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Transforming growth factor β1 messenger RNA in Reed–Sternberg cells in nodular sclerosing Hodgkin's disease

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Abstract

***Aims*—To determine the cellular origin of the most potent cytokine present in Hodgkin's disease, transforming growth factor (TGF) β, the polycellular population of Hodgkin's tissue was studied using in situ hybridisation.**

***Methods*—A biotin labelled oligo-complementary DNA (cDNA) was constructed according to the previously determined sequence for TGFβ1 cDNA. Forty three frozen and paraffin wax embedded tissue samples replaced by Hodgkin's disease or non-Hodgkin's lymphoma, three Reed–Sternberg cell lines, one Ki1 positive lymphoma cell line, and an epithelial cell line were studied for expression of TGFβ1 messenger RNA (mRNA) as well as secretion of the TGFβ1 protein and expression of the CD30 epitope.**

***Results*—The results obtained with the 24 frozen tissue samples confirmed that the TGFβ antigen is found predominantly in the nodular sclerosing Hodgkin's disease (NSHD) subtype. Nineteen paraffin wax embedded tissue samples were used to measure the simultaneous expression of CD30 and TGFβ1 mRNA. The latter was found in eight of eight NSHD samples, two of six mixed cellularity samples, and two of five non-Hodgkin's lymphoma samples. No evidence of fibroblast expression of TGFβ1 mRNA was noted.**

***Conclusions*—Activated lymphocytes in NSHD express TGFβ1 mRNA, but binucleate Reed–Sternberg cells and mononuclear Hodgkin's cells are the primary sources of activated TGFβ in Hodgkin's disease.**

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Keywords: Hodgkin's disease, TGFβ1, in situ hybridisation.

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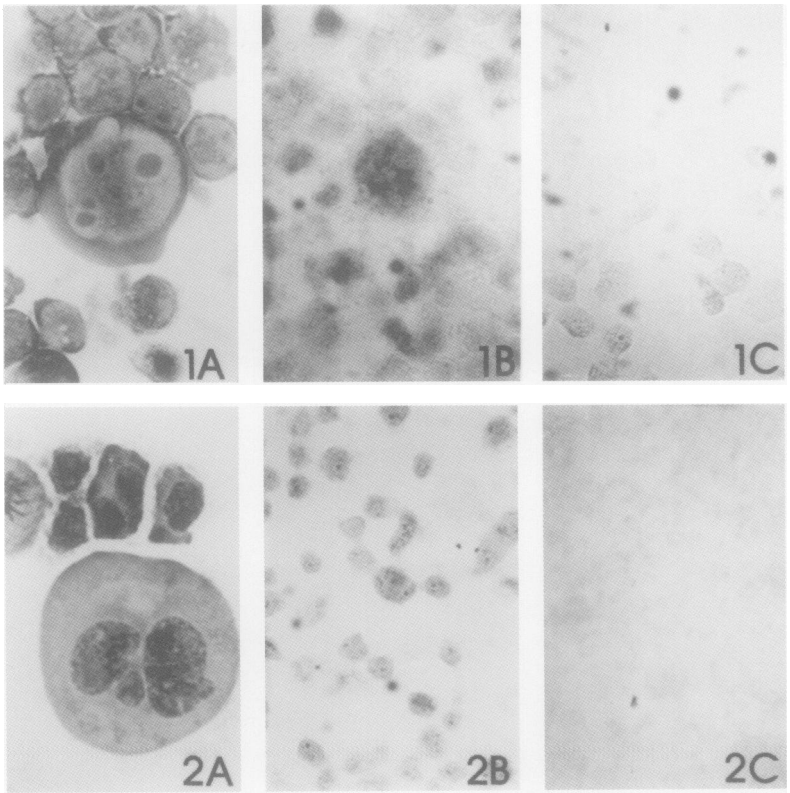

Figures 1 and 2 In situ hybridisation for TGFβ1 mRNA in Reed–Sternberg cells. The left panel (A) of each composite shows the cytospin of each cell line stained with Wright's Giemsa (× 1000). The center panel (B) shows a similar cytospin hybridised with an oligonucleotide probe for TGFβ1. There is no counter stain. The right panel (C) shows control cells treated with sense probe. Figure 1, L-428; fig 2, HDLM (NSHD).

Reed–Sternberg cells produce many cytokines in vitro.[1–12] Of these, transforming growth factor (TGF) β has been studied and also shown to be present in an activated, stainable form in primary tissue[13] and the urine of patients with active disease.[14] The cellular source of this activated Hodgkin's TGFβ in vivo has been questioned.[15] Eosinophils have been proposed as the predominant source.

