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Rapid Communication

Immunohistochemical Evidence of a Role for Transforming Growth Factor Beta in the Pathogenesis of Nodular Sclerosing Hodgkin's Disease

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Transforming growth factor beta (TFG-β) is a multifunctional growth factor that promotes the growth of fibroblasts, collagen synthesis and angiogenesis, and stimulates monocyte migration and activation, but suppresses the growth and differentiation of immune lymphocytes and killer cells. Previously we demonstrated biologic activity for TGF-β in supernatants of fresh Hodgkin's disease (HD) cell cultures and the cell line L428 derived from nodular sclerosing HD. This study was undertaken to find evidence of TGF-β activity directly in tissues affected by HD. Formalin-fixed tissue from 14 patients with HD, including 8 nodular sclerosis, 4 mixed cellularity, 1 lymphocyte predominance, and 1 lymphocyte depletion type were studied by immunoperoxidase technique with antibody CC(1-30) raised against a synthetic polypeptide with the same N-terminal amino acid sequence as TGF-β1. Transforming growth factor beta activity was demonstrated in six cases of nodular sclerosis but not in other histologic types of HD. Staining for TGF-β was found in the cytoplasm of Reed–Sternberg (RS) cells in one case and on the surface of RS cells and their lacunar variants in five cases. Transforming growth factor beta activity associated with the extracellular matrix was localized mainly around blood vessels, zones of necrosis, at the margins of bands of collagen sclerosis, and in areas containing syncytia of RS cells. In two cases TGF-β was associated with collections of epithelioid histiocytes or granulomas. Small lymphocytes, granulocytes, and germinal center cells were unreactive. These results suggest that TGF-β is a growth factor of biologic importance in HD and may be responsible for many of the histologic features, such as nodular sclerosis and granulomas, that may have prognostic significance. (Am J Pathol 136:1209–1214)

Transforming growth factor beta (TGF-β) is a potent multifunctional mediator that suppresses the proliferation of T and B lymphocytes,[1,2] the cytolytic activity and interferon responsiveness of natural killer cells,[3] and the proliferation and differentiation of precursors to killer cells.[4] Transforming growth factor beta also stimulates monocyte migration and activation,[5] fibroblast proliferation, collagen synthesis, and angiogenesis,[6,7] both *in vitro* and *in vivo*. Thus TGF-β could mediate, in part, the clinical immunodeficiency and histologic manifestations of Hodgkin's disease,[8] particularly the nodular sclerosing subtype.

We have shown recently that short-term, partially purified Reed–Sternberg cell cultures from nodular sclerosis secrete a potent growth factor for fibroblasts into the serum-free medium,[9] that the growth factor does not have the biologic properties of IL-1,[10] and that it produces transformationlike growth of fibroblasts in monolayer and soft agar.[9,10] Further study of this growth factor from a Hodg-

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Table 1. *Correlation of TGF-B Expression with Clinical Features, Histology, and Immunophenotype of Reed–Sternberg Cells in 14 Patients with Hodgkin's Disease*

Case	Age	Sex	Histology	Comments	RS-cell phenotype	Stage	TGF-B
1	27	M	NS	Interfollicular syncytial variant with necrotizing granulomas	T	IIB	+
2	39	M	NS		T	IAE	+
3	27	F	NS		non T, non B	IIIA	+
4	59	F	NS	Interfollicular pattern with epitheloid histiocytes; microscopic involvement of spleen	B	III	+
5	32	M	NS	Syncytial variant	non T, non B	IIIB	+
6	37	M	NS	Cellular phase with minimal sclerosis	non T, non B	IA	+
7	34	F	NS		non T, non B	IB	−
8	39	M	NS		non T, non B	IA	−
9	52	M	MC		B	IIIA	−
10	77	M	MC		non T, non B	I	−
11	29	M	MC		T	IIIB	−
12	18	F	MC		T	IIIA	−
13	52	F	LP		B	IA	−
14	24	F	LD		non T, non B	IIIB	−

M, male; F, female; NS, nodular sclerosis; LP, lymphocyte predominant; LD, lymphocyte depleted; MC, mixed cellularity

kin's disease cell line (L428) indicated that it is a unique form of high molecular weight TGF-β active at physiologic pH and not requiring proteases for activation.[11] To determine whether TGF-β is synthesized by fresh noncultured Reed–Sternberg cells *in vivo* and to learn what role TGF-β might play in the pathogenesis of Hodgkin's disease, we report on immunohistochemical studies of TGF-β activity directly in tissues affected by Hodgkin's disease.

