American Journal of Pathology, Vol. 142, No. 1, January 1993

Short Communication

Eosinophils are the Major Source of Transforming Growth Factor-β 1 in Nodular Sclerosing Hodgkin's Disease

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Transforming growth factor-β_1 (TGF-β_1) is a multifunctional cytokine which promotes fibroblast growth and collagen synthesis, but suppresses growth and differentiation of immune lymphocytes and killer cells. Immunohistochemical detection of TGF-β_1 in Hodgkin's disease (HD) has been shown to correlate with the histologic feature of nodular sclerosis, which is associated with a favorable prognosis (American Journal of Pathology 1990,136:1209). In that study, TGF-β_1 was localized mainly at the margins of broad collagen bands (presumably sites of new collagen synthesis) and in areas containing numerous Hodgkin/Reed-Sternberg cells (H/RS). In these areas, TGF-β_1 protein was found on the membrane and occasionally within the cytoplasm of H/RS cells. To determine whether TGF-β_1 is synthesized by H/RS cells or secondarily bound to their membrane and sometimes internalized, we performed* in situ *hybridization (ISH) using 1.5 Kb ^{35}S-labeled anti-sense and sense RNA probes to TGF-β_1. Paraffin-embedded tissues of 10 cases from all histologic types of HD were examined. Somewhat unexpectedly, the major site of TGF-β_1 mRNA was in eosinophils; TGF-β_1 mRNA was not detected in H/RS cells. TGF-β_1 mRNA was found in eosinophils in all cases of nodular sclerosis but not in other types of HD, despite the presence of numerous eosinophils in mixed cellularity cases. The presence of TGF-β_1 mRNA coincided with immunohistochemical detection of TGF-β_1 protein using antibody CC (1–30). These results confirm the role of TGF-β_1 in the histogenesis of nodular sclerosing HD and indicate that eosinophils are the major source of TGF-β_1 in this type of HD. (Am J Pathol 1993, 142:11–16)

Transforming growth factors-β (TGF-β) are members of a family of multifunctional polypeptides that regulate cell growth, differentiation, and morphogenesis (reviewed in ref. 1). The prototype of this family is TGF-β_1, a highly conserved 25-kd disulfide-linked homodimer.[2] TGF-β_1 can stimulate or inhibit growth and differentiation of cells of multiple lineages. The net effect of TGF-β_1 action is determined by interaction with other cytokines and the nature of the target cell. TGF-β_1 stimulates fibroblasts to proliferate and synthesize collagen,[3] attracts and activates monocytes,[4,5] and is chemotactic for human neutrophils.[6] Conversely, TGF-β_1 inhibits proliferation and function of T and B lymphocytes,[7,8] and suppresses the growth and lytic activities of cytotoxic T cells,[9] NK cells,[10] and lymphokine-activated killer cells.[11]

These properties of TGF-β_1 could explain some features of tumor histology (collagen sclerosis, granulomas, abscesses) and clinical manifestations (depressed cellular immunity) of HD. The relative amounts of collagen sclerosis, inflammatory cells,

Presented in part at the 81st Annual Meeting of the United States and Canadian Academy of Pathology, Atlanta, Georgia, March 14–20, 1992.

Supported by the following: Dr. Kadin received grant CD-485 from the American Cancer Society, Atlanta, GA, and grant 2630 R2 from the Council for Tobacco Research, USA, Inc., New York, NY; Dr. Wong, grants DE08680 and DE00318 from the National Institutes of Health; and Dr. Elovic, Physician Scientist Award DE00323.

Accepted for publication September 22, 1992.

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and the cytology of malignant Hodgkin/Reed-Sternberg cells (H/RS) define the four major histologic types of HD: lymphocyte predominance, lymphocyte depletion, mixed cellularity, and nodular sclerosis.[12] It has been hypothesized that these various histologic types are the result of cytokines elaborated by the H/RS cells.[13] Consistent with this hypothesis, eosinophilia in HD has been correlated with the expression of IL-5 mRNA by H/RS cells.[14] Moreover, nodular sclerosis has been associated with immunohistochemically detected TGF-β_1.[15] TGF-β_1 protein was concentrated at the margins of broad collagen bands and in areas with numerous H/RS cells. In those areas, TGF-β_1 protein was found on the surface of H/RS cells and only occasionally within their cytoplasm. To determine whether TGF-β_1 is synthesized by H/RS cells or secondarily bound to their membrane and sometimes internalized, we performed *in situ* hybridization with a probe specific for human TGF-β_1 mRNA.[16] Interestingly, TGF-β_1 mRNA was not detected in H/RS cells, but instead was associated with eosinophils which are frequently present in HD. Expression of TGF-β_1 mRNA seems to be restricted to a subpopulation of eosinophils which are associated with collagen formation in nodular sclerosing HD.

