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# Analysis of antigen receptor genes in Hodgkin's Disease

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## Abstract

***Aim*—To analyse the configuration of the antigen receptor genes in Hodgkin's disease.**

***Methods*—DNA extracted from 45 samples of Hodgkin's disease was analysed using Southern blotting and DNA hybridisation, using probes to the joining region of the immunoglobulin heavy chain gene, the constant region of κ immunoglobulin light chain gene, and the constant region of the β chain of the T cell receptor gene.**

***Results*—A single case of nodular sclerosing disease showed clonal rearrangement of the immunoglobulin heavy and light chain genes, all other samples having germline immunoglobulin genes. The nature of the clonal population in the diseased tissue is uncertain, because the intensity of the rearranged bands did not correlate with the percentage of Reed-Sternberg cells present. The T cell receptor genes were in germline configuration in all the samples.**

***Conclusions*: Antigen receptor gene rearrangement is a rare finding in unselected cases of Hodgkin's disease.**


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The use of molecular biological techniques to detect antigen receptor gene rearrangements is now widely accepted as useful in the study of lymphoproliferative disease. With certain exceptions, reactive conditions can be differentiated from malignant disorders and the B or T cell nature of lymphomas can be determined with reasonable accuracy. The diagnostic usefulness of these techniques is, however, much less certain in Hodgkin's disease; a large number of studies has now been performed which have yielded a bewildering array of conflicting results.[1-38]

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## Methods

Forty five routine diagnostic biopsy specimens from 43 patients with Hodgkin's disease were studied, the only criterion being the availability of sufficient frozen material for DNA extraction. Cases were categorised according to the Rye classification,[39] with modifications[40 41] (table 1). Normal human placenta was used as a germline control.

DNA was extracted using standard proteinase K digestion and phenol-chloroform techniques.[42] DNA (12 μg) from each sample were digested with the restriction enzymes *EcoR1* and *BamH1*, and some samples were also digested with *HindIII*. The resulting fragments were separated by electrophoresis on 0·7% high melt temperature agarose gel and then transferred on to a Hybond N nylon filter by Southern blotting.[43] Probes were labelled with $^{32}P$ dCTP by random priming, and hybridised to the filters by overnight incubation at 65°C in a solution containing 5 × standard saline citrate (SSC), 1 × polyethylene (PE), 10% polyethylene glycol (PEG), and 400 μl denatured salmon sperm DNA. After hybridisation the filters were washed four times in 2 × SSC/0·1% sodium dodecyl sulphate (SDS), followed by four washes in 0·2 × SSC/0·1% SDS, each for 10 minutes at 65°C. Filters were then dried, placed in Saran wrap, and exposed to Kodak X-omat film at −70°C with an intensifying screen. Exposure periods varied between three and 14 days depending on the level of signal obtained and then developed.

The immunoglobulin heavy chain gene probe $J_H$ was a genomic probe of about 2·3 kilobases, and was the *EcoR1-HindIII* fragment of the gene.[44] The κ light chain gene probe $C_\kappa$ was a 0·46 kilobase *EcoR1* fragment containing the constant region.[45] The T cell receptor β chain probe Cβ was a 390 base pair *BglII-BglII* fragment which hybridised with both constant regions, and which was prepared from the 770 base pair *PstI* fragment.[46]

