevent later stages of virus internalisation.

4.1.3 The deletion of the ISU domain from FeLV-A p15E does not improve the antigen, but maintains an identical humoral immune response as observed for immunisation with p15E

Due to the immunosuppressive properties of the transmembrane envelope protein, many researchers and manufacturers argue that p15E should not be added to a FeLV vaccine. Indeed, it has been shown that p15E and a synthetic peptide corresponding to a highly conserved region in the transmembrane envelope protein of all retroviruses (immunosuppressive domain ISU) inhibit mitogen-triggered lymphocyte activation and modulate cytokine production (Hebebrand et al., 1979; Mathes et al., 1979; Denner et al., 1998, Cianciolo et al., 1985). To evaluate the influence of ISU on the immune response, three rats were immunised with a p15E derived recombinant protein lacking the N-terminal located immunosuppressive domain and a C-terminal opposing amino acid sequence (FeLV-A ΔISU p15E). However, no difference was observed in the characterisation of the humoral immune response in comparison to immunisations with the complete p15E ectodomain. The induction of binding antibody

titres ranged from $2.5x10^5$ and $1x10^6$ (Fig. 16A) being similar to those induced by the complete p15E ectodomain. Epitopes in the E1 as well as in the E2 region were detected (Fig. 17) and one serum showed neutralisation of FeLV-A with a titre up to 1:16 (Fig. 16B).

Interestingly the neutralising antiserum (70.1) not only showed the highest titre of binding antibodies but also showed the recognition of the N-terminal E1a and E1b epitopes plus the C-terminal E2a epitope (Fig. 17). The other two antisera (70.2 and 70.3) only detected epitopes within the E1 region and had not such a neutralising activity. Thus it can be assumed that (i) the immunosuppressive domain does not have a major influence on the induction of the humoral immune response against FeLV-A p15E and (ii) that an induction of antibodies specific for epitope regions E1 and additional specific for the epitope region E2 might be necessary for an effective neutralisation of FeLV-A.

4.1.4 Sequences homologue to the epitopes are present as endogenous retroviral sequences in cats

Comparing the sequence of the infectious FeLV-A, able to induce leukemia and immunodeficiency in infected cats, with that of an endogenous proviruses, revealed a strong homology in the epitopes E1a, E1b, E2a and E2b. Only the E2b sequence (DGL instead of GWF) and regions outside of these the epitopes showed differences (Fig. 25). The induction of binding and neutralising antibodies specific for sequences present as endogenous retroviruses in the genome of all cats indicates a lack of tolerance and suggests that expression of the endogenous viral genes during ontogenesis (when discrimination between self and non-self is made) does not occur. Similar observations have been made with human endogenous retroviruses (HERVs), especially HERV-K, which is expressed in human teratocarcinomas (Löwer et al., 1996) and melanomas (Büscher et al., 2005, Muster et al., 2003). Antibodies against HERV-K were found in 45% (45 of 100) of testicular tumour patients, 26% (31 of 120) of lymphoma patients and 38% (3 of 8) of multiparous pregnant women (Löwer et al., 1996). Furthermore, antibodies against the transmembrane envelope protein were found in 22% (13 of 60) of melanoma patients (Muster et al., 2003). Although antibody titres are elevated compared with normal blood donors (3%, 1 of 30), they hardly ever reach the titres seen after infection with exogenous retroviruses such as HIV. Nevertheless, it is intriguing that antibodies are produced at all, since HERV proteins (like the proteins of endogenous retroviruses in cats) can be regarded as self-antigens that should induce tolerance. However, the presence of antibodies suggests that tolerance is not induced and that induction of antibodies specific for endogenous retroviral proteins is possible both by immunisation and by infection with an exogenous but highly related leukaemia virus. Absence of tolerance to retroviral proteins is certainly characteristic for all species carrying endogenous retroviruses.

4.1.5 Combined immunisation with FeLV-A p15E and Leucogen induces an increased response of neutralising antibodies in rats

Immunotherapy studies in AKR mice that spontaneously develop leukaemia showed that although neither antibodies to gp70 nor antibodies to p15E could influence the course of leukaemia development, a combination of the two antibodies was effective (Thiel et al., 1987). In parallel it has been shown for exogenous murine leukaemia virus (MuLV) that immunisation with gp70 and p15E was more effective in inducing neutralising antibodies than was immunisation with gp70 alone (Kleiser et al. 1986), and we therefore studied the ability of FeLV p15E to improve vaccination with Leucogen containing the unglycosylated form of gp70.