This study evaluates the origin of this bifunctional cytokine. The polycellular population of Hodgkin's tissue was studied using in situ hybridisation with simultaneous staining of growth factor antigen and the Ki1 (CD30) antigen. Primary tissue samples replaced by Hodgkin's disease and controls were studied. The results obtained confirmed that TGFβ antigen is found predominantly in the nodular sclerosing Hodgkin's disease (NSHD) subtype. Although activated lymphocytes in NSHD express TGFβ1 mRNA, binucleate Reed–Sternberg cells and mononuclear Hodgkin's cells were the major source.

Methods

Forty three lymph node biopsy specimens were studied, 24 of which were frozen and sectioned for study at 4–6 μm, and the remaining 19 were fixed in 10% neutral buffered formalin and embedded in paraffin wax. Three long term Reed–Sternberg cell lines were studied, L-428, KMH2, and HDLM. A Ki1 positive lymphoma cell culture, Mac-1, was used as a positive

TGFβ expression in Hodgkin's disease tissue sections

Diagnosis	*No. of sections*	*TGFβ1*	*Ki1 (CD30)*	*TGFβ1 mRNA*
Frozen sections				
NSHD	9	7	9	Not interpretable
MCHD	6	1	5	Not interpretable
extranodal HD	1	0	1	Not interpretable
lymphoma	8	2	0	Not interpretable
small lymph (n = 1)		0	0	
follicular, small (n = 4)		0	0	
follicular, mixed (n = 1)		0	0	
diffuse, mixed (n = 1)		1	0	
large cell (with sclerosis) (n = 1)		1	0	
Paraffin wax sections				
NSHD	8	0	8	8 (H/RS, mononuclear HD, activated lymphocytes)
MCHD	6	0	5	2 (lymphocytes)
lymphoma	5	0	0	2 (malignant cells)
follicular, mixed (n = 3)		0	0	0
diffuse, mixed (n = 1)		0	0	1
large cell (with sclerosis) (n = 1)		0	0	1

MCHD = mixed cellularity Hodgkin's disease; H/RS = Hodgkin's/Reed–Sternberg cells; HD = Hodgkin's disease.

control. An epithelial cell line, CCL-64, was used as a negative control.

Anti-TGFβ is a rabbit IgG antibody purified by antigen affinity chromatography (R&D Systems, Minneapolis, Minnesota, USA). The antibody detects TGFβ1, β1·2, β2, β3, and β5 on Western blotting. BER-H2 (anti-Ki1; anti-CD30) recognises Ki1 antigen in formalin fixed tissue (M751; Dako, Carpenteria, California, USA).

A complementary DNA (cDNA) was constructed according to the published sequence for TGFβ1.[16] The 44 base biotin labelled oligonucleotide was as follows: 5′ AC GCA GCA GTT CTT CTC TGT GGA GGA GAA GCA ATA GTT GGT GTC 3′. A control biotin labelled sense probe was also constructed: 5′ TG CGT CGT CAA GAA GAG ACA CCT CCT CTT CGT TAT CAA CCA CAG 3′.

Immunoperoxidase staining was performed on cytospins, deparaffinised tissue sections, and frozen tissue sections. In situ hybridisation was performed on cytospins of cultured cells after fixation in paraformaldehyde (4%) for five minutes. Tissue sections were placed on gelatin coated glass slides treated with diethyl pyrocarbonate. Paraffin wax embedded tissue samples were dewaxed and target sequences were unmasked using weak acid (0·2 M HCl for 20 minutes) and proteinase K/EDTA. Non-specific binding was blocked using transfer RNA (tRNA) (1 mg/ml) and salmon testis DNA (1 mg/ml). In addition to the positive and negative cellular controls three internal negative controls were used: RNase A (Sigma, St Louis, Missouri, USA), unlabelled antisense probe, and labelled sense probe. Heat denatured oligonucleotides were incubated overnight at 37°C with the target slides. The slides were washed and the hybridised biotin probe label was detected using avidin alkaline phosphatase (30 minutes at 20°C) followed by nitro blue tetrazolium buffered by BCIP (5-bromo-4-chloro-3-indolyl-phosphate p-toluidine). After washing, the slides were lightly counterstained with haematoxylin and covered with a coverslip.