Materials and Methods

Lymph node biopsies from 14 patients with Hodgkin's disease were studied. Tissues from all cases were fixed in 10% neutral buffered formalin because previous studies showed that immunoreactivity for TGF-β was best preserved with this fixative.[12]

Transforming growth factor beta activity was detected immunohistochemically with a rabbit polyclonal IgG antibody anti-CC(1-30) raised against a synthetic polypeptide corresponding to the first 30 amino acids of mature TGF-β1, as described by Ellingsworth et al.[12] The CC(1-30) antibody reacts mainly with the secreted, presumably active form of TGF-β1.[13] In preliminary studies, we determined that optimal staining for TGF-β was accomplished by overnight incubation of the anti-CC(1-30) antibody at 4°C with tissue sections adhered to glass slides. The reaction product was detected with a goat anti-rabbit IgG antibody followed by an avidin-biotin-peroxidase complex (ABC) (Vector laboratories, Burlingame, CA). The color reaction was developed with 3, 3-diaminobenzidine tetrathydrochloride and counterstained with methyl green. Before incubation with the anti-CC(1-30) antibody, sections were deparaffinized in xylene and rehydrated in alcohols. Endogenous peroxidase was blocked with 0.3% hydrogen peroxide in methanol. Nonspecific protein binding was blocked by incubation with 5% goat serum in phosphate-buffered saline for 20 minutes. Staining was abolished when the anti-CC(1-30) antibody was preincubated with TGF-β1 bound to a sepharose column or when normal rabbit IgG was substituted for the primary antibody.[13] Positive controls included lymphoma cell lines known to produce biologically active TGF-β.[14]

In each case corresponding hematoxylin and eosin-stained sections were used to determine the histologic subtype of Hodgkin's disease according to the Rye classification.[15] All cases were fully characterized for expression of T-cell, B-cell and Hodgkin's disease associated antigens, as previously described.[16,17]

Results

Table 1 correlates the expression of TGF-β *in situ* with clinical features, histology, and immunophenotypes of Reed–Sternberg cells in each of 14 cases of Hodgkin's disease. The 14 cases of Hodgkin's disease included 8 cases of nodular sclerosis, 4 cases of mixed cellularity, 1 lymphocyte predominance, and 1 lymphocyte depletion type. In all cases, the Reed–Sternberg cells were appropriately positive for Ki-1(CD30) and/or Leu M1(CD15) antigens. In four cases the Reed–Sternberg cells had a T-cell phenotype, in 3 cases, a B-cell phenotype, and in 7 cases neither B- nor T-cell antigens were detected. T- or B-cell

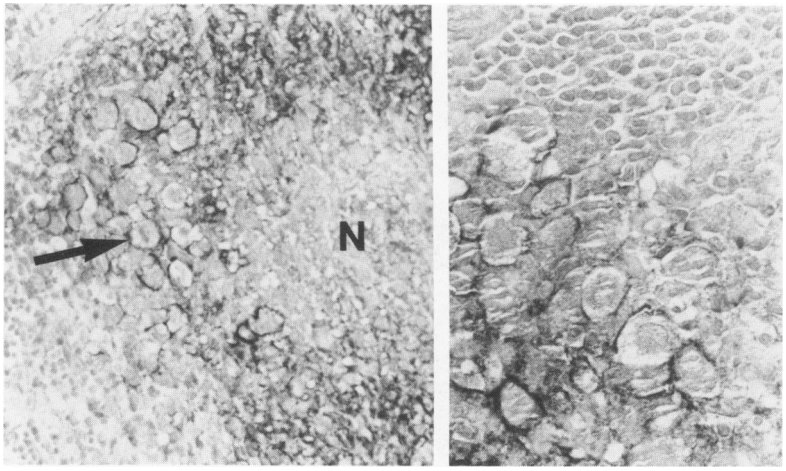

Figure 1. *Composite photograph. The left side shows immunoperoxidase localization of TGF-β to the surface of Reed–Sternberg cells (arrow) and interstitium around zone of necrosis (N) in nodular sclerosing Hodgkin's disease (×180). The right side shows dark surface staining of Reed–Sternberg cells in the lower portion and unstained small lymphocytes above (×400).*

antigens were observed on Reed–Sternberg cells in 3 of 8 cases of nodular sclerosis, and 3 of 4 cases of mixed cellularity Hodgkin's disease. B-cell antigens were found in the one case of lymphocyte-predominance type. These results are consistent with recent immunophenotyping studies of Reed–Sternberg cells in Hodgkin's disease.[16–20]