Materials and Methods

Tissue Processing

Formalin- and B5-fixed, paraffin-embedded tissues from 10 adult patients with HD were studied. Tissues were routinely processed for histology and stained with hematoxylin and eosin or Giemsa. Six cases were subclassified as nodular sclerosing, two as mixed cellularity, one as lymphocyte depletion, reticular subtype (with numerous H/RS), and one as lymphocyte predominance, nodular subtype.

Immunohistochemistry

Tissues were stained with a rabbit polyclonal IgG antibody, anti-CC(1–30), (L. Ellingsworth, Collagen Corp., East Palo Alto, CA), which detects principally extracellular TGF-β_1.[17] Specificity of antibody CC (1–30) was confirmed by absorption with human TGF-β_1 bound to a Sepharose column and by substitution of nonimmune rabbit antibody for the specific antibody.[15,17] Endogenous peroxidase was blocked with 0.3% hydrogen peroxide in methanol (20 minutes). Other details of the immunoperoxidase staining method have been described.[15]

Molecular Probes

For *in situ* hybridization, the human TGF-β_1 (hTGF-β_1) cDNA was a 1.5 kb EcoR1-PstI fragment (G.I. Bell of Chiron Corp., Emeryville, CA). The hTGF-β_1 cDNA was recloned into the plasmid pBSII-SK(−) (Stratagene, La Jolla, CA) such that the sense and anti-sense riboprobes were produced by either T3 or T7 RNA polymerases respectively.

In Situ *Hybridization*

Details of our *in situ* hybridization procedure using ^{35}S-labeled anti-sense and sense hTGF-β_1 riboprobes for the specific detection of TGF-β_1 mRNA in human eosinophils were described previously.[16] We used hybridization conditions that eliminate non-specific annealing of the riboprobes to eosinophils, as previously described.[18–21]

Results

In situ hybridization localized TGF-β_1 mRNA to eosinophils in each of six cases of nodular sclerosing HD. No TGF-β_1 mRNA was detected in other histologic types of HD despite the presence of numerous eosinophils in the mixed cellularity type. Identification of eosinophils as the cells expressing TGF-β_1 mRNA was done by the combined use of bright field microscopy on Giemsa-stained sections and dark field/fluorescence microscopy using appropriate filters. A typical result is shown in Figure 1. Eosinophils expressing TGF-β_1 mRNA were most often concentrated at the periphery of lymphoid nodules containing H/RS cells. Fewer eosinophils expressing TGF-β_1 mRNA were found within these lymphoid nodules. A variable number of eosinophils from each case did not contain TGF-β_1 mRNA.

Eosinophils containing TGF-β_1 mRNA usually were associated with collagen fibers and TGF-β_1 detected immunohistochemically with antibody CC (1–30) (Figure 2A). H/RS cells were not labeled for TGF-β_1 mRNA (Figure 2B). H/RS cells were identified as cells with abundant cytoplasm, large lobated or multiple nuclei, and prominent nucleoli occurring within lymphoid nodules. H/RS cells occasionally were surrounded by eosinophils containing TGF-β_1 mRNA.

Discussion

This study demonstrates that eosinophils are the principal source of TGF-β_1 in nodular sclerosing HD.