Results from immunisation of rats with FeLV-A p15E were confirmed here, showing that with and without simultaneous immunisation with Leucogen epitopes E1a, b and E2a, b were detected (Fig.22). There are three important conclusions from these results. First, sera induced with 100µg or 500µg p15E (groups 55 and 57) always detected the E2b epitope, while the E1b epitope was recognised only by one antiserum of each group (55.2 and 57.1). However, only these two antisera efficiently neutralised FeLV (Fig. 21; Tab.4), indicating a critical role of the E1b epitope in virus neutralisation. Second, the E2 epitopes also seem to be crucial for neutralisation since the serum from animal 60.1 did not recognise E2 and had only a weak neutralisation activity when compared with the sera from two other animals of the same group that recognise E2 (Fig. 21). Third, as mentioned above, only two of the sera from animals immunised with p15E recognised E1b and only these sera were neutralising. On the other hand, sera from all animals immunised with p15E and Leucogen recognised E1b (Fig. 22; Tab.4), indicating that simultaneous immunisation of both antigens increased the recognition of the crucial epitope E1b.

When p15E and Leucogen were injected simultaneously at one site the titre of p45-specific binding antibodies was reduced significantly (Fig. 20; Tab.4). An antigenic dominance of the transmembrane may be a reason for this effect. In addition, p15E may interact with p45, hiding some, but not all epitopes. Since the neutralising activity was not impaired despite the lower titre of binding antibodies, epitopes involved in neutralisation were obviously not hidden.

The results clearly show that immunisation with both antigens induced higher titres of neutralising antibodies suggesting that combination of Leucogen and p15E may be the strategy of the future. In addition, simultaneous immunisation of both antigens increased also the recognition of an epitope in p15E crucial for neutralisation (E1b, Fig. 22; Tab.4). Further more by these findings assumptions from immunisation with FeLV-A ΔISU p15E were supported indicating that the recognition of an N-terminal (E1) and a C-terminal (E2) epitope is essential for an effective neutralisation of FeLV-A.

4.2 FeLV-A p15E vaccine studies in cats

4.2.1 Immunisation with FeLV-A p15E alone can protect cats from productive infection

Our previous findings that immunisation with the transmembrane protein of retroviruses, including HIV-1, PERV and FeLV, can induce virus neutralising antibodies, have here been extended to show that a vaccine comprising FeLV p15E protects a proportion of cats against challenge with live virus.

FeLV is an excellent system in which to develop vaccine strategies for retroviral infections. Although FeLV can establish a persistent viraemia in cats, which leads in most cases to the death of the animal within a few years, the majority of cats exposed to the virus recover naturally. Both virus neutralising antibodies and cytotoxic T cells (CTL) have been implicated in the immune response that leads to recovery (Flynn et al., 2002) and either antibodies (Hoover et al., 1977; Jarrett et al., 1977) or CTL (Flynn et al., 2000) on their own are known to protect cats from experimental infection. Cats that recover from natural infection appear to have a normal life expectancy (Hardy et al., 1984), despite the fact that many retain a latent virus infection in the bone marrow, and possibly other tissues, for many months or years (Pacitti & Jarrett, 1985), and proviral DNA is found in a sizeable proportion of apparently healthy, non-viraemic pet cats (Hofmann-Lehmann et al., 2001). The clinical significance of the retention of this covert infection is not known but the capacity to quantify proviral DNA may have prognostic value in future.

Provirus load is an important parameter characterising disease progression in other retroviral infections (Watson et al., 1997). In HIV and simian immunodeficiency virus (SIV) infection a primary peak of provirus load was described, which decreases with the onset of the virus-specific immune response (Stevenson, 2003). Later, in the asymptomatic phase, provirus load is relatively low, increasing again with progression to AIDS and reaching it highest value with full-blown AIDS. In the present experiment, in non-immunised cats infected with FeLV an acute viremia was observed with a maximum in proviral load at day 30 (Fig. 29). In the animals immunised with Leucogen, provirus load also increased up to day 30 but to a much lower level and then decreased, reaching zero in most cases at day 100. There was a good correlation between provirus load and p27 antigen load. Animals immunised with Leucogen or the combination of Leucogen and p15E had the lowest provirus load and were always p27 antigen negative. Non-immunised animals had the highest provirus load and the highest p27 antigen load. In a previous study, Hofmann-Lehmann et al. (2001) found that cats that had been experimentally infected with FeLV and were antigen negative had a low and decreasing provirus load and increasing antibody titress. These animals developed a regressive contained viremia. On the other hand, animals with high and increasing p27 antigenaemia had a high provirus load and low, if any, antibody titress. These animals developed a progressive persistent viremia. The non-immunised animals in our experiment are characterised by virus load and p27 antigenaemia consistent with a progressive viremia. By contrast, all animals immunised with Leucogen alone or in combination with

