

sions^{15,16} contain mucoglycoprotein, while the electron dense inclusions are highly ordered and paracrystalline¹⁷. The present report describes the immunocytochemical identification of lysozyme at the EM level within giant cells in a human sarcoid lesion. The tissue received was a grey homogeneous nodule 3 cm in diameter resected with adjacent lung from a 68-year-old woman, after hilar lymph node biopsy had shown sarcoidosis suggesting that the lung shadow seen on X-ray was a solitary sarcoid lesion. The receipt of this tissue fresh allowed study by standard transmission EM technique, and by immunoperoxidase staining for lysozyme, using the unlabelled antibody enzyme technique on 6- μ m paraffin sections¹⁸ for light microscopy and a modification of this technique for EM¹⁹. The latter technique involves direct immunostaining of thin sections of glutaraldehyde fixed, non-osmicated material embedded in epon-araldite. The antiserum used was a commercial rabbit antiserum (Dakopatts Ltd).

At light microscope level discrete granular staining was seen in sarcoid giant cells, characteristically in the centre of the cell (figure 1). Appropriate control sections using either normal rabbit serum in place of antilysozyme antiserum, or normal sheep serum in place of sheep anti-rabbit globulin¹⁸ did not show staining. Not all profiles of such giant cells showed positive staining. A relatively small number of positive mononuclear macrophages was present. Thick (1 μ m) plastic sections stained with toluidine blue showed small blue granules in the centre of the giant cells, and conventional electron micrographs of parallel sections stained with 2% OsO₄ and lead citrate showed these to be membrane bound bodies of the type previously described¹⁷. In thin sections stained for lysozyme, many though not all of these dense bodies were specifically stained (figure 2). Staining was never seen outside dense bodies. Within individual experiments, control sections (using controls similar to those used for light microscopy) showed no staining. Specific staining was not identified in mononuclear cells. The tissue contained 51 ± 4 μ g/ml lysozyme/mg wetwt tissue as measured turbidimetrically²⁰. These findings identify for the first time lysozyme in macrophage granules immunocytochemically at the EM level and support strongly the view that macrophages or their derived giant cells are secretory. It is interesting that the major secretory component in this granuloma is the giant cell, and

that relatively few mononuclear macrophages were positively stained. Serum lysozyme levels may be related to the giant cell content of these lesions, and thus change with the progression of the lesion. However, the giant cells in sarcoidosis differ one from another, and the granules within them differ one from another. Further study is required to sort out the nature, degree and significance of such differences.

- 1 Acknowledgment. We are grateful to M.R.C. Canada for financial support, to Dr S. Erlandsen for instruction in technique, and to Dr L. Black for help in obtaining fresh material.
- 2 To whom requests for reprints should be sent.
- 3 M.E. Carson and A.M. Dannenberg, Jr, *J. Immun.* 94, 99 (1965).
- 4 Z.A. Cohn and E. Wiener, *J. exp. Med.* 118, 991 (1963).
- 5 S. Gordon, in: *Mononuclear phagocytes*, in *Immunity, Infection and Pathology*, p. 463. Ed. R. van Furth. Blackwell, Oxford 1975.
- 6 D.Y. Mason and C.R. Taylor, *J. clin. Path.* 28, 124 (1975).
- 7 Q.N. Myrvik, E.S. Leake and B. Fariss, *J. Immun.* 86, 133 (1961).
- 8 Q.N. Myrvik, E.S. Leake and S. Oshima, *J. Immun.* 89, 745 (1962).
- 9 I. Carr, J. Carr, A. Lobo and D. Malcolm, *J. Reticuloend. Soc.*, in press.
- 10 D.N. Mitchell, J.G. Scadding, B.E. Heard and K.F.W. Hinson, *J. clin. Path.* 30, 395 (1977).
- 11 M. Klockars and O. Selroos, *Acta path. microbiol. scand.* 85 A, 169 (1977).
- 12 K.R. Falchuk, J.L. Perrotto and K.J. Isselbacher, *New Engl. J. Med.* 292, 395 (1975).
- 13 R.S. Pascual, J.B.L. Gee and S.C. Finch, *New Engl. J. Med.* 289, 1074 (1973).
- 14 P.E. Perillie, K. Khan and S.C. Finch, *Am. J. med. Sci.* 265, 297 (1973).
- 15 W. Jones Williams, D.A. Erasmus, E.M. Valerie James and T. Davies, *Postgrad. med. J.* 46, 496 (1970).
- 16 E.M. Valerie James and W. Jones Williams, *Thorax* 29, 115 (1974).
- 17 I. Carr and P. Norris, *J. Path.* 122, 29 (1977).
- 18 L.A. Sternberger, P.H. Hardy, Jr, J.J. Cuculis and H.G. Meyer, *J. Histochem. Cytochem.* 18, 315 (1970).
- 19 S.L. Erlandsen, J.A. Parsons and T.D. Taylor, *J. Histochem. Cytochem.* 22, 401 (1974).
- 20 G. Litwack, *Proc. Soc. exp. Biol. Med.* 89, 401 (1955).