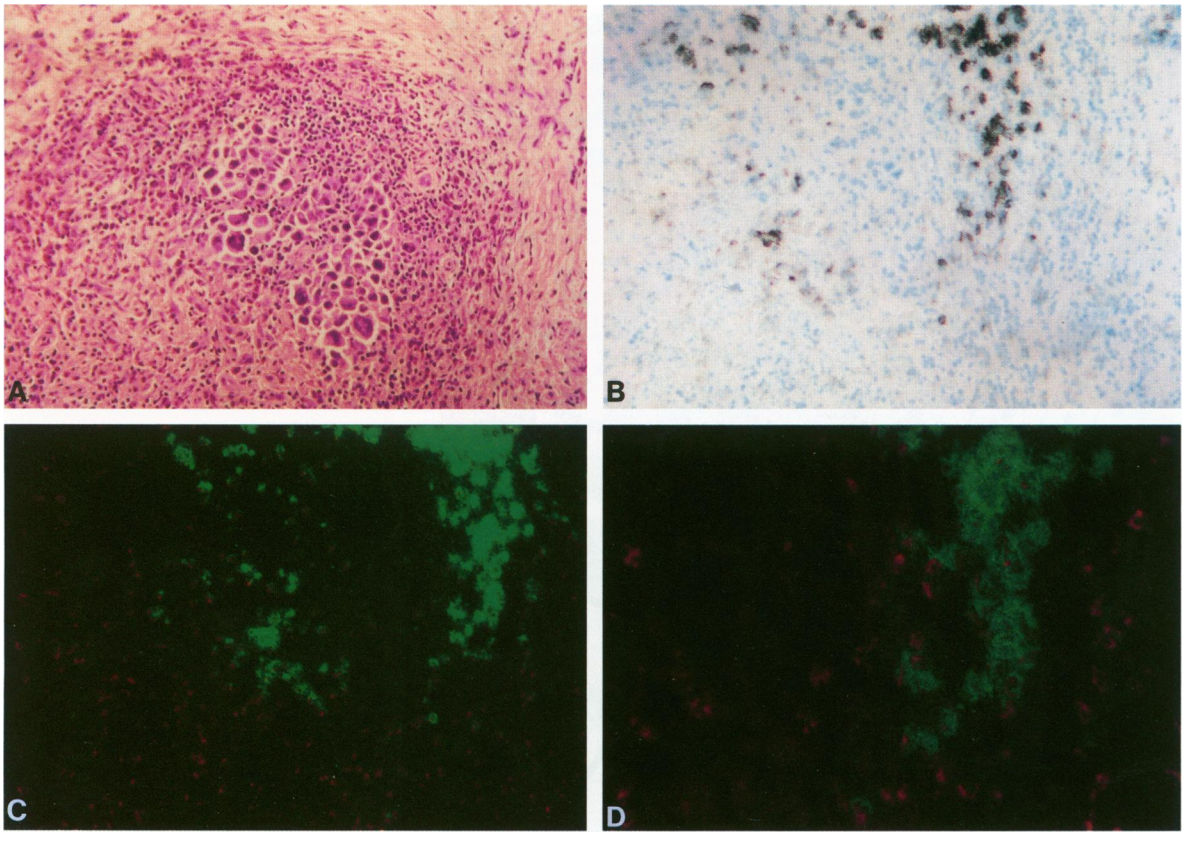

Figure 1. In situ *hybridization detecting TGF-β_1 mRNA with a ^{35}S-labeled hTGF-β_1 anti-sense riboprobe in a typical case of nodular sclerosing HD.* **A:** *Bright field visualization. Note the lymphoid nodule in the center of the field with eosinophils at the periphery and a number of recognizable H/RS giant cells at the center (×240).* **B:** In situ *hybridization labeling for TGF-β_1 mRNA with ^{35}S-labeled human TGF-β_1 anti-sense riboprobe (×120).* **C:** *Composite exposure of the same field as in* (**B**); *rhodamine fluorescence visualization to highlight the eosinophils as brightly red fluorescent cells, followed by dark field visualization to highlight the TGF-β_1 autoradiographic grains (green). Note that the eosinophils in the lower left field do not contain detectable TGF-β_1 mRNA (×120).* **D:** *High magnification (×240) of an area in* **C** *to demonstrate that eosinophils are the cells which contain TGF-β_1 mRNA detected by* in situ *hybridization. Double exposure same as* **C**.

Eosinophils were the only cells found to contain readily detectable TGF-β_1 mRNA. In each case, however, a subpopulation of eosinophils did not contain TGF-β_1 mRNA. This may be explained by intermittent synthesis of this cell product or a functional separation of eosinophil compartments with respect to cytokine synthesis.