A subpopulation, usually a minority of Reed–Sternberg cells and their lacunar variants, were positively stained for TGF-β in 6 of the 14 HD cases. All positive cases were of the nodular sclerosing type. Staining was localized predominantly to the cell surface in five cases (Figures 1 and 2). A perinuclear and cytoplasmic staining pattern was observed in one case (Figure 3). In all positive cases an interstitial pattern of staining in and around Reed–Sternberg cells was also observed. Interestingly, TGF-β staining appeared localized to regions of lymph nodes in the positive cases. Positive staining reactions were found mainly around zones of necrosis (Figure 1), in areas containing nests or syncytia of Reed–Sternberg cells, and at the margins of bands of collagen sclerosis, presumably where new collagen was being made (Figure 4). In two TGF-β–positive cases, an interfollicular pattern of Hodgkin's disease was observed. In two positive cases, a histiocyte/macrophage response was evident. In one of these cases necrotizing granulomas were noted, and in the other case collections of epithelioid histiocytes were found. Transforming growth factor beta activity was found frequently in the perivascular adventitia, a site of collagen deposition in nodular sclerosis.[21] Small lymphocytes and granulocytes were not stained for TGF-β. Similarly, germinal center cells were unreactive.

Discussion

Transforming growth factor beta is a multifunctional growth factor that affects the growth and differentiation of normal and malignant cells. In general TGF-β has a suppressive effect on the growth, differentiation, and function of cells of the immune system.[1–4] Transforming growth factor beta is also a negative growth factor for most epithelial cells and suppresses growth of cell lines derived from human breast and lung cancer.[22,23] Transforming

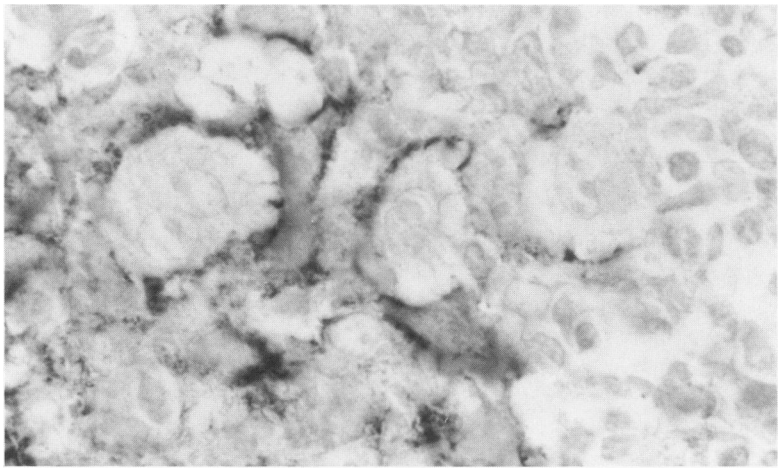

Figure 2. *Higher magnification of TGF-β on the surface of lacunar variants of Reed–Sternberg cell (×1000).*

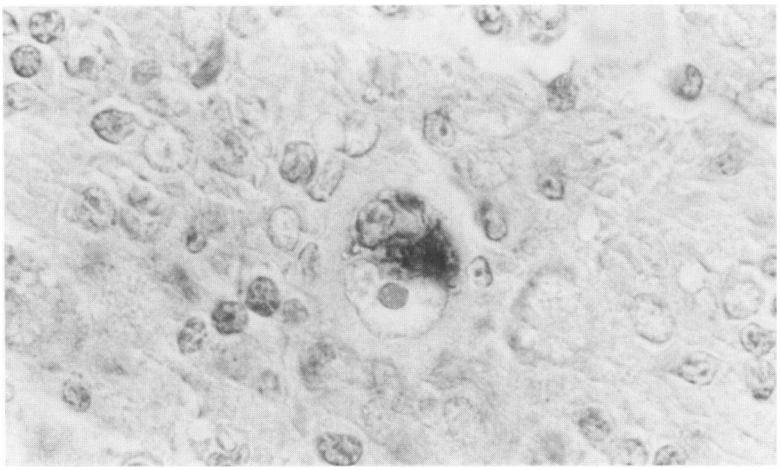

Figure 3. *Localization of TGF-β to the cytoplasm of a Reed–Sternberg cell (×1000).*

growth factor beta stimulates the participation of mesenchymal cells in inflammation and tissue repair. Although TGF-β has been shown to be secreted by many transformed cell lines, including some lymphomas,[14,24] it has not been assayed previously *in situ* in fresh lymphoma tissue.

