7. Appendix

7.1 Chapter 3.3.4, figure 25

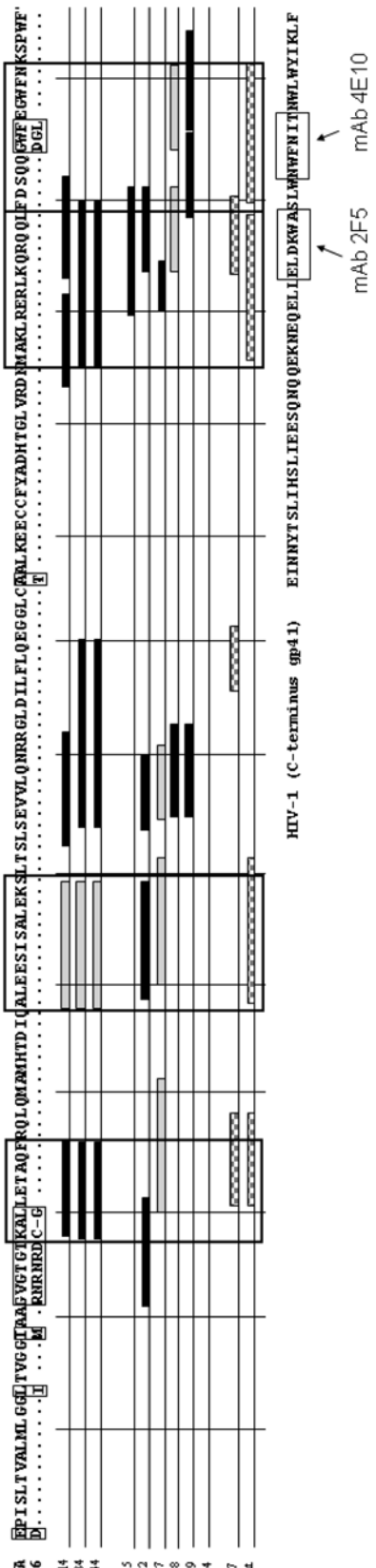

Figure 25 Epitope mapping using sera from immunised and FeLV-infected cats. Summary of the epitopes identified recognised by each serum. Sequences corresponding to the recombinant p15E of FeLV-A, strain Glasgow 1, and the corresponding sequence of an endogenous p15E are given at the top. Cats 14, 34 and 44 were immunised with p15E while the others are representative of FeLV-infected cats. Strong epitopes are marked in black, weak epitopes in grey. Common 2 groups of epitopes are framed (E1a, E1b, E2a, E2b). In addition, epitopes recognised by the serum from goat 27, immunised with p15E, and consensus epitopes recognised by 8 rats immunised with p15E are shown (hatched)6. For comparison, the C-terminal part of the HIV-1 transmembrane envelope protein gp41 and the localisation of epitopes recognised by two monoclonal antibodies (mAb) broadly neutralising HIV-1, (2F5 and 4E10) are shown (framed).

7.2 Primer and probes (Sigma-Genosys, Steinheim, Germany)

1. FeLV-A p15E forward:
   5´-GCGGATCCCTTGAAACAGCCCAGTTCAGACAA-3´

2. FeLV-A p15E reverse:
   5´-CGGAATTCCCAGGGGGACTTGTTGAACCATCC-3´

3. p4:
   5´-ATGCGGCCGCGCAACATTCTTCTTTCAATGCGGCACAGAGTTCAGAAAGGGAGGT-3´

4. p5:
   5´-GCGCGGCCGCAGACTAAAACAGCGGCAACA-3´

5. p5 hybrid I:
   5’-ATGCGGCCGCATTAATTGAAGAATCGCAAAA-3’

6. p5 hybrid II:
   5’-ATGCGGCCGCAGAAAAGAATGAACAAGAA-3’