Interestingly, no TGF-β_1 mRNA was detected in eosinophils of mixed cellularity HD in which sclerosis was not a prominent feature. This suggests that some difference in microenvironmental stimuli may account for the elaboration of TGF-β_1 in nodular sclerosis as opposed to the mixed cellularity type of HD. This stimulus is likely to be some factor which activates eosinophils in specific pathological conditions characterized by tissue fibrosis because eosinophils from patients with hypereosinophilic syndrome[16] and chronically inflamed upper airway tissues[22] were found to express TGF-β_1 mRNA and protein, whereas normal eosinophils do not.

The mechanism by which some eosinophils in HD are activated to produce TGF-β_1 remains to be determined. It is possible that eosinophils are activated by other cytokines to synthesize TGF-β_1 mRNA. Messenger RNA for Interleukin-5, a selective activator of eosinophils,[23] has been demonstrated in H/RS cells of HD patients with eosinophilia.[14] Eosinophil density also has been correlated with T-cell activation in lymph nodes of patients with HD.[24] Activated T cells secrete IL-2[25] and IL-5[26] to which eosinophils can respond. Thus, it will be important to determine whether IL-2, IL-5, and perhaps other cytokines can activate eosinophils to synthesize TGF-β_1 in pathologic conditions with fibrosis, such as nodular sclerosing HD.

Other eosinophil products have been demonstrated to be expressed selectively in lymph nodes of nodular sclerosing HD.[27,28] Lymph nodes with nodular sclerosing histology showed the heaviest infiltration with eosinophils and the most pronounced extra-

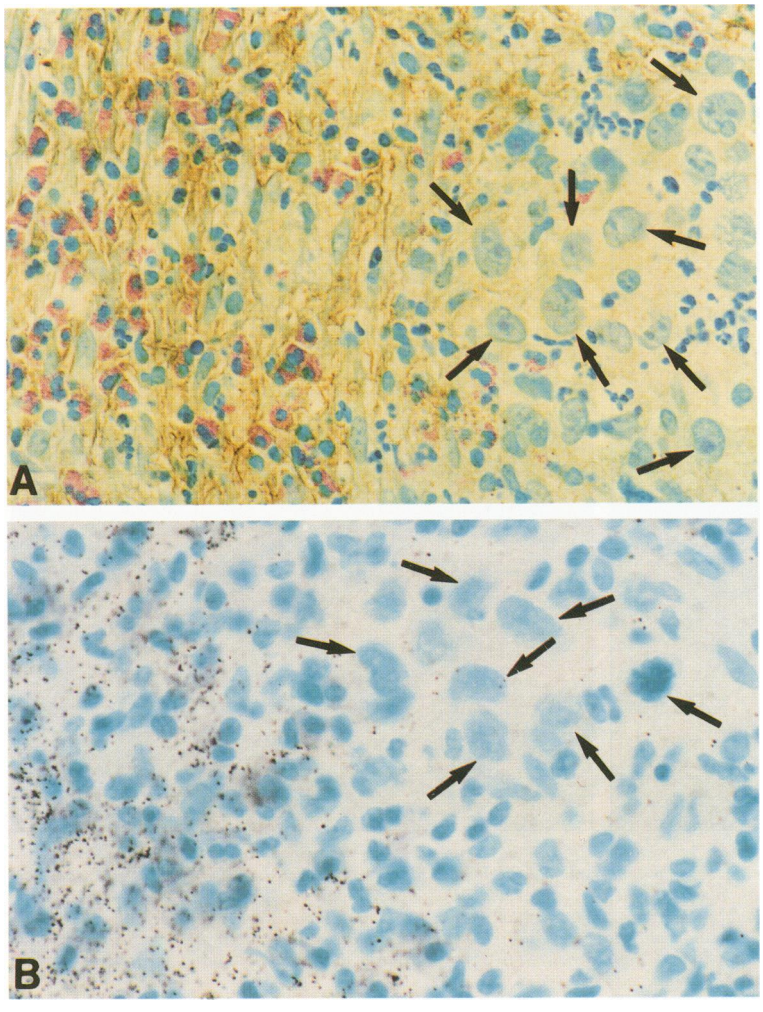

Figure 2. *Localization of TGF-β_1 to eosinophils.* **A:** *Immunoperoxidase with antibody CC$^{1-30}$ and Giemsa counterstain localizing TGF-β_1 protein to collagen fibers around eosinophils at margin of collagen band surrounding H/RS-cells (arrows).* **B:** *H/RS cells do not show hybridization for TGF-β_1 mRNA (×470).*

cellular deposits of eosinophil major basic protein.[27] Extracellular eosinophil peroxidase detected with a monoclonal antibody was distributed in a dendritic pattern in nodular sclerosis, suggesting that extensive eosinophil degranulation in HD may produce tissue damage that results in collagen bands and sclerosis.[28] The presence of these eosinophil products, together with TGF-β_1 mRNA, indicates a primary role for the eosinophil in the histogenesis of nodular sclerosing HD.

