

Practical value of genotypic analysis for diagnosing lymphoproliferative disorders

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SUMMARY The value of DNA hybridisation (using immunoglobulin and T cell receptor gene probes) was assessed during the diagnosis of problematical lymphoid tissue biopsy specimens. In 14 of 18 specimens (78%), which contained a malignant lymphoproliferation of uncertain aetiology, this technique permitted the demonstration of a monoclonal proliferation of B cells (nine cases) or T cells (five cases). In five further lymph node biopsy specimens, in which the differential diagnosis lay between a reactive or malignant process, a clonal proliferation was shown in three cases. DNA analysis is, therefore, of practical value in resolving many of the diagnostic problems that arise in the assessment of lymphoid tissue biopsy specimens.

The availability of cDNA probes to immunoglobulin (Ig) and T cell receptor (TCR) genes provides a powerful tool for showing the clonality of B or T cell lymphoproliferative disorders. Studies with Ig gene probes have confirmed the B cell origin of both common acute lymphoblastic leukaemia¹ and hairy cell leukaemia.² More recently, T cell leukaemias^{3,4} and lymphomas⁵⁻⁷ have been shown to undergo clonal rearrangements of the TCR β chain gene, and this phenomenon has been used to prove the T cell origin of Lennert's lymphoma⁸ and some cases of coeliac associated bowel lymphoma (previously believed to be a histiocytic malignancy).⁹

In spite of the obvious potential value of DNA probes in providing evidence for the B or T cell nature of a lymphoid proliferation it is not yet clear whether the combined use of Ig and TCR gene probes is of practical benefit in resolving the type of diagnostic problems faced by the histopathologist. Most of these difficulties fall into one of two categories: first, biopsy specimens which show a lymphoid neoplasm but in which the B or T cell nature of the lesion cannot be established by immunocytochemical labelling; and secondly, those specimens in which it is difficult

to distinguish between a reactive and a malignant process.

It is clearly of considerable importance to establish how often DNA analysis with Ig and TCR probes will resolve these difficult cases, particularly as many histopathology laboratories with an interest in lymphoma are considering installing the relatively expensive and time consuming technology entailed.

In this paper we report our findings, using DNA analysis, to investigate a series of 23 problem lymphoid biopsy specimens, which had all given rise to difficulty after histological analysis and immunophenotyping.

Material and methods

SAMPLES

The biopsy specimens selected for study were those in which a firm pathological diagnosis was not possible after examination of routinely stained sections and immunohistologically labelled sections. The problems fell into two categories: the cell of origin of a neoplasm was unclear—that is, it could be B cell, T cell, or histiocytic lymphoma or possibly non-lymphoid malignancy; it was impossible to differentiate between a reactive or neoplastic lymphoproliferative process.

Table Results of DNA analysis of various lymph node biopsy specimens

Case No	Histological picture	Diagnostic problem	Genotypic data			
			J _H	C _κ	TCR ^β	Interpretation
1	Non-Hodgkin's lymphoma, pleomorphic	?B or T cell	R	R	G	B cell neoplasm
2	Non-Hodgkin's lymphoma, large + small cell	?B or T cell	R	R	G	B cell neoplasm
3	Non-Hodgkin's lymphoma, large + small cell	?B or T cell	R	R	G	B cell neoplasm
4	Non-Hodgkin's lymphoma, large cell	?B or T cell	G	R	G	B cell neoplasm
5	Non-Hodgkin's lymphoma, pleomorphic	?B or T cell	R	R	R	B cell neoplasm
6	Non-Hodgkin's lymphoma, large cell	?B or T cell	R	R	G	B cell neoplasm
7	Non-Hodgkin's lymphoma, pleomorphic	?B or T cell	G	R	G	B cell neoplasm
8	Non-Hodgkin's lymphoma, pleomorphic	?B or T cell	G	G	R	T cell neoplasm
9	Non-Hodgkin's lymphoma, pleomorphic	?B or T cell	G	G	R	T cell neoplasm
10	Non-Hodgkin's lymphoma, large + small cell	?B or T cell	G	G	R	T cell neoplasm
11	Non-Hodgkin's lymphoma, large + small cell	?B or T cell	G	G	R	T cell neoplasm
12	Non-Hodgkin's lymphoma, pleomorphic	?B or T cell	G	G	G	Inconclusive
13	Non-Hodgkin's lymphoma, pleomorphic	?B or T cell	G	G	G	Inconclusive
14	Non-Hodgkin's lymphoma, large cell	?B or T cell	G	G	G	Inconclusive
15	Non-Hodgkin's lymphoma, pleomorphic	?B or T cell	G	G	G	Inconclusive
16	Malignant process	?Thymoma or non-Hodgkin's lymphoma	R	R	G	B cell neoplasm
17	Lymphoma	?Hodgkin's disease or non Hodgkin's lymphoma	R	G	G	B cell neoplasm
18	Lymphoma	?Hodgkin's disease or non Hodgkin's lymphoma	G	G	R	T cell neoplasm
19	Lymphoid proliferation	?Reactive or malignant	R	R	R	B cell neoplasm
20	Lymphoid proliferation	?Reactive or malignant	R	R	G	B cell neoplasm
21	Lymphoid proliferation	?Reactive or malignant	G	G	R	T cell neoplasm
22	Lymphoid proliferation	?Reactive or malignant	G	G	G	Probably reactive
23	Lymphoid proliferation	?Reactive or malignant	G	G	G	Probably reactive

