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deposits appeared in the membrane and mesangial area. These results are similar to the ones observed by Pizzirani (1989).

In the cases of MPGN types I and II we saw immunocomplex deposits in the subendothelial area in the type I and in subepithelial and mesangial areas in the type II.

We observed important immunological differences between these two types. In the MPGN type I we found deposits of IgG, IgA, IgM and C<sub>3</sub> and in the MPGN type II we principally found IgM (Fig. 2) and a smaller proportion of C<sub>3</sub>. These results correspond with the observations of Rosen (1983).

In conclusion, the results of this study indicate that immunologically the deposits are IgG and C<sub>3</sub> in the initial lesions and IgG and C<sub>3</sub> in

the advanced stages of glomerulonephritis and glomerular sclerosis. IgA did not appear to play a role in the process.

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### IDENTIFICATION OF CANINE AND FELINE MEGAKARYOCYTIC CELLS (NORMAL/LEUKEMIC) BY THE DEMONSTRATION OF GLYCOPROTEIN GPIIB/IIIA AND VON WILLEBRAND FACTOR

*F. Colbatzky, J. Darbès, G. Minkus, W. Hermanns*

It is nearly impossible to discern the immature blast cells of the four hematopoietic cell lines in paraffin sections routinely stained with hematoxylin-eosin or Giemsa. So the reliable identification of promegakaryoblasts and megakaryoblasts is difficult. Besides, some tumours such as myelomas or osteoclastomas can show morphologic characteristics very similar to those of megakaryocytic leukemia.

We, therefore, used air-dried blood films from EDTA-anticoagulated blood, glass slides with platelet films prepared by centrifugation of platelet-rich plasma in a special cytocentrifuge, and cryostat, paraffin as well as plastic sections of normal bone marrow to develop a method for the immunohistochemical demonstration of normal and leukemic megakaryocytic cells in formalin-fixed tissue samples in dogs and cats. In addition, formalin-fixed and paraffin or plastic embedded organ specimens of one cat and two dogs suffering from megakaryocytic leukemia were investigated. The 3 monoclonal antibodies Y2/51 (Dakopatts), CLB-37 (Janssen) and HPL-1 (Sera-Lab) directed against antigenic determinants on the human glycoprotein complex gpIIb/IIIa and a polyclonal antiserum directed against human von Willebrand factor (anti-vWF, Dakopatts) were used.

According to our hitherto performed investigations strong and reliable immunohistochemical staining of normal and leukemic mega-

karyocytic cells could only be achieved with Y2/51 and anti-vWF in paraffin sections. Plastic sections always gave negative results with all antibodies used. The monoclonal antibody CLB-37 detected only human and feline platelets and megakaryocytic cells. The intensity of reaction products was always considerably lower in dogs than in cats. In both species the results with anti-vWF were generally better than with the monoclonal antibodies. In the three cases of megakaryocytic leukemia both megakaryocytes and blast-like tumour cells were stained, the number of positive tumour cells being higher in the cat. All three cases showed a strong labeling of endothelial cells with anti-vWF.

To test the specificity of the monoclonal antibodies in dogs and cats, probes of platelet membranes were prepared for SDS-PAGE. The electrophoretically separated proteins were then blotted to nitrocellulose filters and stained by an indirect immunoperoxidase technique. In both species the monoclonal antibody Y2/51 detected a protein, which was in respect of the molecular weight similar to human gpIIIa (unreducing conditions). As expected, HPL-1, which is directed against the whole glycoprotein complex gpIIb/IIIa, gave negative results. Similarly, the binding site of the gpIIIa specific monoclonal antibody CLB-37 was obviously destroyed in the experimental conditions.

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### DEMONSTRATION BY IMMUNOCYTOCHEMICAL METHODS OF THE T LYMPHOCYTIC ORIGIN OF A FAMILIAL BOVINE THYMIC LYMPHOSARCOMA

*B. Da Costa, S. Djilali, B. Sacré, J. L. Kessler, F. Femenia, A. L. Parodi*

Bovine leukosis is a malignant tumour of lymphoid cells. Four epidemiological and anatomical forms of the disease are known: the adult multicentric form, the cutaneous form, the adolescent thymic form and the multicentric calf form (Bendixen, 1965) (1).

