A quantitative study of α -naphthyl acetate esterase-positive cells in Hodgkin's disease

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SUMMARY The numbers of α -naphthyl acetate esterase (ANAE)-positive cells (other than T cells) have been counted in 32 specimens of Hodgkin's disease and two specimens of histiocytic lymphoma. The different Rye subtypes of Hodgkin's disease contain varying numbers of enzyme-positive cells, ranging from 1.8-16% in the lymphocyte-predominant form to 39.8-47% in lymphocyte-depleted Hodgkin's disease. The percentage of enzyme-positive cells in the mixed cellularity variety was from only 6.5 to 14.6%. In the two specimens of apparently genuine histiocytic lymphoma, the enzyme-positive cells constituted 95.2 and 97.5% respectively of all cells. Thus, the numbers of macrophages and macrophage-like cells in true histiocytic lymphoma are much greater than in Hodgkin's disease.

The classification of Hodgkin's disease into subtypes is of prognostic importance and aids to the accuracy of diagnosis are always of potential value. The most widely accepted classification of Hodgkin's disease in use today is that of the Rye convention,¹ which to a considerable extent makes use of the content of macrophages, "Hodgkin cells" and Reed-Sternberg cells in relation to the proportion of lymphocytes in the affected tissues. Thus, the subtypes range from lymphocyte-predominant variety to the lymphocyte-depleted form.

Macrophages contain a large quantity of lysosomal enzymes, one of which is α -naphthyl acetate esterase (ANAE) which is a convenient marker for macrophages.²⁻⁵ In frozen sections of fixed tissue macrophages stain very strongly for ANAE, although there may be some very weak activity in dendritic reticulum cells.⁶ We have previously reported a quantitative study of ANAE-positive cells in non-Hodgkin's lymphomas, in which we used a multichannel interactive image-analyzer (the Kontron MOP-AMO₃) as a rapid and accurate means of counting cells.⁵ The same technique has now been used to count ANAE-positive cells in Hodgkin's disease, to give an objective value for the percentage of macrophage-type cells in the various subtypes. Two specimens of genuine histiocytic lymphoma are also included.

Material and methods

LYMPH NODES

Thirty-four lymph nodes were examined, from the same number of patients. These were classified as follows: 12 were of the lymphocyte-predominant type of Hodgkin's disease and eight were of the nodular sclerosing variety, whilst seven were of the mixed cellularity subtype and the remaining five showed the features of lymphocyte-depletion (Table). In addition two specimens of high-grade lymphoma composed of large cells with copious, often rather foamy cytoplasm exhibiting phagocytosis and with lobulate large nuclei were included; these were judged on morphological grounds to be probable true histiocytic lymphomas and a preliminary subjective examination of ANAE preparations of these specimens showed a large excess of enzyme-positive cells.

FIXATION, PROCESSING AND ENZYME HISTOCHEMISTRY

These were carried out as described before.⁵

Specimens studied

<i>Histological diagnosis</i>	<i>No</i>	<i>Age range (yr)</i>
Hodgkin's disease:		
lymphocyte-predominant	12	13-58
nodular sclerosing	8	15-33
mixed cellularity	7	22-57
lymphocyte-depleted	5	27-68
Histiocytic lymphoma	2	29 and 65

Fig. 1 A Reed-Sternberg cell showing several areas of enzyme-positivity. ANAE with methyl green counterstain $\times 550$.