Ocular involvement in hamsters transplanted with a human leukemic T-cell line

I. Miyoshi, S. Hiraki, K. Nakamura, T. Tanaka and I. Kimura¹

Department of Medicine, Okayama University Medical School, Okayama 700 (Japan), 30 January 1978

Summary. A leukemic T-cell line (TALL-1) was serially transplanted for 5 passages into newborn hamsters treated with antilymphocyte serum. This cell line was derived from a leukemic patient with clinical evidence of ocular involvement. I.p. implantation of $1-3 \times 10^7$ cells resulted in disseminated growth of tumors in all 15 recipients after 23-41 days and 8 of them showed leukemic infiltration of the uveal tract of one or both eyes.

Ocular involvement in acute lymphoblastic leukemia (ALL) is a known complication for which local radiotherapy is recommended as the treatment of choice^{2,3}. We have experienced a patient who developed ocular and meningeal involvement preceding the leukemic manifestation of a T-cell lymphosarcoma. We wish to report that a leukemic T-cell line (TALL-1)⁴ derived from this patient caused a disseminated disease involving the eyes and other various organs when transplanted into hamsters.

The patient was a 28-year-old male who was admitted to our hospital in April 1975 with generalized lymphadenopathy and left pleural effusion. A diagnosis of T-cell lympho-

sarcoma was made on the basis of lymph node histology and spontaneous rosette formation with sheep erythrocytes by pleural effusion tumor cells. After 4 courses of chemotherapy with adriamycin, vincristine, cyclophosphamide and prednisolone, a complete remission was obtained which lasted for 3 months.

In July 1975, during the latter part of remission, the patient began to complain of visual disturbance of the right eye that progressed to complete blindness in 2 months. Ophthalmoscopy revealed atrophy of the optic nerve. In October 1975, meningeal leukemia developed. Thereafter, the disease manifested a leukemic picture with invasion of the

blood and bone marrow. The patient expired in April 1976. The TALL-1 line was derived from bone marrow aspirated 1 week prior to death.

Cells from this culture line were serially transplanted for 5 passages into newborn hamsters treated with rabbit anti-hamster thymocyte serum, according to the method previously described⁵. I.p. implantation of $1-3 \times 10^7$ cells gave rise to progressive growth of tumors in all 15 recipients after 23-41 days. There were massive mesenteric and retroperitoneal tumors with or without ascites. Ocular lesions were observed in one or both eyes of 8 of them. TALL-1 cells infiltrated the ciliary body and choroid. In a few instances, the entire eyeballs were encircled by uveal infiltration with impingement on the optic nerve. Leukemic infiltrations were also present in the liver, gallbladder, kidneys, spleen, lymph nodes, thymus, bone marrow, brain, spinal cord and meninges. The majority of these animals showed leukemic cells in the peripheral blood, although leukocyte counts were not usually elevated.

T-cell ALL is characterized by massive leukemic infiltration and a poor prognosis as compared with a more common type of null-cell ALL⁶. It is interesting that even in

heterologous hosts human leukemic T-cells behaved as in the donor patient and became widely disseminated in many organs including the eyes. Southam et al.⁷ observed similar eye lesions in rats transplanted i.v. with cultured Burkitt's lymphoma cells. Our hamster system would be useful as a chemotherapeutic model of human T-cell leukemia-lymphoma.

- 1 Supported by a Cancer Research Grant from the Ministry of Health and Welfare of Japan.
- 2 A.R. Rosenthal, P.R. Egbert, J.R. Wilbur and J.C. Probert, *J. pediat. Ophthalm.* 12, 84 (1975).
- 3 K.H. Murray, F. Paolino, J.M. Goldman, D.A.G. Galton and C.F.J. Grindle, *Lancet* 1, 829 (1977).
- 4 I. Miyoshi, S. Hiraki, T. Tsubota, I. Kubonishi, Y. Matsuda, T. Nakayama, H. Kishimoto, I. Kimura and H. Masuji, *Nature* 267, 843 (1977).
- 5 I. Miyoshi, S. Hiraki, I. Kubonishi, Y. Matsuda, H. Kishimoto, T. Nakayama, T. Tanaka, H. Masuji and I. Kimura, *Cancer* 40, 2999 (1977).
- 6 I. Tsukimoto, K.Y. Wong and B.C. Lampkin, *New Engl. J. Med.* 294, 245 (1976).
- 7 C.M. Southam, J.H. Burchenal, B. Clarkson, A. Tanzi, R. Mackay and V. McComb, *Cancer* 23, 281 (1969).