p15E and 3 of 6 animals immunised with p15E alone are characterised by a regressive, contained viremia (transiently antigenaemic or p27 antigen-negative).

All of the cats in the present experiment became provirus positive after FeLV challenge, indicating that neither the commercial vaccine Leucogen nor p15E protected from provirus acquisition and minimal viral replication. This result agrees with findings in another study with two other vaccines: Eurifel, a canarypox-vectored live vaccine containing FeLV-A *env*, *gag* and *pol,* and Fel-O-Vax, an inactivated whole virus FeLV vaccine, using similar methods (Hofmann-Lehmann et al., 2005). These vaccines also did not induce sterilising immunity. However, vaccinated cats that resist challenge develop a very powerful immune response and are likely to be solidly protected from further infection, and free from FeLV-related disease. Although half of the cats immunised with p15E were protected from a productive infection they showed a significantly higher provirus load in the peripheral blood than cats immunised with Leucogen or with Leucogen and p15E until the end of the study.

In all cats immunised with Leucogen, p15E or a combination of both, binding antibodies were found (Fig. 27). Interestingly, cats immunised with Eurifel (Hofmann-Lehmann et al., 2005) or experimental FeLV DNA vaccines (Hanlon et al; 2001; O'Donovan et al., 2005), which also conferred protection from viremia, did not develop antibodies at all. Antibodies developed only after challenge, indicating that the challenge virus had grown in the vaccinated cats. In contrast, animals immunised with Fel-O-Vax, had detectable FeLV specific antibodies prior to challenge and showed afterwards a strong anamnestic response (Hofmann-Lehmann et al., 2005). In the animals protected from antigenaemia after immunisation with Leucogen, p15E or a combination of both, the neutralising activity of their sera correlated with protection. The protected cats showed the highest levels of neutralisation activity four weeks after immunisation (except cat 51.3) and at day 100 after the challenge (Fig.27C, Fig. 28), while for the non-immunised control cats or the cats that were not protected by immunisation, significantly lower levels of neutralising antibodies were observed (Fig. 30). These data confirm previous findings showing that cats with contained viremia displayed a more pronounced humoral immune response (binding antibodies measured in an ELISA) than cats that became viraemic (Hofmann-Lehmann et al., 2001). Animals immunised with Leucogen alone did not have neutralising antibodies prior to virus exposure (Fig. 30). This result agrees with data obtained when cats were immunised with Eurifel or Fel-O-Vax (Hofmann-Lehmann et al., 2005). Most interestingly, 2 of 6 animals immunised with p15E alone (Fig. 30) and both animals immunised with the combination of Leucogen and p15E (Fig. 30) had neutralising antibodies before challenge. This is the first time that neutralising antibodies prior to challenge have been described. Cats immunised with Leucogen alone (Fig. 30) or with Eurifel or Fel-O-Vax (Hofmann-Lehmann et al., 2005) did not have detected neutralising antibodies prior to virus exposure. On the other hand, in the present experiment, neutralising antibodies appeared in all animals, including non-immunised animals after week 10.

Although there was no correlation between protection and the neutralising activity three days before the challenge (i.e. 4 months after the boost immunisation) (Fig. 28), there was a good correlation