Specimens were collected directly after surgical removal and were cut into slices 2 mm thick, fixed in cold (4°C) formol-calcium for 48 h and transferred to gum-sucrose solution. Frozen sections were cut on a cryostat at $6-8\ \mu\text{m}$ and stained using a simultaneous azo-coupling method for ANAE.⁴ Tissue slices were also fixed in 10% formol-saline at room temperature, dehydrated and embedded in paraffin wax. Sections were cut at $2-3\ \mu\text{m}$ and stained by Harris's haematoxylin and eosin for diagnosis and typing according to the Rye classification.¹

CELL COUNTING PROCEDURE

This was performed in the manner previously described, using the Kontron MOP-AMO₃ image-analyser.⁵ Five hundred lymphoid cells were counted and stored on one channel of the microprocessor and the numbers of ANAE-positive cells in the same fields were counted and stored on another channel. The latter were calculated as a percentage of the former by the MOP-AMO₃. T lymphocytes, containing punctate ANAE-activity^{4,7} were not counted. Microscope fields were selected randomly, although in nodular sclerosing Hodgkin's disease, areas consisting of more than 20% fibrous tissues were excluded from the count. Thus, only densely cellular tissue was counted in each specimen. All cells were counted within each field.

Results

In the specimens of Hodgkin's disease, macrophages, ranging from the rounded "tingible-body" type to branching forms, stained strongly for ANAE, with occasional very weakly enzyme-positive highly branching structures representing so-called "dendritic reticulum cells." The staining response of "Hodgkin's histiocytes" was rather variable, in some instances showing a small localised area of enzyme-positivity; but in general being negative for ANAE. Reed-Sternberg cells also usually lacked ANAE but in some instances one or more small rather ill-defined positively-reacting areas were seen (Fig. 1).

Fig. 2 Lymphocyte-predominant Hodgkin's disease showing few enzyme-positive macrophages ANAE with methyl green counterstain $\times 250$.

Fig. 3 (a) *Nodular sclerosing Hodgkin's disease. Enzyme positive macrophages are more numerous than in the lymphocyte-predominant subtype. ANAE with methyl green counterstain $\times 400$. (b) Lymphocyte-depleted subtype of Hodgkin's disease. Esterase-positive macrophages are numerous. ANAE with methyl green counterstain $\times 250$.*

In the two specimens of lymphoma thought to be of the truly histiocytic type, the strongly ANAE-positive macrophages were mainly of the rounded non-branching type and very few of the tumour cells were negative for the enzyme.

In the specimens of Hodgkin's disease, ANAE-positive cells constituted 1.8–16% of all cells in the lymphocyte-predominant group (Fig. 2), appearing to cluster in two ranges, from 1.8–5.2% and from 12.5–16%, respectively. Nodular sclerosing specimens contained a rather larger number of ANAE-positive cells, from 13.6–22.1% (Fig. 3a), whilst enzyme-positive cells constituted 6.5–14.6% of mixed cellularity specimens. The lymphocyte-depleted variety contained the greatest number of esterase-positive cells of the types of Hodgkin's disease, ranging from 39.8–47.7% of all cells (Fig. 3b). Figure 4 shows the numbers of ANAE-positive cells in the specimens of Hodgkin's disease and histiocytic lymphoma.

The two specimens of presumed true histiocytic lymphoma contained 95.2 and 97.5% ANAE-positive cells respectively, the few negative cells appearing to be small lymphocytes (Fig. 5). As the majority of cells were strongly positive for the enzyme and varied little in size or appearance it was not possible to distinguish between the malignant cells themselves and possible "reactive" macrophages in the tumours.

Fig. 4 The numbers of ANAE-positive cells (other than T lymphocytes) in histiocytic lymphoma and the subtypes of Hodgkin's disease (LP = lymphocyte-predominant, NS = nodular sclerosing, MC = mixed cellularity, LD = lymphocyte-depleted and HL = histiocytic lymphoma).

