Alternatives to in vivo animal experimentation : development of a novel in vitro assay to study neutrophil-endothelium interaction

Autor(en): **Holden, W. / Zwahlen, R. / Holub, M.**

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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.

Institut für Immunologie, GSF, Munich, FRG

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

G. Hoffmann-Fezer, I. Thum, C. Ackley, J. Mysliwietz, 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 $DC4^+$ 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 $CD4^+$ 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 $fCD4^+$ cells and 11.36% of $fCD8^+$ celle (table), whereas seropositive cats had 26% $fCD4^+$ and 17.2% $fCD8^+$ 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.

	$CD4^{\pm}$ %	abs/µl	$CD8^{\pm}$ %	abs/µl	CD4/CD8 ratio
seropositive n = 20	$26.05^{\pm}10.02$	$733^{\pm}461$	$17.2^{\pm}7.9$	$477^{\pm}382$	$1.6^{\pm}0.83$
seronegative n = 22	$37.32^{\pm}14.11$	$1305^{\pm}761$	$11.36^{\pm}3.98$	$379^{\pm}153$	$3.4^{\pm}1.6$

Institute of Veterinary Pathology, University of Berne, Switzerland

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

W. Holden, R. Zwahlen, M. Holub

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

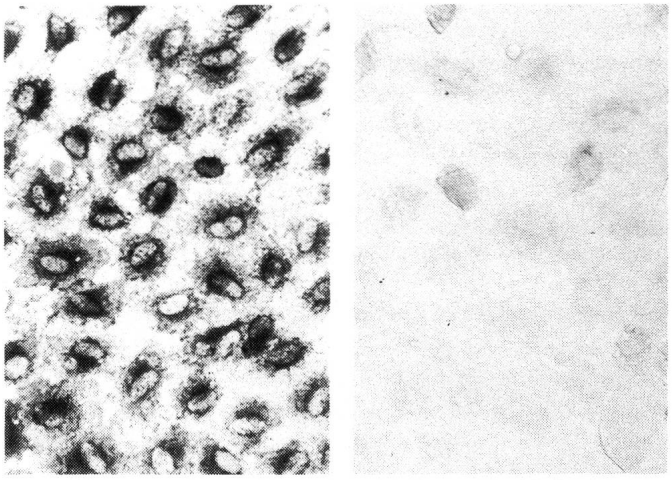

Fig. 1: Immunohistochemical evidence of Factor VIII on confluent endothelial cell culture. Left: positiv, right, negativ control.

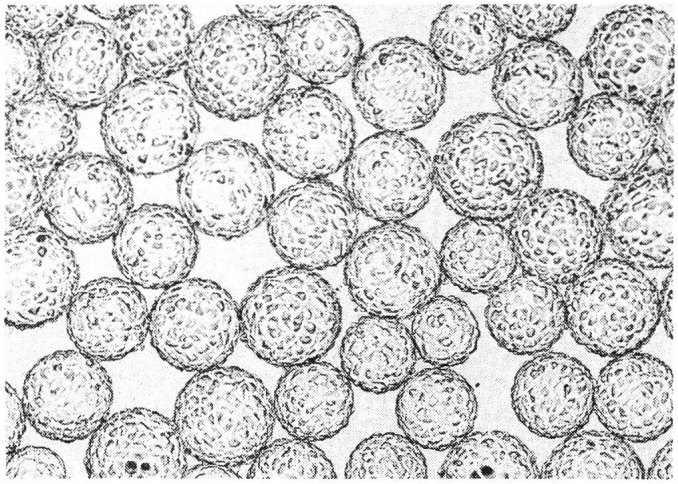

Fig. 2: Confluent endothelial cell culture on Cytodex microcarriers.

(Cytodex 3, Pharmacia) suspended in stirrer-bottles. A magnetic stirrer allowed modulation of the rotation time and speed. When the microcarriers were totally covered with EC (Fig. 2), they were rinsed in serum free medium, aliquoted into tubes; then were treated with C5a (1:250) or 10^{-9} M platelet activating factor (PAF). Two tubes remained untreated, indicating the basal adherence values. After the treatment, the covered microcarriers were loaded in borosilicate glass columns and kept at 37°C. PMN were isolated from bovine whole blood containing the anticoagulant ACD (Acid Citrate Dextrose) in a 9:1 ratio. After centrifugation, the plasma layer, buffy coat and top half of the erythrocytes were discarded. The red cell fraction was then subjected to flash hypotonic lysis with cold distilled water, followed by reconstitution of isotonicity with hypertonic saline solution and centrifugation. Separation of neutrophils from eosinophils was achieved on a discontinuous Percoll gradient, separating the cells according to their density. The PMN pellet was then resuspended in a balanced salt solution and its concentration adjusted to 5x10 6 cells/ml. The PMN vitality was assessed by Trypan blue dye exclusion.

Adherence assay: The columns, loaded with the EC-covered microcarriers, were drained of their medium to the top of the microcarrier-layer; the PMN-suspension, untreated or treated (510 µM dexamethasone or 3.3 mM phenylbutazone, respectively) was then loaded on the columns. A sample of PMN-suspension was taken before and after passage through the columns, allowing the calculation of adherence. The results thus obtained gave values of 56% for basal adherence, while C5a stimulated cells presented an enhanced adherence of 110% of the control value. Incubation of PMN with dexamethasone reduced the adherence to 34%, and with phenylbutazone to 8% of the control value. EC stimulated with 10^{-9} M PAF presented an adherence of 98% of the control. Preincubation of PMN with dexamethasone and phenylbutazone reduced those values to 39%, and 11% respectively. These results indicate clearly that the effects of antiinflammatory drugs can be reproduced in a physiological isolated system. This novel system is now being tested more extensively. It shoul help reducing the number of experimental animals used in assessing pharmacological and toxicological data on new drugs.

Institut of Veterinary Pathology, University of Berne, Switzerland

BOVINE CARDIOMYOPATHY: PATHOMORPHOGENETIC AND BIOCHEMICAL STUDIES IN YEARLING STEERS

F. König, R. Zwahlen, J. Schaller, U. Kämpfer, D. Roth, A. Tontis, H. Luginbühl

In Switzerland bovine cardiomyopathy (BCMP) is a serious problem of SimmentalxHolstein crossbred cattle, causing severe economic losses in the dairy industry. While the morphologic features of the advanced stages of this invariably fatal disease have been described in detail, information on development of lesions and on the pathogenesis of the disease still remains scant.

Material and methods

In the present study we analyzed organs from 172 yearling steers (group A: 78 crossbred steers, genetically disposed to BCMP; group B: 70 crossbred steers, of genetic low risk for BCMP; and group C [controls]: 24 pure Simmental steers). None of the selected steers showed clinical signs of illness. The following methods were used for morphological, and in selected animals, for additional biochemical analyses: qualitative and semiquantitative randomized histologic evaluation of BCMP related lesions in heart, lung, liver and kidney; biochemical analysis of the myocardial amino-acid pattern in selected animals of our study population (12 controls and 3 animals with histologically confirmed myocardial degeneration and fibrosis) and, additionally, biochemical analysis of 6 adults (positive control) with clinically manifest BCMP by acid hydrolysis and HPLC.