between protection and the titres of neutralising antibodies in sera obtained immediately after the second, booster immunisation (except cat 51.3). This result suggests that the ability to induce protection and the efficacy of a vaccine might be tested immediately after immunisation by estimating the titre of neutralising antibodies at that time. Later the titre of neutralising antibodies decreased rapidly and could not be detected at challenge. Interestingly, the titre of neutralising antibodies induced by p15E declined less rapidly than those induced by Leucogen. This result may be due to the neutralising antibodies in each group of cats being directed at a different target in the virus: p15E in the case of the cats immunised with p15E and gp70 in the case of the cats immunised with Leucogen. In addition, it was clearly shown that the titre of neutralising antibodies, but not binding antibodies, is a critical parameter for the prediction of antibody mediated protective immunity.
In capter 3.3.2 in which the immunisation of rats with FeLV p15E or Leucogen or a combination of both is described, the combination induced significantly higher titres of neutralising antibodies compared to immunisation with single antigens (p15E or Leucogen alone). Consequently, it was anticipated that vaccination with the combination might improve the quality of the response to challenge. However, cats immunised with the combination did not have higher titres of neutralising antibodies than cats immunised with p15E alone (Fig. 27, Fig. 29).
These observations have essential implications for the development of antiretroviral vaccines including a vaccine against HIV-1. First, protection against challenge by vaccination against retroviruses using the transmembrane protein is possible in an outbred population. Secondly, the question of whether the induction of sterilising immunity against retroviruses is necessary to protect vaccinates from developing active persistent infection and disease is open. A HIV vaccine that did not induce sterilising immunity, but protected from viremia and disease would be a great success. However, cells with persistent integrated proviruses might finally start to produce virus, e.g. by immune activation, which might be followed by viremia, disease and virus transmission. Therefore a vaccine able to induce sterilising immunity would be of great advantage. Adoptive transfer studies using broadly neutralising antibodies directed against gp120 and gp41 of HIV- 1 (2F5 and 4E10) showed that sterilising immunity in a retroviral system is possible (Ruprecht et al., 2003). Better immunisation strategies, including combination of different viral targets and a better presentation of the transmembrane envelope protein may increase the neutralising activity of the induced antisera.

4.3 Induction of neutralising antibodies against HIV-1 gp41 in rats by immunisation with p15E/ gp41 hybrid protein I

According to the protein folding model shown in chapter 3.6, Fig.31A which is based on previous publications demonstrating interactions between the N-terminal helix and the C-terminal helix of retroviral transmembrane envelope proteins (Eckert and Kim 2001; Gallo et al., 2003) the E1 epitope in the N-terminal helix is located opposite to the 2F5/4E10 epitope domain in the C-terminal helix.

These findings support the data showing that antibodies neutralising PERV or FeLV induced by immunisation with their p15E recognised an N-terminal and a C-terminal epitope region exposed in close proximity after interaction of both helices during intramolecular conformational changes (Fiebig et al., 2003).

The data obtained here indicates the possibility of induction of antibodies neutralising HIV-1 of the type 2F5 and 4E10 using p15E of FeLV with the inserted E2 (2F5/4E10 epitope) domain of gp41 of HIV-1. This is the first report showing induction of 2F5/4E10-like neutralising antibodies despite numerous attempts by several other laboratories (Tian et al., 2001; Lu et al., 2000; McGaughy et al., 2004). In addition, a new epitope domain in gp41 recognised by the antisera induced by immunisation with the p15E/gp41 hybrid protein was described (QNQQEKNEQELLELDKW). The localisation near the 2F5 epitope suggests that epitopes within this domain are the target of the neutralising antibodies.. Recently binding of 2F5 as well as 4E10 to cardiolipin was shown and a polyspecific autoreactivity was proposed for these antibodies (Haynes et al., 2005). Although in preliminary experiments we were unable to confirm these data at least for 2F5, the newly induced neutralising antibodies have to be investigated for their ability to bind cardiolipin. Most importantly, using a p15E/gp41 hybrid protein, antibodies of the 2F5/4E10-type neutralising laboratory and primary strains of HIV-1 were easily induced, suggesting that they do not represent autoimmune antibodies and that the conformation of the antigen is of great importance. As gp41 derived peptides or gp41 proteins were not able to induce neutralising antibodies against HIV-1 (Tian et al., 2001; Lu et al., 2000; McGaughy et al., 2004), it has to be assumed that the conformation of the hybrid antigen allows the induction of such antibodies. However it remains unclear if the neutralising activity depends on a single antibody population. At least one of the monoclonal antibodies generated here (chapter 3.7, mAb3E4E3) from a rat with a HIV-1 neutralising serum recognises an epitope near by the main serum epitope on gp41, but does not show any neutralisation of the virus. These findings might offer a new way for proceeding towards an HIV-1 vaccine based on the induction of broadly neutralising antibodies.