In none of these HD cases was TGF-β_1 mRNA detected in H/RS cells. This result was somewhat unexpected since the HD cell line L428 derived from H/RS cells was found to produce biological activity for TGF-β_1.[29] However, further analysis revealed that L428 cells in culture produce an unusually high molecular weight (350 kd) form of TGF-β which is active at physiological pH.[29] TGF-β_1 normally occurs as a latent low molecular weight (25 kd) peptide bound to α_2-macroglobulin.[30] TGF-β synthesized by L428 cells may not be recognized by the probe for TGF-β_1 used in our study.

Nodular sclerosis is associated with a relatively good prognosis compared with mixed cellularity and lymphocyte depletion HD.[31] The mechanism of nodular sclerosis is not well understood. These results suggest that nodular sclerosis may be due to the synthesis and secretion of TGF-β_1 by eosinophils in some patients with HD. However, these results do not readily explain the relatively lower frequency of nodular sclerosis in underdeveloped countries[32] or in patients with AIDS.[33] Perhaps a primary defect of eosinophils or a deficiency of the interleukins necessary to activate eosinophils to produce TGF-β_1 contributes to the altered histology of HD in these patients.

The favorable prognosis of nodular sclerosis may be associated with the formation of collagen bands surrounding the malignant H/RS cells. A favorable prognosis also has been found for non-Hodgkin's

lymphomas with fibrosis.[34] The findings of this study suggest another possible explanation for the favorable prognosis of nodular sclerosis. It seems that the TGF-β_1 found on the surface and sometimes within the cytoplasm of H/RS cells is not a product of those cells but instead is made by eosinophils and secondarily bound to the membrane of H/RS cells. Since H/RS cells seem to be derived from activated lymphocytes,[13] and TGF-β_1 has an antiproliferative effect on activated lymphocytes,[7,8] this cytokine may have an antiproliferative effect on H/RS cells in nodular sclerosing HD.