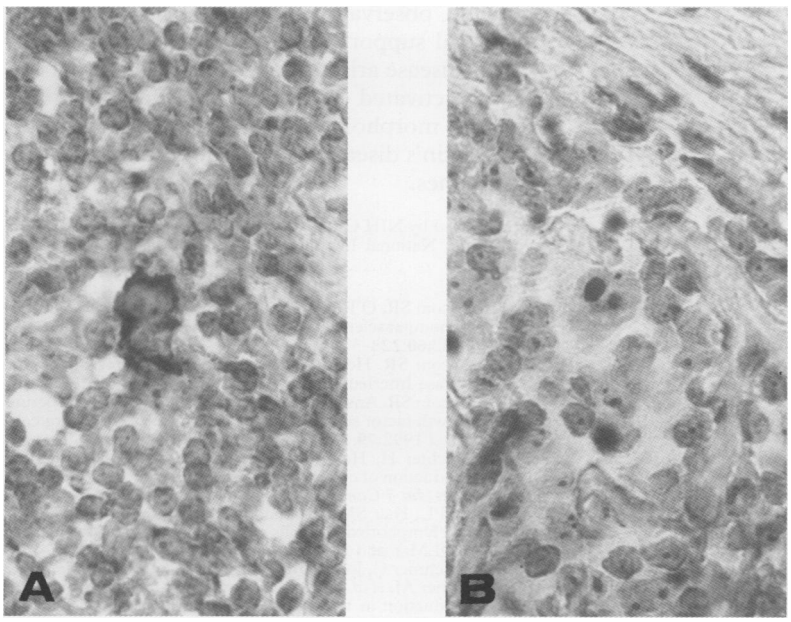

Figure 3 In situ hybridisation for TGFβ1 mRNA in NSHD. Panel A shows strong expression of mRNA by a Reed–Sternberg cell. Control panel B has been hybridised with a sense probe for TGFβ1 mRNA. The Reed–Sternberg cell with giant nucleoli is negative (× 630).

Results

Figures 1 and 2 show L-428 and HDLM Reed–Sternberg cells positive for TGFβ1 mRNA on in situ hybridisation. L-428 and HDLM (NSHD) had higher quantities of mRNA per cell than KMH2 (mixed cellularity Hodgkin's disease) (data not shown). Ki1 positive control lymphoma cells also expressed TGFβ1 mRNA, confirming previous reports.[17] Epithelial cells in G0 did not express TGFβ1 mRNA.

The anti-TGFβ antibody used in the present study was prepared against purified TGFβ1 and detected TGFβ only in frozen tissue. The observations confirmed the previous report of predominant NSHD expression. In seven of nine NSHD lymph nodes TGFβ antigen could be identified within the lymph node in extracellular sites, particularly adjacent to collagen fibrosis. The results of staining for TGFβ and Ki1 antigen in 24 frozen lymph nodes are summarised in the table.

In situ hybridisation for TGFβ1 mRNA was performed in 19 formalin fixed tissue samples. The NSHD subtype expressed TGFβ1 mRNA more strongly than the other lymph node tissue samples studied. Eight of eight NSHD lymph node samples contained Hodgkin's cells expressing TGFβ1 mRNA. The results are summarised in the table and presented in figs 3 and 4.

Some lymph node fields contained large numbers of eosinophils with granules that bound the TGFβ1 mRNA oligonucleotide probe. Control sections treated with RNase or a sense probe continued to bind TGFβ1 antisense mRNA. This false positive binding is illustrated in fig 5.

Discussion

Production of cytokines by Reed–Sternberg cells and mononuclear Hodgkin's cells has been

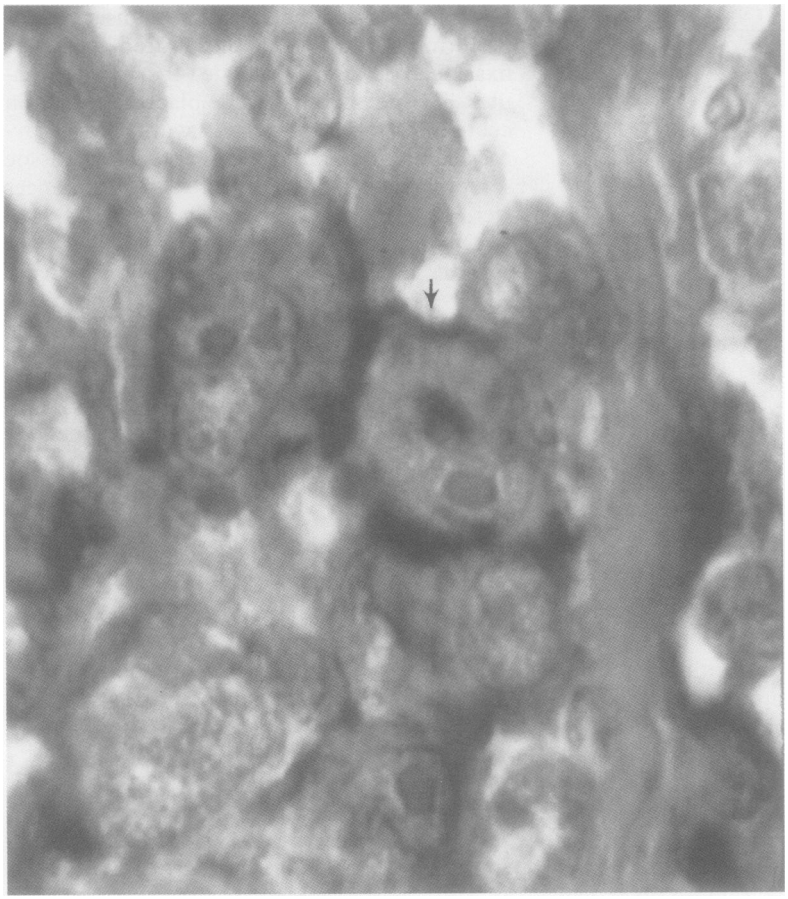

Figure 4 In situ hybridisation for TGFβ1 mRNA in NSHD. All of the classic Reed–Sternberg cells were negative. Many mononuclear Hodgkin's cells were positive. This field shows a mononuclear Hodgkin's cell (arrow) expressing mRNA for TGFβ1 adjacent to a collagen band (× 1000).

