able to suggest that A particles may represent a precursor or a formative stage of the C particles.

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2 G. Yasuzumi and S. Higashizawa, Gann *47*, 527 (1956).
3 M. Friedländer and D.H. Moore, Proc. Soc. exp. Biol. *92*, 828 (1956).
4 A.F. Howatson and E.A. McCulloch, Nature, Lond. *181*, 1213 (1958).
5 D.F. Parsons, E.B. Darden, D.L. Lindsley and G.T. Pratt, J. biophys. biochem. Cytol. *9*, 353 (1961).
6 A.J. Dalton, M. Potter and R.M. Merwin, J. nat. Cancer Inst. *26*, 1221 (1961).
7 G.H. Smith, H.B. Andervont and T.B. Dunn, J. nat. Cancer Inst. *44*, 657 (1970).
8 A.O. Myking and A. Åbro, Acta path. microbiol. scand. *82A*, 571 (1974).
9 W.R. Adams and A.M. Prince, J. biophys. biochem. Cytol. *3*, 161 (1957).
10 T. Kodama and M. Kodama, J. nat. Cancer Inst. *50*, 707 (1973).
11 E.C. Chew, in: Summary Programme and Abstracts of Papers, p. 119. 3rd Asian Cancer conference, Manila 1977.
12 C. De Guili, H. Hanafusa, S. Kawai, S. Dales, J.H. Chen and K.C. Hsu, Proc. nat. Acad. Sci. USA *72*, 3706 (1975).
13 A.J. Dalton, J. nat. Cancer Inst. *52*, 483 (1972).
14 M.C. Bibby and G.M. Smith, Br. J. Cancer *35*, 743 (1977).
15 A.J. Dalton and F. Haguenau, Ultrastructure of Animal Viruses and Bacteriophages - An Atlas. Academic Press, New York.

The EM immunocytochemical demonstration of lysozyme in macrophage giant cells in sarcoidosis

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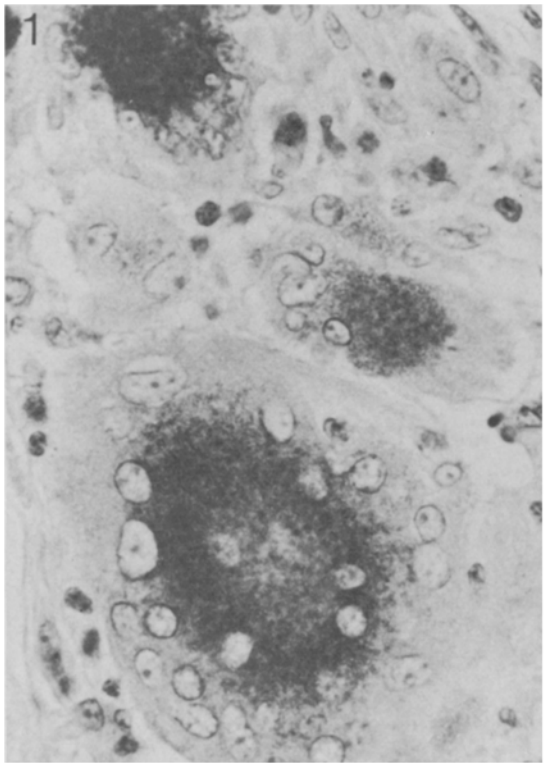
Summary. The giant cells (multinucleate macrophages) of human sarcoidosis have been shown by the unlabelled antibody immunoperoxidase technique at electron microscope level to contain lysozyme within cytoplasmic granules.

It is now widely held that macrophages are secretory cells producing a wide variety of substances - hydrolytic enzymes, elastase, collagenase and lysozyme among others[3-8]. Evidence has recently been submitted that in an animal model granuloma the aggregate of macrophages may act as an endocrine gland secreting lysozyme into the blood and lymph, and causing an elevation of serum lysozyme[9]. In human sarcoidosis[10] a similar situation probably exists. Granuloma macrophages have been shown immunocytochemically to contain lysozyme[11], and serum lysozyme levels are elevated in sarcoidosis and other granulomatous lesions[12-14].

Ultrastructural examination of sarcoid granulomas has shown the component macrophages to contain several types of inclusion of which electron dense and electron lucent types are of present interest. The electron lucent inclu-



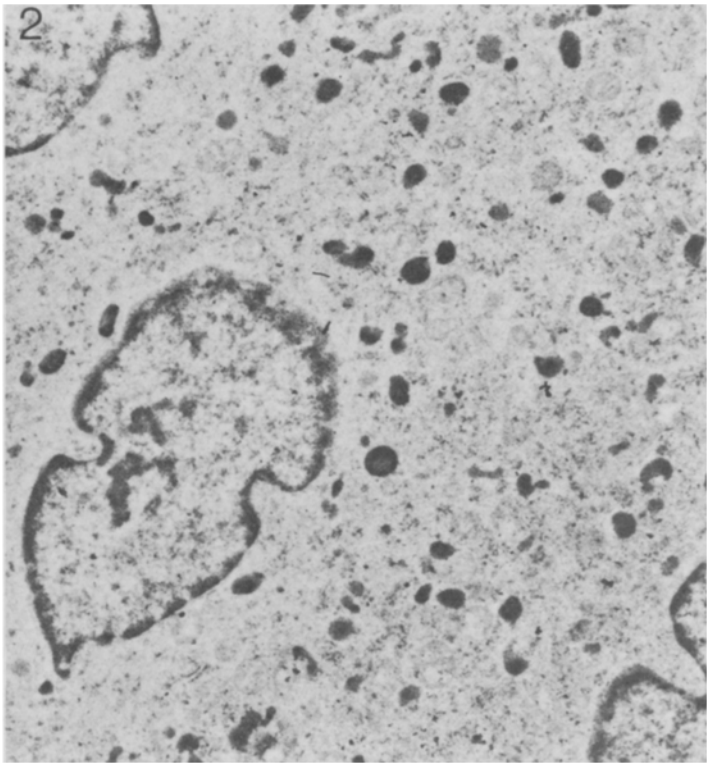


Fig. 1. Light photomicrograph of giant cells in sarcoid lesion stained by immunoperoxidase using antilysozyme antiserum. There is a granular deposit in the centre of the giant cells. 6 µm paraffin section. × 624. Fig. 2. Electron micrograph of giant cell in sarcoid lesion stained by immunoperoxidase using antilysozyme antiserum. There is strong positive staining in many but not all lysosomes. Mitochondria do not stain. × 11,500.

sions[15,16] contain mucoglycoprotein, while the electron dense inclusions are highly ordered and paracrystalline[17].

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-µm µm paraffin sections[18] for light microscopy and a modification of this technique for EM[19]. 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 globulin[18] 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 µm) 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% OsO_4 and lead citrate showed these to be membrane bound bodies of the type previously described[17]. 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 µg/ml lysozyme/mg wetwt tissue as measured turbidimetrically[20]. 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.

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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

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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 $1\text{-}3\times 10^7$ 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 raditherapy 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 lymphosarcoma 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