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The results document that atrazine has a toxicity for gills and renal and hemopoietic tissue also in fish. Even relatively low concentrations comparable to those in some surface waters may induce distinct lesions at chronic exposure.

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### **T-LYMPHOCYTE SUBPOPULATIONS IN FIV-POSITIVE AND -NEGATIVE CATS**

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#### K. Hartmann, W. Kraft

Strong similarities have been shown between feline immunodeficiency virus infection (FIV) and the human AIDS-complex concerning virus structure as well as clinical and epidemiological manifestations (Pedersen, 1989; Yamamoto et al., 1989).

The human counterpart HIV is known to infect predominantly DC4<sup>+</sup> T helper lymphocytes and cells of the monocyte/macrophage lineage (Detels et al., 1983; Levy, 1985; for review Sattentau et al., 1988). Gradual reduction in CD4<sup>+</sup> T helper subpopulation absolute numbers as well as in percentage is one of the most striking immunological consequences of HIV infection. We wondered if T-helper and T-suppressor lymphocytes were also changed in FIV-infected cats.

Therefore we have examined peripheral blood lymphocytes of FIV antibody positive domestic cats using recently developed monoclonal antibodies against cat CD4 homologue (Ackley et al., 1990) and cat CD8 homologue (Klotz et al., 1986).

The most prominent clinical symptoms of cats included in this study were: chronic infections of the mouth, chronic upper respiratory infections, fever, chronic infections of the skin, inappetence, weight loss and in some cats, neurological signs or uremia. Control animals were FIV and FeLV seronegative cats without signs of disease.

Immunofluorescence analysis of mononuclear cells of peripheral blood was performed as two-colour staining using goat-anti-cat-Ig-PE for demonstration of B-cells together with either FITC-labeled anti-fCD4 or FITC-labeled anti-fCD8. Lymphocytes were analysed by automated flow cytometry (FACScan, Becton Dickinson, Mountain View, CA). FACS analyses of peripheral blood lymphocytes of healthy seronegative cats revealed a percentage of 37% of fCD4<sup>+</sup> cells and 11.36% of fCD8<sup>+</sup> celle (table), whereas seropositive cats had 26% fCD4<sup>+</sup> and 17.2% fCD8<sup>+</sup> labeled lymphocytes, all of which

were negative for feline Ig. The percentage of  $Ig^+B$ -lymphocytes in seropositive cats were not different from those of seronegative animals.

When the absolute numbers of  $fCD4^+$  and  $fCD8^+$  cells were calculated, there was a similar appearance of figures, however an increase in  $fCD8^+$  cells was less prominent than a decrease in  $fCD4^+$  cells.

Changes in T-cell subsets in FIV-antibody positive, clinically affected cats show a clear tendency of decreased  $fCD4^+$  cells and of an increase in  $fCD8^+$  lymphocytes. These alterations result in a fCD4/fCD8 ration of 1.6 in FIV-positive cats compared with a ratio of 3.4 in control animals. A relationship between severity of illness and low  $fCD4^+$  cells counts (as demonstrated in HIV-infection) could be revealed. But there were also low  $fCD4^+$  values in cats without severe clinical symptoms. A correlation between virus antigen positivity and  $fCD4^+$  counts could not be detected, since virus positivity is not yet available. The alterations in T-cell subsets are a further evidence for a strong similarity between human AIDS and FIV induced feline AIDS-like disease concerning the pathogenesis. Therefore FIV induced disease is an animal model better suitable for treatment and immunization approaches than for example, SIV infections.

Table: Percent and absolute numbers of  $fCD4^+$  and  $fCD8^+$  cells in FIV seropositive and seronegative cats.

	CD4 <sup>±</sup> %	abs/µl	CD8 <sup>±</sup> %	abs/µl	CD4/CD8 ratio
seropositive $n = 20$	26.05 <sup>±</sup> 10.02	733 <sup>±</sup> 461	17.2 <sup>±</sup> 7.9	477 <sup>±</sup> 382	1.6 <sup>±</sup> 0.83
seronegative n = 22	37.32 <sup>±</sup> 14.11	1305 <sup>±</sup> 761	11.36 <sup>±</sup> 3.98	379 <sup>±</sup> 153	3.4 <sup>±</sup> 1.6

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## ALTERNATIVES TO IN VIVO ANIMAL EXPERIMENTATION: DEVELOPMENT OF A NOVEL IN VITRO ASSAY TO STUDY NEUTROPHIL-ENDOTHELIUM INTERACTION

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Adhesion of neutrophil granulocytes (PMN) to endothelial cells (EC) is an essential event in the triggering of further steps in the inflammation process. A substantial number of drugs such as steroidal or non-steroidal antiinflammatories are used either therapeutically or prohphylactically. Therefore, it is important to study the mechanisms of action of those drugs and their eventuel cytotoxic effects on the endothelium.

We developed a novel physiological model, based on endothelial cell culture to study those cellular interactions in conditions simulating the *in vivo* situation. Cultures of EC were initiated from bovine aortas obtained at the local slaughterhouse. The cells were isolated enzymatically with collagenase 0.2%. The primary cultures were fed with growth medium containing 10% fetal calf serum and antibiotics. After the first passage the cells were fed with a medium free of antibiotics to preserve the integrity of the receptors. The cells were passaged until purity: the endothelial nature was confirmed morphologically by the typical «cobblestone pattern» and immunohistochemically by the presence of factor VIII (Fig. 1). In the final stage of the culture, the EC were grown on collagen-coated microcarriers