Light chains in Mediterranean lymphoma

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SUMMARY Four cases of Mediterranean lymphoma, in two of which serum α chains had been identified, were investigated with immunohistochemical techniques. In addition to α chains of α 1, subclass, monotypic light chains were identified in infiltrating tumour cells in all four patients and in plasma cells in two. The pattern of staining was in keeping with loss of light chain occurring with plasma cell differentiation and strongly suggested that the plasma cells and tumour cells shared a common clonal origin. In two patients concentrations of dendritic reticulum cells were identified in the tumour. These results support the suggestion that Mediterranean lymphoma is a tumour of follicle centre cells which undergoes plasma cell differentiation as a result of exposure to luminal antigen.

Mediterranean lymphoma, also known as immunoproliferative small intestinal disease, is a variant of primary small intestinal lymphoma, which shows striking geographical variation in incidence.^{1,2} While most cases have been reported from the Middle East, sporadic reports have appeared from other countries, with a substantial number of cases reported from South Africa.^{3,4} The finding of an abnormal α heavy chain protein in association with many of the cases^{5,6} has given rise to the term α chain disease, and the synthesis, but not necessarily the secretion, of α chain by plasma cells in Mediterranean lymphoma appears to be characteristic.⁷ The disease has a long natural history paralleled by the histological features, which consist essentially of a heavy plasmacytic infiltrate of the lamina propria gradually giving way to frank lymphoma.⁸ The precise relation between the plasma cells and the lymphoma is uncertain, but recently Isaacson and Wright⁹ have proposed a direct relation with the suggestion that the histogenesis of Mediterranean lymphoma simulates the normal pathways of gut associated lymphoid tissue. Immunohistochemical studies have provided the basis for these suggestions but have been hampered by the quality of the material available. Much of the material has come from abroad already embedded in paraffin blocks, having been subjected to a variety of fixatives. A recent opportunity to study four cases of Mediterranean lymphoma in which the tissue had been optimally fixed has, we believe, shed further light on the his-

togenesis of Mediterranean lymphoma and helped to explain the curious protein abnormalities seen in this disease.

Patients and methods

Table 1 summarises the principal clinical features of the patients. All four patients were young men aged between 18 and 29. They all presented with diarrhoea and weight loss and, with lymphoma as the clinical diagnosis, came to laparotomy. In three of the four patients the small intestine looked normal externally, and in all patients mesenteric lymph nodes were enlarged. Full thickness jejunal biopsy and lymph node biopsy were carried out in each case. Routine protein immunoelectrophoresis, supplemented with immunoselection methods, showed free α chains in the serum samples of patients 2 and 3 but not in the serum of patient 1. Standard immunoelectrophoresis failed to show free α chains in patient 4.

Tissue from all four patients was fixed for 24–48 h in buffered formol-saline and embedded in paraffin. Sections cut at 5 μ m were stained routinely with haematoxylin and eosin and by the indirect or peroxidase-antiperoxidase (PAP) immunoperoxidase techniques using the antisera listed in Table 2. Where indicated tissues were treated with trypsin before immunostaining.¹⁰ Staining was carried out for the major heavy and both light immunoglobulin chains using polyclonal antisera raised in rabbits. Sections were also stained with monoclonal antibodies to the two subclasses of α heavy chain and to C3b receptor.¹¹

Table 1 *Clinical features of the four patients with Mediterranean lymphoma*

Patient no.	Country of origin	Age (yr)	Sex	Presenting features	Serum α chains	Findings on laparotomy	Tissue
1	South Africa	26	M	Diarrhoea, weight loss, clubbing	Negative	Normal small bowel, mesenteric lymphadenopathy	Full thickness jejunal biopsy, mesenteric lymph node
2	South Africa	18	M	Steatorrhoea, weight loss,	Positive	Normal jejunum, mesenteric lymphadenopathy	Full thickness jejunal biopsy, mesenteric lymph node
3	South Africa	29	M	Diarrhoea, weight loss,	Positive	Thickened jejunum, mesenteric lymphadenopathy	Full thickness jejunal biopsy, mesenteric lymph node
4	Sudan	25	M	Diarrhoea, peripheral neuropathy	Negative	Normal jejunum, mesenteric lymphadenopathy	Full thickness jejunal biopsy, mesenteric lymph node

Table 2 *Antibodies used in study*

Antibody	Source	Specificity
Polyclonal rabbit anti- κ	Dako*	κ Ig light chain
Polyclonal rabbit anti- λ	Dako	λ Ig light chain
Polyclonal rabbit anti- α	Dako	α heavy chain
Polyclonal rabbit anti- μ	Dako	μ heavy chain
Polyclonal rabbit anti- γ	Dako	γ heavy chain
Monoclonal mouse anti- $\alpha 1$	Seward†	Heavy chain of IgA1
Monoclonal mouse anti- $\alpha 2$	Seward	Heavy chain of IgA2
Monoclonal mouse anti-C3b receptor	ICRF‡	Dendritic reticulum cells§

*Dakopatts A/S, Copenhagen, Denmark.