Discussion

Macrophages are always present in malignant lymphomas and may be demonstrated by virtue of their large content of lysosomal hydrolytic enzymes, including ANAE.²⁻⁵ The ANAE reaction is also very weakly positive in dendritic reticulum cells^{5,6} and

Fig. 5 Histiocytic lymphoma. The majority of cells are strongly ANAE-positive. ANAE with methyl green counterstain $\times 250$.

punctate in form in some T lymphocytes, especially when the ANAE reaction is carried out at an acid pH.⁷ As a means of enumerating macrophages, therefore, the ANAE reaction is useful, since the reaction in dendritic reticulum cells is very weak and the characteristic configuration of the reaction product in T cells enables their exclusion from a counting procedure. The Kontron MOP-AMO₃ has provided a rapid and accurate means of counting ANAE-positive cells (other than T cells) in specimens of HD and two specimens of true histiocytic lymphoma. There is little difference between the content of ANAE-positive cells of lymphocyte-predominant, nodular sclerosing and mixed cellularity subtypes of Hodgkin's disease, the enzyme-positive cells forming a minority of the total cells, even in the mixed cellularity type. A strikingly high proportion of ANAE-positive cells was observed however in lymphocyte-depleted Hodgkin's disease. Another feature of note is the apparent presence of two groups of specimens within the lymphocyte-predominance subtype, one with 1.8–5.2% and one with 12.5–16% enzyme-positive cells. It is suggested that the latter group represent the "L and H" variety of lymphocyte-predominant Hodgkin's disease, with a relatively higher content of macrophages of the epithelioid variety. The two specimens of suspected true histiocytic lymphoma consisted almost entirely of ANAE-positive cells, in contrast to the specimens of Hodgkin's disease.

In a previous study⁸ of differential cell counts in subtypes of diffuse Hodgkin's disease using haematoxylin and eosin-stained paraffin sections of nine lymph nodes, it was found that the cellular basis of the Rye classification was generally supported. However, it was noted that the lymphocyte content in lymphocyte-depleted Hodgkin's disease was greater than in the mixed cellularity subtype. In addition mononuclear cells (including fibroblasts and macrophage-type cells) were found to be much more numerous in mixed-cellularity Hodgkin's disease than in other types of diffuse Hodgkin's disease. We have confirmed that there are anomalies in the Rye classification of Hodgkin's disease, although by the use of ANAE as a means of demonstrating macrophage-type cells we have arrived at different results to those in the above study. This may be related, in part, to the nature of ANAE-positive cells themselves; for example, supposedly neoplastic "Hodgkin histiocytes" were found to show variable staining for ANAE, generally being negative, in contrast to non-neoplastic macrophages which show intense staining. Thus, ANAE-positive staining may select a population of cells different to that described as macrophage-like in haematoxylin and eosin-stained preparations. Reed-Sternberg cells were also

often negative for ANAE and thus excluded from the count.

The relation between macrophages presumed to be non-neoplastic, "Hodgkin histiocytes" and Reed-Sternberg cells remains uncertain. The variability of ANAE-staining in the latter two cell types^{2,9,10} serves to confuse their possible relation to non-neoplastic macrophages. However there are morphological similarities between the nuclei in these cell types, and Katz¹¹ recently showed, in specimens of Hodgkin's disease in vitro, the presence of ultrastructurally atypical cells with the functional attributes of macrophages. In addition, it has been suggested on the basis of studies of metalophilia that Reed-Sternberg cells and Hodgkin cells are related to dendritic histiocytes.¹²

Our present studies show that by using ANAE to demonstrate them, macrophage-like cells form a minority of all cells in Hodgkin's disease, although in lymphocyte-depletion specimens they may account for nearly 50% of the cell count. These findings are of interest in relation to our demonstration that ANAE-containing cells may constitute up to approximately 20% of all cells in high-grade malignancy non-Hodgkin's lymphomas.⁵ It is again stressed that a diagnosis of true histiocytic lymphoma, on the basis of macrophage content, should be undertaken with great caution, as our two specimens of true histiocytic lymphoma contained 95.2 and 97.5% of ANAE-positive cells respectively. No significant difference in appearance or staining intensity of the ANAE-positive macrophages was noted in the specimens of histiocytic lymphoma. Thus it was impossible to differentiate between the malignant cells and reactive macrophages which were presumably present.

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