Localisation of CD10 to biliary canaliculi by immunoelectron microscopical examination

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Abstract
Common acute lymphoblastic leukaemia antigen (CALLA) was first characterised in lymphoid leukaemic cells. The antigen is present in different stages of lymphoid cell differentiation as well as in subsets of myeloid cells, and further studies have also shown its presence in non-lymphoid tissues. The recent cloning and sequencing of the gene permitted deduction of its amino acid sequence which is identical with the human membrane-associated enzyme, neutral endopeptidase. Strong immunostaining for CALLA was detected in the human liver with a canalicular pattern. Immunoelectron microscopy also confirmed that the antigen was localised only in the area of the bile canaliculi. Although the function of neutral endopeptidase in the canaliculi is unknown, this antigen may prove useful in the study of biliary function and diseases.

Common acute lymphoblastic leukaemia antigen (CALLA) was originally identified and characterised as a cell surface molecule on acute lymphoblastic leukaemic cells, and was assigned the cluster of differentiation number 10 (CD10) during the First International Workshop on Human Leukocyte Differentiation Antigens.

Although original descriptions suggest that the protein is expressed only in immature lymphoid cells and their malignant counterparts, it soon became evident that the antigen is more widely distributed in different normal and neoplastic tissues. These include neutrophils, fibroblasts, renal epithelia, breast myoepithelial cells, fetal intestine, melanomas, gliomas, retinoblastomas and carcinomas of breast and colon.

Methods
Fresh normal liver parenchyma was selected from necropsy and biopsy specimens from staging laparotomy for malignant lymphoma, and lobectomy for oriental recurrent pyogenic cholangitis. A block, 1 cm³, was mounted in OCT compound (Lab-Tek division, Miles Laboratories, Inc., Naperville, Illinois, USA), snap-frozen in liquid nitrogen, and stored at −70°C. Another adjacent fresh tissue block, 0·5 × 0·5 × 0·2 cm in size, was immediately fixed for two hours in periodate-lysine-paraformaldehyde (PLP) fixative at 4°C, rinsed in phosphate buffered saline (pH 7·4), and transferred to 30°C, sucrose in phosphate buffer for “cryoprotection” (about one hour).

Monoclonal antibodies, J5 (Coulter Immunology, Hialeah, Florida, USA), and Anti-CALLA (Becton Dickinson Immunocytometry Systems, Mountain View, California, USA) are murine monoclonal antibodies of IgG2a subtype raised against tumour cells from a patient with non-T cell acute lymphoblastic leukaemia, and both recognise CALLA. A monoclonal antibody of the same IgG subtype (anti-Leu-1, Becton Dickinson) was used as negative control.

IMMUNOSTAINING FOR ELECTRON MICROSCOPY
The cryoprotected tissue was rapidly frozen in liquid nitrogen and 8 μm cryostat sections were cut and mounted on slides coated with gelatin. The slides were air dried for 30 minutes at room temperature and were further fixed in PLP for 15 minutes. A modified streptavidin-biotin peroxidase complex (SABC) method of immunostaining using the monoclonal murine antibody J5 as the primary antibody, a biotinylated sheep-anti-mouse immunoglobulin, and the SABC was used. For ultrastructural detail, sections should be fixed for 10 minutes in 2·5%, glutaraldehyde before treating with diaminobenzidine (unpublished observations). The sections were then post-fixed in 1% osmium tetroxide for one hour, washed, and dehydrated through graded alcohols and propylene oxide. Poly/Bed 812 Epoxy resin (Polysciences, Inc., Warrington, Philadelphia, USA) was dropped on to the sections and heated overnight at 60°C for polymerisation. The sections were “peeled-off” from the glass slides and the area of interest was selected and mounted on a dummy block with Epoxy glue. Ultrathin sections were cut and stained with uranyl acetate and lead citrate before examination under a JOEL JEM-100SX electron microscope at 80 kilovolts.

IMMUNOSTAINING FOR LIGHT MICROSCOPY
Immunostaining was performed on 6 μm cryostat sections cut from the frozen block using a modified alkaline phosphatase anti-alkaline phosphatase (APAAP) method. To ensure that the signals detected were not endogenous alkaline phosphatase activity, immunostaining using the streptavidin-biotin peroxidase complex method, similar to that...
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used for immunoelectron microscopy, was also performed.

Results
Immunostaining of the frozen sections showed consistent localisation of the antigen in a pattern that suggested canalicular binding (fig 1). No staining was observed when the first antibody J5 was either omitted or replaced by negative control antibody (Anti-Leu-1). Comparable results were obtained by the SABC technique. The same pattern of staining was noted when the antibody Anti-CALLA was used instead of J5, suggesting that the molecule recognised was actually CD10.

Immunostaining for electron microscopical examination showed predominant localisation of the granular, electron dense, chromogen product along the surfaces of microvilli in the bile canaliculi. The adjacent lateral surfaces of hepatocytes outside the tight junctions were not stained (fig 2). The staining was rather variable in intensity in different canaliculi, suggesting that the antigen may be distributed unevenly.

Discussion
The expression of CD10 in lymphoid cells is stage limited. It is expressed in early lymphoid cells as well as in their leukaemic counterparts where it indicates more favourable prognosis. The antigen is also present in follicular centre B cells, and this is sometimes useful in identifying malignant lymphomas of follicular centre cell origin.

As knowledge accumulates, it becomes apparent that some of the so-called haemic tissue specific antigens are in fact more widely distributed. Such realisation not only leads to a re-evaluation of the use of these markers in diagnostic pathology, but also allows them to be used for the study of normal and abnormal cell function.

We observed that CD10 is consistently demonstrable in the hepatocytes in a canalicular distribution. The reactivity of anti-CD10 with biliary structures was also reported by Voigt et al. As staining is successful with two antibodies, it is unlikely that it is due to cross reaction with another antigen. The distribution on surfaces of microvilli is consistent with the predominantly surface location of the antigen in other tissues. Such location also suggests that the antigen may have a role in canalicular transport.

The isolation of the cDNA clones specific to CD10 from a pre-B ALL cell line and human kidney cDNA library, and the sequencing of the clones, has predicted a peptide sequence which is 100% identical with the human membrane-associated enzyme, neutral endopeptidase.

The enzyme has been characterised in human kidney,22 skin and lung fibroblasts,23 placental microvilli,25 epithelial cells from prostate and epididymis,26 neutrophils,27 and brain,28 most of which express CD10. Different functions have been attributed to the enzyme according to the location,29 and there is evidence that it may be required for normal chemotaxis in neutrophils.30

The enzyme has not been biochemically characterised in the liver. The complexity of the canalicular domain and its richness in enzyme content make it difficult to detect the specific enzyme activity.

The inclusion of hepatocytes as one of the CD10 bearing tissues could affect our application of antibodies against this antigen. It is not known whether the antigen is preserved in regenerative, dysplastic, or neoplastic states of hepatocytes, and its expression might prove useful in setting more objective criteria for their distinction. It has been used in breast lesions, identifying myoepithelial cells using the antigen as a marker. On the other hand,
caution is required in interpreting staining for CD10 in the liver for detection of secondary tumour disease, such as detection of ALL."

Lastly, the antigen may prove to be another serum enzyme marker for canicular or hepatic lesions, as other well recognised canicular enzymes, such as alkaline phosphatase, have been used and it deserves further investigation.