†Seward Laboratory, Bedford, UK.

‡Imperial Cancer Research Fund.

§In formalin fixed, paraffin embedded tissue.

Results

The histological appearances in routinely stained sections were characteristic of Mediterranean lymphoma and similar in all cases. A dense infiltrate of mature plasma cells was present in the upper lamina propria with a band like and nodular lymphoid infiltrate of lymphoid cells below. This lymphoid infiltrate consisted of a mixture of follicle centre cells, centrocytes and centroblasts, and immunoblasts, which invaded upwards into the lamina propria producing characteristic lympho-epithelial lesions.^{9,12} Lymph nodes showed either total replacement by a mixture of plasma cells and follicle centre cells or a perifollicular infiltrate by these cells with preservation of sinusoidal architecture.

Table 3 *Results of immunohistochemistry*

Patient no.	Antibody to				
	$\alpha 2$	$\alpha 1$	κ	λ	C3b receptor
1	Minority of plasma cells	Majority of plasma cells	Plasma cells, immunoblasts and follicle centre cells	Negative	Dendritic reticulum cells in lymphoid nodules
2	Minority of plasma cells	Majority of plasma cells	Negative	Plasma cells, immunoblasts and follicle centre cells	Negative
3	Minority of plasma cells	Majority of plasma cells	Immunoblasts and follicle centre cells	Negative	Dendritic reticulum cells in lymphoid nodules
4	Minority of plasma cells	Majority of plasma cells	Immunoblasts and follicle centre cells	Negative	Negative

Fig. 1a

Fig. 1b

Fig. 1c

Fig. 1 Intestinal mucosa of patient 1 stained (a) for $\alpha 1$ chain, (b) for $\alpha 2$ chain, and (c) with anti-C3b receptor. The majority of plasma cells contain $\alpha 1$ chain with a scattering of strongly staining reactive $\alpha 2$ plasma cells. In (c) C3b receptor positive processes of dendritic reticulum cells surround large neoplastic cells at the base of the mucosa. (a) and (b) Immunoperoxidase $\times 100$. (c) $\times 400$.

Fig. 2a

Fig. 2b

Fig. 2c

Fig. 2 Mucosa from patient 2 showing lymphoepithelial lesions stained (a) for $\alpha 1$ chain, (b) for κ chain, and (c) for λ chain. Plasma cells stain strongly and uniformly for $\alpha 1$ chain, less intensely and unevenly for λ chain, and are negative for κ chain. No cytoplasmic immunoglobulin is seen in the follicle centre cells. Immunoperoxidase. $\times 640$.

Fig. 3a

Fig. 3b

Fig. 3c

Fig. 3 Lymph node infiltrate from patient 4 stained (a) for $\alpha 1$ chain, (b) for κ chain, and (c) for λ chain. $\alpha 1$ chain is present in lymphoid cells (follicle centre cells and immunoblasts) and an occasional plasma cell but none is seen in plasma cells. Large lymphoid cells contain κ chain. Stain for λ chain is negative. Immunoperoxidase. $\times 800$.

small minority of plasma cells (Fig. 3). Antisera to all other heavy chains including immunoglobulin $\alpha 2$ showed scattered positively staining plasma cells in the intestine and lymph nodes. A corresponding number of plasma cells showed strong staining for both light chains.

Discussion

Previous immunocytochemical studies of Mediterranean lymphoma using polyclonal antisera have shown α chains in plasma cells but of unspecified subclass.^{8 12 13} Given that serum α chains in Mediterranean lymphoma have invariably been shown to be of $\alpha 1$ subclass our finding of a predominance of $\alpha 1$ heavy chains in plasma cells is not surprising. Some workers claim to have shown α chains in pleomorphic tumour cells,¹⁴ but others have found that the invasive tumour cells consistently lack cytoplasmic immunoglobulin.^{12 13} There have been a few reports of monotypic light chain associated with α chain in the serum of patients with Mediterranean lymphoma.^{15 16} Preud'homme *et al*¹⁷ showed surface and cytoplasmic IgA κ in cell suspension preparations of lymphoid cells from the intestine and mesenteric lymph nodes of a patient with Mediterranean lymphoma; the plasma cells contained only α chains. Apart from this single exception immunohistochemical studies have consistently failed to show light chains in Mediterranean lymphoma.