described by many investigators. Tumour necrosis factor, colony stimulating factors (CSFs) (interleukin-3 (IL-3) and granulocyte-macrophage CSF (GM-CSF)), interferon-γ, IL-4, IL-5, IL-6, IL-8, IL-9, TGFβ, macrophage CSF, and leukaemia inhibitory factor have all been described as products of Hodgkin's/Reed–Sternberg cells in vitro.[1–11 17 18] Evidence presented here, and by others, suggests that the small Ki1 positive mononuclear Hodgkin's cells are the primary source of biologically active cytokines and that the classic multinucleated Reed–Sternberg cell is a non-dividing cell with low concentrations of newly synthesised mRNA.[12 19] Some Reed–Sternberg cells contained TGFβ1 mRNA.

The polycellular population of a Hodgkin's disease lymph node contains many cells, either recruited directly by cytokines from Hodgkin's/Reed–Sternberg cells, or recruited by secondary events. Activated lymphocytes responding to IL-3, interferon-γ, IL-4, and IL-6 are potential sources of activated TGFβ.[20] The normal lymphocyte activation process takes place over 24 hours, firstly by TGFβ mRNA expression, then TGFβ receptor expression, and lastly by secretion of TGFβ.[20] The current study suggests that these events take place in vivo in Hodgkin's tissue.

Eosinophils are recruited into Hodgkin's mixed cellularity lymph nodes, at least partially, by the secretion of IL-5 by Hodgkin's/Reed–Sternberg cells.[7] The sparse eosinophils within the NSHD lymph node could be a third source of TGFβ,[15] in addition to Hodgkin's/Reed–Sternberg cells and lymphocytes. The nature of eosinophil TGFβ, active or inactive, has not been evaluated and in situ hybridisation studies using the intact 1050 base pair cDNA[16] could be misleading because of the inability of this large probe to hybridise with all sites. The findings reported here suggest that eosinophils produce false positive binding of TGFβ1 mRNA.

In summary, in situ hybridisation using a biotin labelled oligonucleotide for TGFβ1 shows that mononuclear Hodgkin's cells and some Reed–Sternberg cells are a source of TGFβ, particularly in NSHD. The biological actions of TGFβ1 are well described and the ability of activated lymphocytes[20] and cloned Hodgkin's/Reed–Sternberg cells[1 12 17] to secrete physiologically active TGFβ has been shown in vitro. In vivo, elimination of urinary TGFβ has been shown after induction of remission in NSHD.[14]

The observations reported here lend additional support to the hypothesis that Hodgkin's disease arises as a result of transformation of an activated cell that, in turn, induces many of the morphological and clinical features of Hodgkin's disease via the elaboration of potent cytokines.

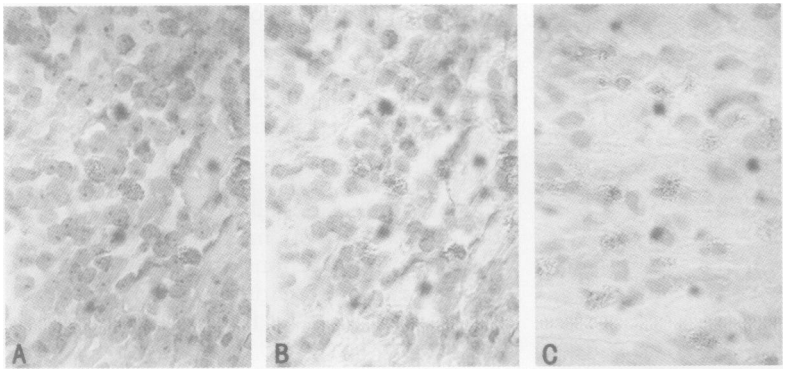

Figure 5 In situ hybridisation for TGFβ1 mRNA in NSHD. A cluster of eosinophils can be seen. Panel A was hybridised with TGFβ1 antisense probe. Panel B was hybridised with TGFβ1 sense probe. Panel C was pretreated with RNase before hybridisation with the antisense probe. All panels show eosinophils and suggest false positive staining for TGFβ1 mRNA (× 630).

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