In this study, immunologically reactive TGF-β was found in most cases of nodular sclerosis but not in other histologic types of Hodgkin's disease, in agreement with our previous observations using short-term cell cultures from lymph nodes involved by Hodgkin's disease, non-Hodgkin's lymphomas, and reactive cells.[10] The presence of TGF-β activity in the perinuclear cistern and cytoplasm of some Reed–Sternberg cells in one case is of particular interest because the CC(1-30) antibody used here usually detects the secreted, presumably active form of TGF-β.[13] These results are consistent with our characterization of a unique TGF-β active at physiologic pH in the L428 cell line derived from nodular sclerosing Hodgkin's disease.[11]

The multifocal distribution of TGF-β activity in lymph nodes, staining Reed–Sternberg cells in some areas but not in others, may be due to a requirement for a minimum concentration of growth factor most often achieved in areas containing nests or syncytia of Reed–Sternberg cells. Variable staining also could be due to inactivation of secreted growth factor,[25,26] and/or to different rates of growth factor synthesis by subclones of Reed–Sternberg cells. We found significant differences in the amount of TGF-β activity produced by Hodgkin's cell subclones *in vitro*.[27]

We suggest that the histologic features of nodular sclerosis may occur, at least in part, in response to the biologic effects of TGF-β. Thus TGF-β immunostaining was concentrated at the margins of broad collagen bands and around blood vessels that appear to be sites of new collagen synthesis.[6,21] Similarly, the chemotactic effect of TGF-β on monocytes could be responsible for the collections of epithelioid histiocytes and formation of granulomas observed in two cases.[5]

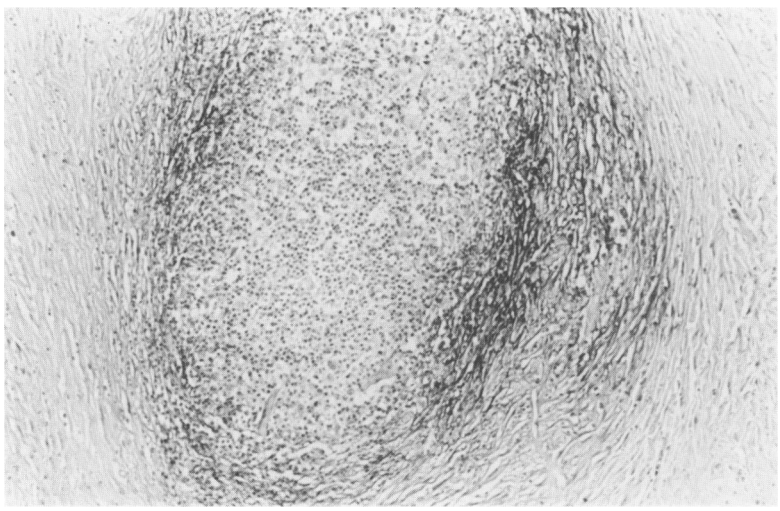

Figure 4. *Transforming growth factor beta staining fibers at the interface between a lymphoid nodule and surrounding bands of collagen (×100).*

Transforming growth factor beta is known to suppress the proliferation of nonmalignant activated lymphocytes.[1-5] In this and previous studies, we and others have shown that some Reed–Sternberg cells express lymphocyte surface markers and are presumably of lymphoid origin. Furthermore, these cells have an activated phenotype that may include expression of receptors for IL-2 (Tac antigen) and transferrin (T9).[28] IL-2 receptors are expressed but down regulated by TGF-β in mitogen-activated lymphoid cells,[1] leading to suppression of IL-2–promoted growth. We have shown that malignant activated lymphocytes (Ki-1 + lymphomas) have a partial or complete loss of response to TGF-β, depending on the availability of TGF-β receptors.[14] The L-428 cell line derived from advanced nodular sclerosing Hodgkin's disease secretes approximately 1 fM TGF-β cell/day but is not inhibited by TGF-β and has no receptors for either TGF-β or IL-2.[14] Thus it would seem that Reed–Sternberg cells, if they are of lymphoid origin, have, to some degree, lost their ability to be regulated by TGF-β, contributing to the malignant behavior of Hodgkin's disease.

The presence of activated TGF-β identified *in situ* on the cell membrane and in the cytoplasm of Reed–Sternberg cells from patients with nodular sclerosing Hodgkin's disease suggests that these Reed–Sternberg cells are secreting activated TGF-β continuously *in vivo*. Because some human malignancies, such as glioblastomas, also produce an active form of TGF-β that directly suppresses the generation of lymphokine activated killer cells *in vitro*,[29] we suggest that the production of TGF-β by Reed–Sternberg cells is responsible not only for some of the histologic findings of nodular sclerosing Hodgkin's disease but perhaps also for some aspects of the immunosuppressive state commonly observed in patients with Hodgkin's disease.[30]

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