All samples had the C_λ genes in a non-rearranged configuration.
G = germline configuration; R = rearranged configuration.

Fresh unfixed biopsy specimens were obtained from 23 cases studied by the histopathology departments participating in this study. Specimens were obtained from lymph nodes (n = 17), skin (n = 3), spleen (n = 2) and lung (n = 1). The material was snap frozen and stored at -70°C. All biopsy specimens were classified according to conventional morphological criteria and were also analysed immunohistologically, by staining cryostat sections with monoclonal antibodies, as previously described.¹⁰ Controls consisted of samples of tissue from 28 cases of carcinoma and peripheral blood from 36 healthy caucasian volunteers.

DNA ANALYSIS

DNA was extracted from frozen tissue biopsy specimens (or peripheral blood leucocytes) by routine methods and digested with restriction endonucleases.¹¹ The DNA fragments were subjected to electrophoresis in 0.8% agarose gels and transferred to nitrocellulose filters by Southern blotting. Filters were hybridised with ³²P labelled probes specific for Ig and TCR genes,¹² washed under appropriate con-

ditions, and subjected to autoradiography. The four probes used in this study were an Ig heavy chain joining region J_H probe (C76R51A), an Ig κ chain constant region C_κ probe (pUCR17C_κ), an Ig λ chain region C_λ probe (Chr 22 λ5),¹³ and a TCRβ chain gene probe (Jurkat β2).¹⁴

Immunoglobulin genes Aliquots of all DNA samples were digested with BamHI, EcoRI, and Bgl II before hybridisation with the J_H probe. DNA was digested with BamHI and Bgl II before C_κ hybridisation and with Hind III and EcoRI before C_λ probe hybridisation. All control samples showed identical restriction fragment patterns with the above enzymes and probes, with no DNA polymorphisms or rearrangements being observed.

T cell receptor β chain gene Aliquots of all DNA samples were digested with BamHI, EcoRI, and Hind III before hybridisation with the TCRβ chain gene probe. All control samples showed identical patterns with all enzymes.

A rearrangement was defined as having occurred if, after digestion with restriction endonucleases, a new band was detected on hybridisation. Where possible,

all rearrangements were shown using at least two different restriction enzymes to exclude the possibility of abnormal patterns being caused by DNA polymorphism.

Results

The table summarises the results of DNA analysis with Ig gene and TCR β chain gene probes. Difficulties in diagnosis were divided into two categories as outlined below.

MALIGNANT LYMPHOPROLIFERATIONS OF UNCERTAIN CELL ORIGIN

In this category 15 cases were diagnosed histologically as non-Hodgkin's lymphomas, which could not be unequivocally assessed as being of either B or T cell origin after immunohistological staining (staining for κ and λ light chains had shown no clear pattern of light chain restriction). A clonal B cell population was shown by DNA analysis in seven cases (cases 1–7), in four of which numerous T cells had been shown by immunohistological studies (leading to a suggested diagnosis of a T cell neoplasm). Case 5 showed both TCR β and Ig gene rearrangements, but as light chain gene rearrangement seems, from our own work and other data,¹ to be restricted to B cell neoplasms, it is likely that this case was of B rather than T cell lineage. Four cases were shown to be T cell derived non-Hodgkin's lymphomas, while in four cases there was no evidence of a B or T cell clone.

