Presence of 3-fucosyl-N-acetyllactosamine shown by monoclonal antibody AGF 4.48 in Reed-Sternberg cells

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SUMMARY A series of 50 specimens of Hodgkin's disease and 10 of reactive follicular hyperplasia were examined by means of indirect immunoperoxidase staining with a monoclonal antibody AGF 4.48: this is known to bind to 3-fucosyl-N-acetyllactosamine, which, in particular, is expressed by granulocyte series cells. Most Reed-Sternberg and many Hodgkin's cells were labelled by the antibody after pretreatment with neuraminidase. Routinely processed paraffin wax embedded sections proved suitable for staining. The findings were comparable with those reported by others with monoclonal antibodies to various other granulocyte markers. This technique is of potential diagnostic value.

Staining with mouse monoclonal antibody AGF 4.48 has been shown in granulocytic cells from the promyelocyte stage of development onwards,1 together with various other cell types.2 More recently, AGF 4.48 has been used, with variable success, for labelling granulocytic sarcomas.3 AGF 4.48, like certain other monoclonal antibodies, which label granulocyte series cells,4-6 reacts with 3-fucosyl-N-acetyllactosamine. The ability of the antibody to stain certain cells and tissues and the intensity of the response is enhanced by pretreating the tissues with the enzyme neuraminidase.7

In view of the recent studies, which have shown determinants in Reed-Sternberg cells that are shared with those of granulocytes,8-11 we examined a series of specimens of Hodgkin's disease with an immunoperoxidase technique that uses AGF 4.48. The results were compared with those obtained on staining with the monoclonal antibody Leu M1.

Material and methods

Tissues examined

Fifty specimens of Hodgkin's disease from the same number of patients were recovered from the files of the East Birmingham histopathology department. These comprised 10 specimens of lymphocyte predominant disease, 20 of the nodular sclerosing subtype, and 10 showing a mixed cellularity pattern. In addition, 10 cases of lymphocyte depleted Hodgkin's disease were included. Furthermore, 10 "reactive" hyperplastic lymph nodes were examined. Another 10 specimens comprising five T cell non-Hodgkin's lymphoma and five large cell B lymphoma were included; the nature of these had been confirmed previously by monoclonal anti-B and anti-T antibodies.

Paraffin sections, 4 µm thick, were prepared from these blocks, which had been prepared from material fixed in 10% formol saline. After dewaxing and having been taken to Tris buffer (pH 6-8) the following procedures were adopted.

Immunohistochemistry

Hybridoma cell culture supernatant was concentrated to give a working preparation. The sections were incubated in neuraminidase (from Clostridium perfringens, Sigma, Dorset, England) at a concentration of 1 U/ml for one to two hours at room temperature. After this procedure the sections were washed in Tris buffer (pH 6-8). Endogenous peroxidase was blocked with hydrogen peroxide in methanol, and a standard indirect peroxidase sequence was applied as AGF 4.48 antibody was applied at room temperature in a chamber on a "rocker" for 30 minutes. After a thorough wash in Tris buffer peroxidase conjugated sheep antimouse α and μ heavy chain antiserum (Serotec) was applied. After a further thorough wash the sec-
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Fig. 1 Numerous granulocytes in dilated blood vessel in a case of nodular sclerosing Hodgkin's disease. (Peroxidase stain for AGF 4-48 after neuraminidase.) × 200.

Fig. 2 Hodgkin's cell in a case of lymphocyte predominant Hodgkin's disease. (Peroxidase stain for AGF 4-48 after neuraminidase.) × 850.

Fig. 3 Reed-Sternberg cell from a case of lymphocyte predominant Hodgkin's disease. (Peroxidase stain for AGF 4-48 after neuraminidase.) × 850.

3-fucosyl-N-acetyllactosamine was detected in Reed-Sternberg cells of cases of nodular sclerosing Hodgkin's disease. The staining was developed with 3, 3'-diaminobenzidine, counterstained with Mayer's haemalum, dehydrated, cleared, and mounted in synthetic medium.

Initially, 10 of the specimens underwent the staining sequence with AGF 4-48 antibody but were not pretreated with neuraminidase. In addition, all 50 specimens were stained by a monoclonal antibody Leu M1 (Beckton-Dickinson, United Kingdom), using the peroxidase sequence as above.

**Results**

**AGF 4-48**

No staining was obtained in any of the initial 10 specimens after reaction with the AGF 4-48 antibody in the absence of pretreatment with neuraminidase. In all specimens, however, after preincubation with the

<table>
<thead>
<tr>
<th>Type of disease</th>
<th>No of specimens positively stained and total number of specimens with AGF 4-48</th>
<th>No of specimens positively stained and total number of specimens with Leu M1</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hodgkin's disease:</strong></td>
<td></td>
<td></td>
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<tr>
<td>Lymphocyte predominant</td>
<td>10/10</td>
<td>0/10</td>
</tr>
<tr>
<td>Nodular sclerosis</td>
<td>20/20</td>
<td>20/20</td>
</tr>
<tr>
<td>Mixed cellularity</td>
<td>10/10</td>
<td>10/10</td>
</tr>
<tr>
<td>Lymphocyte depletion</td>
<td>10/10</td>
<td>10/10</td>
</tr>
<tr>
<td><strong>Non-Hodgkin's lymphomas:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Large B cell type</td>
<td>0/5</td>
<td>0/5</td>
</tr>
<tr>
<td>T cell type</td>
<td>0/5</td>
<td>0/5</td>
</tr>
</tbody>
</table>

Specimens examined with numbers of such specimens containing Reed-Sternberg or Reed-Sternberg like cells reacting with monoclonal antibody AGF 4-48 after pretreatment with neuraminidase and Leu M1 antibody.
enzyme, localisation was observed in all of the 50 specimens of Hodgkin's disease and in the reactive lymph node group (Table).

