Immunochemical demonstration of J chain: a marker of B-cell malignancy

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SUMMARY Many B-cell lymphomas can be shown to contain cytoplasmic immunoglobulin which is characteristically monotypic with respect to light chains. In Hodgkin's disease, however, the Reed-Sternberg cells have been shown to contain both immunoglobulin light chains. This finding, which is also present in some other lymphomas, has been used as evidence both for and against a B-cell derivation of these cells. J chain is present in normal immunoblasts irrespective of the class of immunoglobulin being synthesised and, thus, should be present in tumour cells that synthesise cytoplasmic immunoglobulin. In a series of lymphomas, in which the cells could be shown to contain immunoglobulin, J chain was present only in those tumours exhibiting a monotypic light chain staining pattern. J chain was not present in Reed-Sternberg cells and other cells staining polytypically for light chains. Demonstration of J chain is thus a useful marker for B-cell lymphomas; its absence in Reed-Sternberg cells indicates that the immunoglobulin in these cells is not synthesised by them and cannot be used as evidence for their derivation from B-cells.

Membrane and cytoplasmic immunoglobulin of malignant lymphomas derived from B-cells, including plasma cell tumours, is characteristically monotypic with respect to light chains (Taylor, 1978; Warnke and Levy, 1978). In some malignant lymphomas, however, the tumour cells have been shown to be polytypic, containing both kappa (K) and lambda (λ) light chains in their cytoplasm (Taylor, 1974; Hertel *et al.*, 1977; Taylor, 1978; Taylor *et al.*, 1978). In some instances this has been interpreted as evidence of the polyclonal nature of the tumour (Hertel *et al.*, 1977), but, where serial sections have shown both light chains in the same cell, it has been suggested that this is a manifestation of disordered immunoglobulin synthesis (Taylor, 1974) or that the immunoglobulin has been phagocytosed or taken up from the environment in some other way, perhaps in the form of immune complexes (Curran and Jones, 1978; Isaacson and Wright, 1978a). Polytypic immunoglobulin staining is characteristic of the tumour cells in malignant histiocytosis of the intestine and of the Reed-Sternberg cell of Hodgkin's disease (Taylor, 1974; Curran and Jones, 1978). In the former, the malignant cells have been clearly shown to be derived from histiocytes (Isaacson and Wright, 1978a and b; Isaacson *et al.*, 1979), and their polytypic immunoglobulin content

could be explained by phagocytosis, but the derivation of the Reed-Sternberg cell remains uncertain. A histiocytic origin is favoured by some workers (Kaplan and Gartner, 1977; Long *et al.*, 1977; Kadin *et al.*, 1978) but others have produced evidence from ultrastructural studies that Reed-Sternberg cells synthesise immunoglobulin (Diebold *et al.*, 1977; Bernuau *et al.*, 1978).

J chain is a polypeptide, around which IgA and IgM molecules polymerise, and is produced by immunoglobulin synthesising cells (Kaji and Parkhouse, 1974). Brandtzaeg (1976b), using immunofluorescence, has shown that J chain is present in most circulating immunoblasts synthesising cytoplasmic immunoglobulin, regardless of its class. He found that, in tissue sections, J chain was absent from mature (that is, reactive) plasma cells containing IgG and monomeric IgA and stained weakly or not at all in reactive plasma cells containing dimeric IgA unless unmasked by protein denaturation; IgM-containing plasma cells stained positively for J chain (Brandtzaeg, 1976a). Brandtzaeg (1974) demonstrated J chain in immunoglobulin synthesising cells in follicular centres of human tonsils and in the cells of an IgG plasmacytoma (Brandtzaeg and Berdal, 1975), and Kaji and Parkhouse (1974) were able to extract J chain from all classes of mouse myeloma. These findings suggest that J chain should be demonstrable in all plasmacytomas and lym-

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phomas synthesising cytoplasmic immunoglobulin. The absence of J chain in malignant lymphoma cells containing immunoglobulin would be strong evidence that the immunoglobulin had not been synthesised by the cells but had been taken up from the environment. Conversely, if J chain is present in Reed-Sternberg cells and other cells staining polytypically, this would suggest that they are indeed synthesising immunoglobulin and hence are derived from B-cells.

Material and methods

A series of formalin-fixed, paraffin-embedded sections of malignant lymphomas and plasmacytomas (Table) was stained for all classes of immunoglobulin heavy and light chains and for J chain using the modified bridge immunoperoxidase technique (PAP method) with prior trypsinisation (Curran and Gregory, 1977). In the cases of Hodgkin's disease, serial sections to include the same Reed-Sternberg cells were stained for IgG (γ heavy chain) and J chain. To show the comparative staining of mature plasma cells and immature immunoglobulin synthesising cells of follicular centres, serial sections of human tonsil were stained for all three classes of immunoglobulin heavy chain and J chain. Anti J chain serum was obtained from Nordic Immunological Laboratories and specificity was shown by abolition of all positive staining by prior absorption of the antiserum with purified J chain (Nordic Immunological Laboratories). Further evidence of specificity was provided by absorbing the antiserum with denatured IgM, which also abolished all positive staining (Brandtzaeg, 1975).