In addition to these 15 cases, there were three cases in which the difficulty lay in distinguishing between a non-Hodgkin's lymphoma and a malignant process of non-lymphoid origin. In the first biopsy specimen (case 16) it was not possible to differentiate between a non-Hodgkin's lymphoma and a thymoma on morphological criteria, as leucocyte common antigen staining was negative. A clonal rearrangement of both Ig heavy and κ light chain genes, however, provided objective evidence that the tumour was B cell lymphoma. In two further cases the diagnosis lay between non-Hodgkin's lymphoma or Hodgkin's disease. Case 17 was a lymphoma arising in a patient with a history of Hodgkin's disease: at relapse the tumour had the appearance of Hodgkin's disease but seemed to contain a focus of high grade large cell non-Hodgkin's lymphoma, with the phenotypic pattern of a B cell neoplasm. DNA analysis confirmed the B cell origin of this new tumour. In case 18 the differential diagnosis after morphological and immunohistological examination lay between a T non-Hodgkin's lymphoma and Hodgkin's disease with a large number of reactive T cells. Genotypic analysis on this case resolved the problem and provided objective evidence for a T cell neoplasm.

LYMPHOID PROLIFERATIONS

In cases 19 to 23 it was impossible to differentiate a reactive from a malignant process. In cases 19 and 20 clonal rearrangements of both heavy and light Ig chain genes showed the presence of B cell lymphoma. In case 21 a T cell skin lymphoma was shown and in the last two cases DNA analysis showed a germline pattern with both Ig and TCR β probes and was hence inconclusive.

Discussion

This study indicated that DNA analysis of tissue biopsy specimens from malignant lymphoproliferations of uncertain cell origin using Ig and TCR gene probes yielded an answer in most cases. In nine of 18 malignant lymphoid processes the neoplasm was shown to be of B cell origin. In four of these tumours the difficulty in diagnosis seems to have been caused by the presence of large numbers of reactive T cells. The results of our study suggest that this may be a relatively common cause of difficulty in interpreting immunohistological analysis of lymphoma biopsy specimens. The cases probably correspond to the cases of "pseudo-T cell" non-Hodgkin's lymphoma, described by Arnold *et al.*¹⁵ These authors proved the existence of a monoclonal B cell proliferation in such tumours, as did the findings in our own study, by showing Ig gene rearrangement.

The availability of probes against the TCR β chain gene now facilitates analysis of lymphoid neoplasms for evidence of possible T cell origin.^{3–5} From the findings in this study, however, it seems that cases of T non-Hodgkin's lymphoma, which give rise to diagnostic difficulty through masking by reactive normal cells, may be less common than B cell lymphoma (only five of 18 biopsy specimens were of T cell origin masked by reactive B cells).

The cases in which no rearrangement could be shown were apparently all malignant (on morphological criteria) and showed histological and antigenic features (expression of the leucocyte common antigen) of lymphoma. As all B cell neoplasms seem to show Ig gene rearrangement these lymphomas are unlikely to be of B cell origin. It is thus conceivable that they are T cell neoplasms in which the β chain gene has not undergone rearrangement (because they derive from an early maturation stage), or, alternatively, they may be of non-lymphoid origin, such as true histiocytic neoplasms. Further studies of tumours of this type are required to resolve these questions.

In addition to the 18 cases of lymphoma of uncertain origin, we studied DNA from five lymph node biopsy specimens in which there was dispute as to whether the histological picture was of a reactive

lymphoid proliferation or a low grade lymphoma. A clonal lymphoid expansion was shown in three cases, supporting a diagnosis of lymphoma. This is in keeping with the rapid downhill course in cases 19 and 21. In the two other cases no clonal rearrangements were detected, and although negative information of this type is open to several interpretations, the results support a reactive rather than a malignant lymphoid proliferation. This conclusion was supported by clinical follow up, in that both patients remained disease free after 20 and 24 months (the first received chemotherapy for six months and the second was not treated).

Finally, it should be noted that, although this study has shown the value of gene rearrangement studies in resolving diagnostic problems, the magnitude of this problem in diagnostic practice is relatively small. This is reflected in the fact that these cases were collected from six different centres. We estimate that less than 5% of lymph node biopsy specimens present difficulties in diagnosis after evaluation by histological and immunohistological procedures. It could therefore be argued that the most cost effective procedure is to establish facilities for DNA analysis in a limited number of centres. The present study entailed testing many biopsy specimens which had been sent on dry ice from different centres (in this country and abroad), and the logistical problems of transporting this type of material were relatively small. Consequently, centralisation of DNA studies in a few specialist centres is preferable to attempts to perform these techniques in a large number of smaller laboratories.

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