The observation of 216 cases of the thymic form of bovine leukosis during a short period in some areas of France prompted us to

undertake studies of these unusual tumours. A genetic survey demonstrated that all the leukotic calves were sired by the same bull. The occurrence has been estimated to 3% among the offspring of the bull (2).

### Material and methods

A complete study was undertaken in 38 tumour cases by hematology, serology, light and electronic microscopy and immunocytochemi-

stry. Immunocytochemical study was performed with a panel of monoclonal antibodies on frozen tissue sections by a peroxidase staining method. Following monoclonal antibodies were used: BoT2 (4), BoT4 (5), and BoT8 (6). The other monoclonal antibodies were obtained in our laboratory after immunization of mice with the TLS cells: M1 (presumably BoT5), M23 (pan T-cell) and M24 (presumably anti class II MHC antigen).

Terminal deoxynucleotidyl transferase (TdT), a marker for immature thymic lymphocytes was detected by indirect immunofluorescence assay.

### Results and discussion

The tumour was classified as a lymphoblastic malignant lymphoma according to the adapted Kiel classification (3).

No positive labelling on tumour sections was obtained with either B-lymphocyte markers (CIg and SIg) or T-lymphocyte markers BoT2, BoT4 and BoT8. Nevertheless, three monoclonal antibodies obtained in our laboratory: M1, M23 and M24 gave positive labelling. Furthermore, all tested TLS were TdT positive. Our results suggest the T-lymphoid origin of the TLS (BoT5+, and BoT7+). By comparison with human T cell ontogeny the tumour cells presented

an immature phenotype: class II antigen+, TdT+, BoT2-, BoT4- and BoT8-, which can be related to the prothymocyte stage of T cell differentiation (7).

### Conclusion

In conclusion, we describe herein an unusual familial TLS in bovine species which developed in a large scale in the offspring of a bull. For the first time, the T-lymphoid origin and the immature phenotype of a thymic lymphosarcoma in bovine species were demonstrated using cell membrane immunomarkers and enzymatic activity.

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## SEARCHING LITERATURE FOR VETERINARY PATHOLOGY, II. BUILDING A BIBLIOGRAPHIC DATABASE FOR EVERYDAY USE IN VETERINARY DIAGNOSTIC PATHOLOGY

Th. A. M. Elsinghorst, A. H. H. M. Mathijssen

A description is given of the creation of a bibliographic file for use in veterinary diagnostic pathology. The file is filled by an «intermediary documentalist» as mentioned in part I (previous presentation). The filling of the file was carried out in the following ways:

A. Retrospectively:

1. By downloading from Medline on CD-ROM (Compact Disc Read-Only-Memory), 1976–1989
2. Manually from existing files (cards, reprints, photocopies etc.)

B. Weekly updating, mainly from Current Contents on diskette, editions: Life Sciences and Agriculture, Biology & Environmental Sciences.

The vast majority of the records were extracted from CD-ROM using the following search profiles:

I. Years 1981–1989:

- a. Explode C22 (Animal Diseases), with the subheadings pathology and pathophysiology
- b. Explode C22, limited to reviews, with all subheadings
- c. Relevant MESH-terms for techniques used in (veterinary) pathology
- d. All references from the journal *Seminars in Diagnostic Pathology*

II. Years 1976–1980:

Explode C22, limited to reviews, with all subheadings

The total yield using these search profiles was approximately 12000 references (= 0.4% of the total contents of the CD-ROM discs).

The journal coverage from Current Contents on diskette was determined by the end-users (i.e. 16 staff members of the Department of Veterinary Pathology). The following journals were selected:

- I. 40 journals of the category PATHOLOGY
- II. 80 journals of the category VETERINARY MEDICINE
- III. 80 journals of 26 other categories

From the weekly «Current Contents Yield» (about 900 references), an average of 60 articles were selected (*by the staff-members/endusers*) and added to the diagnostic file. After 3–6 months the selected references were extracted from Medline CD-ROM (if present!), including abstracts and the Major MESH-terms, and brought to the main diagnostic file. The selected Current Contents references which were not present on CD-ROM (a minor part) were added at the diagnostic file as well.

A description is given of the steps which are needed for transporting the selected records from Medline CD-ROM and Current Contents into the personal database manager: *downloading*, *conversion* of the format of the records, and finally *importing* them into the personal retrieval system.

The file is continuously updated.

Experiences of the endusers in their daily work in the Department of Veterinary Pathology will be presented.