In all specimens treated with neuraminidase definite focal and intense staining was observed in at least 80% of all Reed-Sternberg cells and in the same proportion of Hodgkin's cells. In the Reed-Sternberg cells the reaction product was usually observed as a focal cluster of activity between the two nuclei of the cells, or centrally, surrounded by multiple nuclei. In the Hodgkin cells activity was observed as a similarly sized area adjacent to the nucleus. There was no difference between the incidence of staining of Reed Sternberg and Hodgkin's cells in the different Rye subtypes. Staining was not seen in other cells, apart from granulocytes (Figs. 1-4).

In the reactive lymph nodes activity was observed in occasional granulocytes and in numerous cells in the interfollicular areas, some of which appeared to be somewhat stellate in structure. In these the staining was not focal but occurred diffusely in the cytoplasm.

The large B cell lymphomas and T cell neoplasms, previously shown to be of such lineages and containing Reed-Sternberg like cells, were uniformly negative on attempts to react with AGF 4-48 after pretreatment with neuraminidase (Figs. 5 and 6).

**LEU M1**

Staining of Reed-Sternberg and Hodgkin's cells was consistently obtained in all specimens of Hodgkin's disease, with the exception of the lymphocyte predominant variant: such cells were consistently negative. Granulocytes reacted strongly in all Rye subtypes.

The Reed-Sternberg like cells in large B cell lymphomas and T cell tumours were not stained with the monoclonal antibody.

**Discussion**

AGF 4-48, a murine monoclonal antibody, was initially described as a granulocyte marker, labelling cells from the promyelocytic stage of maturity onwards, and reacting strongly with the HL60 promyelocytic cell line in man. Like certain other antibodies, including VEP 8 and 9, My1, and Ig10, AGF 4-48 reacts with the carbohydrate chain...
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3-fucosyl-N-acetylactosamine, which can be described as follows: β-D-galactose 1→4 (α-L-fucose 1→3) N-acetyl-D-glucosamine.

AGF 4-48 has recently been shown to label several cell types other than granulocytes, as might be expected with an antibody directed against a small carbohydrate group. Indeed, it has also been shown that this staining may be enhanced in terms of intensity and width of cellular expression by preincubating sections with neuraminidase. The reasons for this phenomenon could be two fold. Firstly, haematopoietic groups, which are terminal in their chains, may be sialylated by their reaction with neuraminic acid "residues." Such a combination could, clearly, prevent the antigen in question from binding with its antibody. Secondly, the antigen could simply be hidden by steric hindrance, or by neuraminic acid "residues", or both. Interestingly, in this study neither Reed-Sternberg nor Hodgkin's cells were reactive with AGF 4-48 in the absence of pretreatment with neuraminidase. The problems that could occur as a result of "masking" 3-fucosyl-N-acetyllactosamine have been discussed previously in detail.

The ontological nature of Reed-Sternberg and Hodgkin's cells is not understood, and numerous candidates have been proposed. These include cells of histiocytic origin, T cells and B cells of a transformed type, and reticulum cells. They have been shown to lack S100 protein, a marker for interdigitating dendritic cells.

Notwithstanding these suggestions, several recent studies have shown localization of granulocyte marking determinants in Reed-Sternberg and Hodgkin's cells. For example, Stein et al. used the monoclonal antibodies TÜ9 and 3C4 in a study of Hodgkin's disease. These antibodies labelled granulocyte series cells, Reed-Sternberg, and Hodgkin's cells. 3C4 has also been shown to react with a wide variety of non-lymphoid cells. More recently, granulocyte markers have been discussed in relation to Hodgkin's disease, and TÜ9 and 3C4 have been found to be of value in the diagnosis of this condition; the antibody Ki-1, which reacts against a very small population of lymphoid cells, has also been described. The monoclonal antibody Leu M1 has also been investigated as a marker of Reed-Sternberg and Hodgkin's cells, being known to label granulocytes; in this study Leu M1 was used to stain these cells in paraffin sections very efficiently, although both lymphocyte predominant specimens were negative.

More recently, the monoclonal antibody 3C4 has been studied in Hodgkin's disease and compared with the staining responses with Leu M1. Both of these antibodies labelled Reed-Sternberg cells, but, interestingly, these structures were not stained in the lymphocyte predominant specimens. In our study, however, AGF 4-48 reacted with Reed-Sternberg cells in all subtypes.

In practical terms AGF 4-48 with neuraminidase pretreatment seems to be a diagnostically useful means of detecting Reed-Sternberg and Hodgkin's cells. The presence of the determinant in 80% rather than 100% of such cells is not necessarily a problem, as, even in lymphocyte predominant Hodgkin's disease, sufficient cells are usually present for their ready recognition. Furthermore, it is possible, in view of the focal nature of the labelling in these large cells, that non-optimal sections may miss the region of activity.

The presence of granulocyte marking determinants within Reed-Sternberg and Hodgkin's cells does not, of course, necessarily imply that they are of granulocytic origin. Shared epitopes do not imply common ontogeny. Indeed, many of these determinants may be hidden and present in many differing cell populations.

It is important to note that no reactivity was observed with AGF 4-48 in Reed-Sternberg like cells in confirmed B cell and T cell non-Hodgkin's lymphomas. These findings agree with those previously reported using the monoclonal antibodies Leu M1 and 3C4.

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References


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