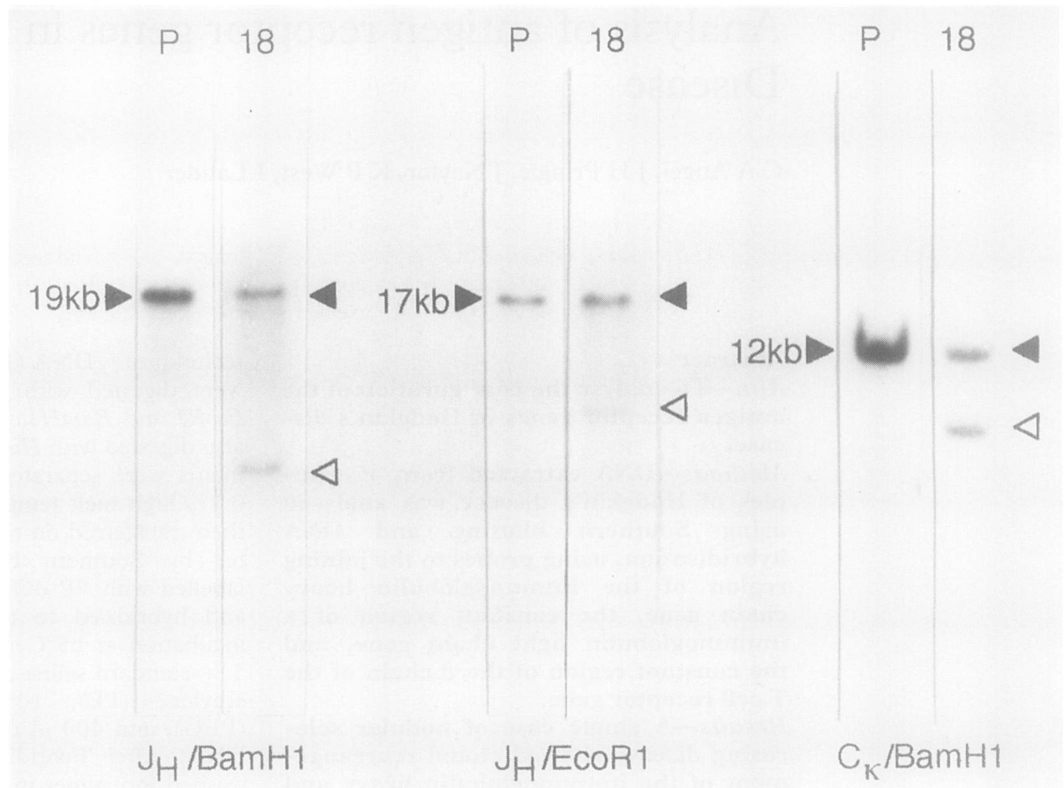
To ensure that no gel artefact, partial digestion artefact, genetic polymorphism or other phenomenon was misinterpreted as gene rearrangement, great care was taken when the autoradiographs were examined. For example, a non-germline band on an *EcoR1* digest with the $J_H$ probe was only interpreted with confidence as a rearrangement when a non-germline band was also present

*Table 1 Histological subtypes of Hodgkin's disease cases studied*

| *Histological subtype* | *Number* |
|---|---|
| LPN | 3 |
| LPD | 1 |
| NS1 | 19 |
| NS2 | 16 |
| MC | 6 |
| Total | 45 |

LPN = lymphocyte predominant nodular; LPD = lymphocyte predominant diffuse; NS1 = nodular sclerosing grade 1; NS2 = nodular sclerosing grade 2; MC = mixed cellularity.

*Case 18 probed with the $J_H$ and $C_K$ probes and showing non-germline bands consistent with clonal immunoglobulin gene rearrangement. Hind III digested DNA probed with $J_H$ is not shown. (P = placental DNA, ▶ = germline band, ▷ = non-germline band.)*




with at least one other restriction enzyme. When a non-germline band appeared that failed to meet this criterion, the experiment was repeated using a freshly prepared DNA sample and, where it was thought necessary, prolonged digestion. Many non-germline bands were seen to represent gel artefacts or products of incomplete digestion when this procedure was followed. Where possible, polymorphisms were excluded by the examination of unaffected tissue, although this was frequently impossible to obtain.

## Results

DNA extracted from 44 of the biopsy specimens showed no evidence of immunoglobulin or T cell receptor $\beta$ chain gene rearrangement. A single case (case 18) showed apparent rearrangement of the immunoglobulin genes, non-germline bands being present when DNA restricted with all three enzymes was probed with $J_H$, and when *BamH1* restricted DNA was probed with $C_\kappa$ (figure). The TCR$\beta$ chain gene was in germline configuration. The density of the non-germline bands in each of the *BamH1* probings was consistent with a clonal population representing about 40% of the total DNA present. The band seen with *EcoR1* was less intense compared with the residual germline DNA present, but this apparent inconsistency does not necessarily cast doubt on the integrity of the finding, as many technical factors lead to variations in the efficiency of transfer during Southern blotting.[47]

Case 18 was an example of nodular sclerosing (NS) Hodgkin's disease, and Reed-Sternberg cells and their mononuclear variants accounted for no more than 5% of the total population. Immunohistology analysis showed that 30–40% of the background lymphoid population in the tissue comprised small B lymphocytes, but it was not possible to detect monotypia on cryostat section immunohistochemistry. There was no B marker positivity or immunophenotypic evidence of clonality within the Reed-Sternberg cell population. Clinically, the patient was a 28 year old woman who presented with cervical lymphadenopathy and night sweats and who was staged as 1B. She was treated with upper mantle irradiation and was well when seen 21 months after presentation.