Results

The results of staining the series of lymphomas and plasmacytomas are summarised in the Table. Cytoplasmic J chain could be demonstrated in routine paraffin sections with remarkable clarity. Unlike staining for immunoglobulin, there was almost no background staining or staining of serum in blood vessels. In those tumours in which a monotypic staining pattern was present, J chain could be demonstrated in the immunoglobulin containing cells (Figs 1 and 2). This was true regardless of the class of immunoglobulin being produced by the tumour. Polytypic tumour cells, including Reed-Sternberg cells, characteristically stained most strongly for IgG, κ , and λ , and weakly or not at all for IgA and IgM. J chain could not be demonstrated in polytypic cells, and this was further confirmed in serial sections of Reed-Sternberg cells where adjacent sections of the same cell stained positively for IgG but negatively for J chain (Fig. 3).

In sections of human tonsil, the distribution of cells staining positively for J chain was strikingly different from cells staining positively for the three classes of immunoglobulin heavy chain (Fig. 4). Apart from scattered reactive plasma cells, probably containing IgM, J chain staining was confined to follicular centres where immunoblasts and other immature immunoglobulin synthesising cells are situated. Reactive IgA and IgG plasma cells did not stain for J chain.

Discussion

These results support Brandtzaeg's (1976b) hypo-

Table Staining patterns of 18 cases of malignant lymphoma

Case	Immunoperoxidase staining of tumour cells	
	Immunoglobulin	J chain
1 Follicular centre cell lymphoma	M κ	+
2 " " " "	M κ	+
3 " " " "	M λ	+
4 " " " "	G λ	+
5 Lymphoplasmacytoid lymphoma	M κ	+
6 Immunoblastic sarcoma	M κ	+
7 Plasmacytoma	G λ	+
8 " "	G κ	+
9 Myeloma	A κ	+
10 " "	M κ	+
11 " "	G κ	+
12 " "	K	+
13 Malignant histiocytosis of intestine	G $\kappa\lambda$ *	-
14 " " " "	G $\kappa\lambda$ *	-
15 " " " "	G $\kappa\lambda$ *	-
16 Hodgkin's disease (RS cells)	G $\kappa\lambda$	-
17 " " " "	G $\kappa\lambda$	-
18 " " " "	G $\kappa\lambda$	-

*Weak staining for IgA and IgM

Fig. 1 Case 6. Extramedullary plasmacytoma (Gλ). Adjacent sections of the same microscopic field show tumour cells staining strongly for IgG (a) and J chain (b). Immunoperoxidase × 250.

Fig. 2 Case 4. Follicular centre cell lymphoma (Gλ). Stains for IgG (a) and J chain (b) both show strongly positive tumour cells. Immunoperoxidase × 250.

Fig. 3 Case 16. Hodgkin's disease. Serial sections of Reed-Sternberg cells (arrows on right) stain strongly for IgG (a) but negatively for J chain (b). Immunoperoxidase $\times 400$.

thesis that the capacity for J chain synthesis exists in most immature B-cells synthesising cytoplasmic immunoglobulin, hence enabling the cells to switch their heavy chain synthesis during the early phase of clonal differentiation. The staining pattern of sections of human tonsil is similar to that obtained (but not illustrated) by Brandtzaeg using immunofluorescence (Brandtzaeg, 1974; 1976b) and indicates that J chain should be a marker of B-cell neoplasms synthesising cytoplasmic immunoglobulin of any heavy chain class. The results obtained in the cases of immunoglobulin synthesising malignant lymphomas confirm this and show that J chain is a useful marker of these tumours. Absence of J chain in tumours derived from histiocytes, the cells of which stain strongly for IgG and κ and λ light chains, is further evidence that in these cases the immunoglobulin has been ingested. Likewise, it would appear that the immunoglobulin in Reed-Sternberg cells is not synthesised by them. Polytypic staining of malignant lymphomas is being reported with increasing frequency (Isaacson and Wright, 1978a; Taylor, 1978; Taylor *et al.*, 1978; Isaacson *et al.*, 1979), and it is important to be able to separate

those cells synthesising immunoglobulin from cells taking it up from the environment. While staining for J chain achieves this, the exact mechanism whereby immunoglobulin enters the tumour cells is still uncertain. Besides phagocytosis, the possibilities include pinocytosis, binding to Fc receptors, antibody directed against a tumour cell antigen, and simple diffusion into damaged and dead cells. Therefore, in the absence of other clear histiocytic characteristics, the demonstration of polytypic immunoglobulin in a tumour cell is not, on its own, evidence of a histiocytic derivation.

Since histiocytes and Reed-Sternberg cells both possess Fc receptors for IgG (Jaffe *et al.*, 1977; Kadin *et al.*, 1978) and since IgG is by far the predominant immunoglobulin in polytypic cells, it seems likely that these receptors are the way by which immunoglobulin enters the cells. It could be argued that since the cells of many B-cell lymphomas have Fc receptors for IgG (Jaffe *et al.*, 1977) they could take up extraneous immunoglobulin, thus masking their true monotypic nature. Such cells, however, would contain J chain. While this study does not completely resolve the controversy

Fig. 4 Serial sections of human tonsil stained for IgA (a), IgG (b), IgM (c), and J chain (d). Mature plasma cells and immature immunoglobulin synthesising cells of follicular centres stain for immunoglobulin while J chain staining is confined to follicular centre cells with the exception of scattered plasma cells probably containing IgM. Immunoperoxidase $\times 40$.

over the derivation of Reed-Sternberg cells, it does indicate that the immunoglobulin within these cells,

often cited as evidence of their B-cell origin, is not synthesised by them.

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