7. FeLV-RTQ:

   sense primer:
   5´-TCAAGTATGTTCCCATGAGATACAA-3´

   antisense primer:
   5´-GAAGGTCGAACTCTGGTCAACT-3´

   FeLV-A probe:
   5`-6Fam-TTAAGCACCTGGGCCCCGGC-Dabcyl-3´

8. HIV-RTQ:

   68i: SK68i M:
   5'-GGARCAGCIGGAAGCACIATGG-3´

   69i: SK69i M:
   5´-CCCCAGACIGTGAGITICAACA-3´

   HIV-1 probe:
   5´- 6Fam-TGACGCTGACGGTACAGGCCAGAC-Dabcyl-3´

9. PERV-RTQ:

   PERV real s: 5'-TCCAGGGCTCATAATTTGTC-3´

   PERV real_as: 5'-TGATGGCCATCCAACATCGA -3´

   PERV probe: 5’-6Fam-AGAAGGGACCTTGGCAGACTTTCT –Dabcyl-3’

7.3 Amino acid and nucleotide sequences of recombinant constructs without CBP

1. FeLV-A p15E ectodomain:

CTTGAAACAGCCCAGTTCAGACAACTACAAATGGCCATGCACACAGACATCCAGGCCCTAGA
AGAATCAATTAGTGCCTTAGAAAAGTCCCTGACCTCCCTTTCTGAAGTAGTCTTACAAAACA
GACGGGGCCTAGATATTCTATTCTTACAAGAGGGAGGGCTCTGTGCCGCATTGAAAGAAGAA
TGTTGCTTCTATGCGGATCACACCGGACTCGTCCGAGACAATATGGCCAAATTAAGAGAAAG
ACTAAAACAGCGGCAACAACTGTTTGACTCCCAACAGGGATGGTTTGAAGGATGGTTCAACA
AGTCCCCCTGGTTT

LETAQFRQLQMAMHTDIQALEESISALEKSLTSLSEVVLQNRRGLDILFLQEGGLCAALKEE
CCFYADHTGLVRDNMAKLRERLKQRQQLFDSQQGWFEGWFNKSPWF

2. FeLV-A ΔISU p15E:

CTTGAAACAGCCCAGTTCAGACAACTACAAATGGCCATGCACACAGACATCCAGGCCCTAGA
AGAATCAATTAGTGCCTTAGAAAAGTCCCTGACCTCCCTTTCTGAACTCTGTGCCGCATTGA
AAGAAGAATGTTGCGCGGCCGCAAGACTAAAACAGCGGCAACAACTATTTGACTCCCAGCAG
GGATGGTTTGAAGGATGGTTCAACAAGTCCCCCTGGGAATTCTAG

LETAQFRQLQMAMHTDIQALEESISALEKSLTSLSELCAALKEECCAAARLKQRQQLFDSQQ
GWFEGWFNKSPWF

3. p15E/gp41 hybrid protein I:

CTTGAAACAGCCCAGTTCAGACAACTACAAATGGCCATGCACACAGACATCCAGGCCCTAGA
AGAATCAATTAGTGCCTTAGAAAAGTCCCTGACCTCCCTTTCTGAACTCTGTGCCGCATTGA
AAGAAGAATGTTGCGCGGCCGCATTAATTGAAGAATCGCAAAACCAGCAAGAAAAGAATGAA
CAAGAATTATTGGAATTAGATAAATGGGCAAGTTTGTGGAATTGGTTTAACATAACAAATTG
GCTGGAATTCTAGA

LETAQFRQLQMAMHTDIQALEESISALEKSLTSLSELCAALKEECCAAALIEESQNQQEKNE
QELLELDKWASLWNWFNITNWLEF

4. p15E/gp41 hybrid protein II:

CTTGAAACAGCCCAGTTCAGACAACTACAAATGGCCATGCACACAGACATCCAGGCCCTAGA
AGAATCAATTAGTGCCTTAGAAAAGTCCCTGACCTCCCTTTCTGAACTCTGTGCCGCATTGA
AAGAAGAATGTTGCGCGGCCGCAGAAAAGAATGAACAAGAATTATTGGAATTAGATAAATGG
GCAAGTTTGTGGAATTGGTTTAACATAACAAATTGGCTG

LETAQFRQLQMAMHTDIQALEESISALEKSLTSLSELCAALKEECCAAAEKNEQELLELDKW
ASLWNWFNITNWL