Binding bovine immunoglobulins to anterior lobe cells of the hypophysis¹

W.H. Boyd, A. Peters and G. Morris

Biomedical Sciences, University of Guelph, Guelph, (Ontario, Canada), 16 February 1978

Summary. Cells of the anterior lobe of the bovine hypophysis, show an affinity for immunoglobulins IgG, IgA and IgM. The phenomenon either provides a message to stimulate cellular secretion or to excite mechanisms associated with the synthesis and release of Ig's or their determinants.

Routine histological preparations (H&E, PAS, AF, orange G) of pituitary anterior lobe tissue show a compact arrangement of cells grouped into chromophils (basophils and acidophils) and chromophobes on the basis of their affinity or lack of affinity for various dyes. Viewed under white light, after fluorescent staining, useful criteria for identifying anterior lobe cells are the size and shape of the nucleus and the general cytological variations.

Because of the compact arrangement of anterior lobe cells, neither cryostat nor paraffin sections of glandular tissue proved suitable for study. We found that the best information was gained from isolated single and small groups of cells, secured from a 'cell drop technique' developed in our laboratory.

Materials and methods. Anterior lobe tissue from 4 to 5 fresh bovine glands was harvested², placed in 5 ml of 0.005 M phosphate buffered saline (PBS pH 7.2) and minced with a scalpel into pieces 0.5 mm³ or less. The mixture was poured through 3 layers of gauze into a centrifuge tube. The gauze was rinsed again with 2 ml of PBS and the mixture centrifuged for 5 min. Following this procedure, the supernatant was discarded, and the pellet with whitish appearing intermediate lobe cells on the top and dark brown red blood cells below, was fixed (5-10 min) by gently adding 2 ml of either cold Carnoy's or buffered formalin. The top or intermediate lobe cell layer was carefully pipetted off and placed in a test tube with the addition of a sufficient amount of fixative to secure the desired density.

After a period of refrigeration (1 h at -4°C) a variable number of cells were resuspended with a pipet. A small drop of suspended cells was placed on precleaned microscopic slides and allowed to air dry. The type of anterior lobe cell was identified by staining directly with conjugated (FITC) rabbit anti-bovine IgG, IgA and IgM; indirectly

(sandwich technique) with rabbit anti-bovine IgG, IgA, IgM and conjugated (FITC) goat as well as sheep anti-rabbit serum³.

In the direct staining method, the controls were treated with rabbit anti-bovine IgG, IgA, IgM and incubated (1 h) in a moistened chamber at room temperature. In the indirect method, the controls were treated with a solution of equal parts, rabbit anti-bovine IgG, IgA and IgM and conjugated (FITC) rabbit anti-bovine IgG, IgA and IgM. Following the staining procedures, slides were gently rinsed ($\times 2$) with PBS, allowed to dry and mounted in PBS (10%) glycerin (90%) and covered.

Sections were examined under white and fluorescent light ($\times 300$) using the Wild microscope (M 20) mounted on the transmitted-light base 111. The Wild HBO-200 mercury vapour lamp proved suitable for blue light and FITC fluorescence with blue-light fluorescence exciting filter \varnothing 32 mm (126 103 BG12); blue-light barrier filter \varnothing 32 mm (202 202 OG1c); and barrier filters for UV-(GG 13c) and blue-light (OG 1c). The cells were photographed ($\times 480$) on Kodak Tri-X-135 film (ASA 400) and enlarged ($\times 6$).

Results and discussion. Microscopically, the 2 morphologically distinct basophils, (beta and delta)⁴ usually recognized after routine staining, could be identified with limited clarity after fluorescent staining. Basophils, stained directly and indirectly with conjugated (FITC) rabbit anti-bovine IgG, IgA and IgM, showed many of the characteristics of the delta cell. One constant feature was the size of the nucleus (4.35 μ m to 4.51 μ m); well within the limits of similar cells stained routinely.

Stained with FITC/IgM and viewed under white light (figure 1, A), the basophilic cell appeared to be in a resting state i.e., there was no sharp cytoplasmic outline, the cytoplasmic granules seemed fused into homogeneity, and the nucleus was crenated and pycnotic. Under fluorescent