The presence of light chains in the four patients reported here may be related to the African origin of the patients since one of us (PGI) was unable to show light chains in patients with Mediterranean lymphoma studied previously, all of whom were from the Middle East.^{9 12 18} In all other respects, however, both clinically and histologically, these four cases are indistinguishable from Middle Eastern Mediterranean lymphoma. We believe that the essential difference lies, not in the origin of the patients but in the nature of the material, which in each case consisted of carefully excised full thickness intestinal and mesenteric node biopsies, all of which were optimally fixed. This contrasts with the material studied previously, which consisted principally of endoscopic biopsies and surgical resections, most of which had been treated with unknown fixatives for prolonged periods.

In follicle centre cell lymphomas all of the cells usually synthesise monotypic surface immunoglobulin, which can be shown immunohistochemically in cryostat sections.¹⁹ In paraffin sections of these tumours, however, monotypic immunoglobulin synthesis can be shown in only two thirds of patients as cytoplasmic immunoglobulin in a minority of

tumour cells.²⁰ By analogy, therefore, it can be inferred that follicle centre cells in Mediterranean lymphoma express surface immunoglobulin of either $\alpha 1\kappa$ or $\alpha 1\lambda$ isotype, although the presence of surface IgM rather than surface IgA cannot altogether be excluded; the study of cryostat sections of Mediterranean lymphoma would be necessary to do this. While the $\alpha 1$ heavy chain appears to be poorly expressed in the cytoplasmic immunoglobulin of follicle centre cells the reverse appears to be true in plasma cells, where light chain is more weakly expressed than $\alpha 1$ heavy chain and is usually not demonstrable at all.

The precise relation between the plasma cells and infiltrating lymphoma in Mediterranean lymphoma has long been a subject of debate. The phase of plasma cell infiltration may precede by many months or years that of frank tumour, and some workers believe that the plasma cell infiltrate is not malignant but represents a setting in which lymphoma occurs. Attempts to prove a continuous clonal relation between plasma cells and infiltrating tumour using anti-idiotypic antibodies would be fruitless since the serum α chain is abnormal, lacking the variable portion. The presence of monotypic light chain in our patients, particularly in the two patients in whom it was present in both infiltrating tumour and plasma cells, is a strong argument for both having originated from the same clone.

These findings support the argument put forward by Isaacson and Wright⁹ that Mediterranean lymphoma is a tumour of mucosal follicle centre cells which are antigen driven to show extreme plasma cell differentiation following the normal pathways of gut associated lymphoid tissue.²¹ Occasional reports of a follicular pattern in Mediterranean lymphoma further substantiate this hypothesis.^{13 22} Clear evidence that the band like and nodular lamina propria infiltrate in Mediterranean lymphoma has a follicular basis is provided by our finding of nodular concentrations of dendritic reticulum cells, the processes of which embraced large abnormal tumour cells in two patients. Failure to stain dendritic reticulum cells in the other two patients was probably related to slight variations in fixation time since the antigen in question is particularly sensitive in this way. If, however, as has been proposed by others,^{23 24} the surface IgA on the follicle centre cell consists of α chain alone then the suggestions of Isaacson and Wright are not tenable since the receptor antibody on antibody forming cells must be complete and include the variable region in order for the cell to be transformed by antigen; α chain in Mediterranean lymphoma is not only not linked to light chain but lacks the variable region. The presence of light chains in cells of Mediterranean lymphoma suggests

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that the malignant follicle centre cells do bear complete surface immunoglobulin, thus permitting response to antigen at this stage. To this effect it is relevant that the administration of antibiotics alone can result in disappearance of the plasma cells in Mediterranean lymphoma.

The sequence of events in Mediterranean lymphoma would appear to be that a malignant clone of follicle centre cells emerges with complete immunoglobulin on the surface of the cells. This immunoglobulin is capable of reacting with a lumenal (probably bacterial) antigen and plasma cell differentiation occurs. It is at this stage that disordered immunoglobulin synthesis occurs with loss of light chain, which may persist to some degree in plasma cells, and synthesis of an abnormal $\alpha 1$ heavy chain. This sequence of events is similar to that occurring in the mouse heavy chain disease model.²⁵ Here there is an internal deletion of the Fd portion of the IgA1 molecule, which interferes with bonding of heavy and light chains and results in the synthesis of abnormal $\alpha 1$ heavy chain. The fate of the light chains in Mediterranean lymphoma is uncertain. Since Bence-Jones proteinuria is not a feature of Mediterranean lymphoma it is likely that they are not secreted. Why $\alpha 1$ rather than $\alpha 2$ heavy chain synthesis is characteristic of Mediterranean lymphoma remains to be explained, as does the underlying defect of immunoglobulin synthesis. The geographical distribution of Mediterranean lymphoma would support suggestions^{26,27} that associated immune deficiency, which may have a genetic basis, could be implicated.

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