## Discussion

This study has shown that most cases of Hodgkin's diseases show no antigen receptor gene rearrangements when routine methods of investigation are used, a finding which is largely in keeping with those of earlier studies. In a review of 38 publications which examined 492 samples[1–38] antigen receptor genes were in germline configuration in 394 (80·1%), immunoglobulin gene rearrangements were reported in 68 (13·8%), and T cell receptor gene rearrangements in 31 (6·3%) (table 2).

Clonal immunoglobulin gene rearrangement seems to be a rare finding when studies

*Table 2 Review of published findings*

| *Reported finding* | *References* |
|---|---|
| Ig gene rearrangement | 4,6,7,9,10,15,20,22,23,24,25,26,27, 29,30,32,33,34,35,36,37,38 |
| TCR rearrangement | 2,3,4,8,16,17,22,23,24,25 |
| No rearrangement | 1,5,11,12,13,14,18,19,21,28,31 |

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concentrate on unselected cases of Hodgkin's disease, as in the present study. When biopsy specimens are selected for high numbers of Reed-Sternberg and Hodgkin's cells, gene rearrangement is more frequently found, occurring in 20 of 77 cases studied (26%),[6 7 30 36] and frequent rearrangement has also been shown when samples are enriched for Reed-Sternberg cells.[10] This might tend to suggest that the rearrangements are occurring in the Reed-Sternberg cells themselves, although the authors of some of these papers remain unconvinced.[6 30 36] The high degree of selection in these studies led to the inclusion of recurrent, presumably treated cases of disease, together with cases with highly atypical aggressive behaviour. Such cases are not representative of Hodgkin's disease as a whole. In the present study the intensity of the rearranged bands did not correlate with the number of Reed-Sternberg cells present, but was in keeping with the percentage of B cells present in the background population. Where other studies have addressed this aspect, similar findings have been apparent.[15 26 27 33 34] It has been suggested, therefore, that these rearrangements arise from clonal populations of B cells in the cellular background. Kodura *et al* combined karyotypic analysis with antigen receptor gene rearrangement studies and showed that of four cases with clonal karyotypic abnormalities, only one had rearranged immunoglobulin genes, and the density of the bands did not correlate with the percentage of Reed-Sternberg cells present.[26] Four other cases had rearranged genes but normal karyotypes. They suggested that the cytogenetic clone was non-lymphoid and also that there were minor clonal populations of B cells in diseased tissues. It seems unlikely that Reed-Sternberg cells, long held to be the malignant element in Hodgkin's disease, are solely responsible for the clonally rearranged immunoglobulin genes detected in diseased tissues, although they may represent a component of these clonal proliferations.

Only 6·3% of all cases studied have shown T cell receptor $\beta$ chain gene rearrangements. It is well known that rearrangements of the $\gamma$ chain gene in isolation are unreliable markers of lineage or clonality[18 48] and it is now clear that many of the early publications in which frequent T cell receptor gene rearrangements were reported had interpreted non-germline bands using TCR $\gamma$ probes as evidence of clonality.[2 8] Furthermore, it is now becoming apparent that DNA extracted from Hodgkin's disease tissue shows evidence of polyclonal T cell rearrangements,[18 34] in keeping with the predominance of T cells in most cases of Hodgkin's disease. Some have suggested that the Reed-Sternberg cells themselves form part of this polyclonal T cell population in Hodgkin's disease.[4]

Molecular analysis of this nature may be of some use as an adjunct to the diagnosis of Hodgkin's disease. For example, when the differential diagnosis lies between Hodgkin's disease and non-Hodgkin's lymphoma, absence of detectable rearrangements would tend to confirm a diagnosis of Hodgkin's disease. Clear evidence of T cell receptor gene rearrangement seems to be rare in Hodgkin's disease and such a finding might lead one in the direction of T cell non-Hodgkin's lymphoma. It is clear that gene rearrangement analysis can only supplement other diagnostic procedures, and that the results should always be considered in the context of the clinical, histological, and immunohistological background. Immunoglobulin gene rearrangement seems to occur in otherwise typical Hodgkin's disease, and cannot be used to distinguish Hodgkin's disease from B cell non-Hodgkin's lymphoma.

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