References

1. Massague J: The TGF-β family of growth and differentiation factors. Cell 1987, 49:437–438
2. Derynck R, Jarrett JA, Chen EY, Eaton DH, Bell JM, Assoian RK, Roberts AB, Sporn MB, Goeddel DV: Human transforming growth factor-beta complementary DNA sequence and expression in normal and transformed cells. Nature 1985, 316:701–705
3. Roberts AB, Sporn MB, Assoian RK, Smith JM, Roche NS, Wakefield LM, Heine UI, Liotta LA, Falanga V, Kerhl JH, Fauci AS: Transforming growth factor type beta: Rapid induction of fibrosis and angiogenesis *in vivo* and stimulation of collagen formation *in vitro*. Proc Natl Acad Sci USA 1986, 83:4167–4171
4. Wahl SM, Hunt DA, Wakefield LM, McCartney-Francis N, Wahl LM, Roberts AB, Sporn MB: Transforming growth factor-beta induces monocyte chemotaxis and growth factor production. Proc Natl Acad Sci USA 1987, 84:5788–5792
5. Welch GR, Wong HL, Wahl SM: Selective induction of Fc RIII on human monocytes by transforming growth factor-β. J Immunol 1990, 144:3444
6. Reibman J, Meixler S, Lee TC, Gold LI, Cronstein BC, Haines KA, Kolasinski SL, Weissmann G: Transforming growth factor β1, a potent chemoattractant for human neutrophils, bypasses classic signal-transduction pathways. Proc Natl Acad Sci USA 1991, 88:6805–6809
7. Kehrl JM, Wakefield LM, Roberts AB, Jakowlew S, Alvarez-Mon M, Derynck R, Sporn MB, Fauci AS: Production of transforming growth factor beta by human T lymphocytes and its potential role in the regulation of T cell growth. J Exp Med 1986, 163:1037–1050
8. Kehrl JM, Roberts AB, Wakefield L, Jakowlew S, Sporn MB, Fauci AS: Transforming growth factor beta is an important immunomodulating protein for human B lymphocytes. J Exp Med 1986, 137:3855–3860
9. Ranges GE, Figari IS, Espevik T, Palladino Jr, MA: Inhibition of cytotoxic T cell development by transforming growth factor β and reversal by recombinant tumor necrosis factor alpha. J Exp Med 1987, 166:991
10. Rook AB, Kehrl JH, Wakefield LM, Roberts AB, Sporn MB, Burlington DB, Lane HC, Fauci AS: Effects of transforming growth factor β on the functions of natural killer cells: depressed cytolytic activity and blunting to interferon responsiveness. J Immunol 1986, 136:3916–3920
11. Kasid A, Bell GI, Director EP: Effect of transforming growth factor-β on human lymphokine-activated killer cell precursors. Autocrine inhibition of cellular proliferation and differentiation to immune killer cells. J Immunol 1988, 141:690–697
12. Lukes RJ, Butler JJ, Hicks EB: Natural history of Hodgkin's disease as related to its pathologic picture. Cancer 1966, 19:317–344
13. Stein H, Mason DY, Gerdes J, O'Cononor N, Wainscoat J, Pallesen G, Gatter K, Falini B, Delsol G, Lemke H, Schwarting R, Lennert K: The expression of the Hodgkin's disease associated antigen Ki-1 in reactive and neoplastic lymphoid tissue: evidence that Reed-Sternberg cells and histiocytic malignancies are derived from activated lymphoid cells. Blood 1985, 66:848–858
14. Smoszuk M, Nansen L: Detection of interleukin-5 messenger RNA in Reed-Sternberg cells of Hodgkin's disease with eosinophilia. Blood 1990, 75:13–16
15. Kadin ME, Agnarsson BA, Ellingsworth LR, Newcom SR: Immunohistochemical evidence of a role for transforming growth factor beta in the pathogenesis of nodular sclerosing Hodgkin's disease. Am J Pathol 1990, 136:1209–1214
16. Wong DTW, Elovic A, Matossian K, Nagura N, McBride J, Chou HY, Gordon JR, Rand TH, Galli SJ, Weller PF: Eosinophils from patients with blood eosinophilia express transforming growth factor β1. Blood 1991, 78:2702–2707
17. Flanders KC, Thompson NL, Cissel DS, Obberghen-Schilling E, Baker CC, Kass ME, Ellingsworth LR, Roberts AB, Sporn MB: Transforming growth factor β1: Histochemical localization with antibodies to different epitopes. J Cell Biol 1989, 108:653–660
18. Fox CH, Kotler D, Tierney A, Wilson CS, Fauci AS: Detection of HIV-1 RNA in the lamina propria of patients with AIDS and gastrointestinal disease. J Infect Dis 1989, 159:467
19. Patterson S, Gross J, Webster ADB: DNA probes bind nonspecifically to eosinophils during *in situ* hybridization: carbol chromotrope blocks binding to eosinophils but does not inhibit hybridization to specific nucleotide sequences. J Virol Methods 1989, 23:105
20. Wong DT, Weller PF, Galli SJ, Elovic A, Rand TH, Gallagher GT, Chiang T, Chou MY, Matossian K, McBride J, Todd R: Human eosinophils express transforming growth factor-alpha. J Exp Med 1990, 172:673–681
21. Elovic A, Galli SJ, Weller PF, Chang ALC, Chiang T, Chou MY, Donoff RB, Gallagher GT, Matossian K, McBride J, Tsai M, Todd R, Wong DTW: Production of transforming growth factor-alpha by hamster eosinophils. Am J Pathol 1990, 137:1425–1434
22. Ohno I, Lea RG, Flanders KC, Clark DA, Banwatt D, Dolovich J, Denburg J: Eosinophils in chronically

inflamed human upper airway tissues express transforming growth factor β1 Gene (TGFβ1). J Clin Invest 1992, 89:1662–1668
23. Lopez AF, Sanderson CJ, Gamble JR, Campbell HD, Young IG, Vadas MA: Recombinant human interleukin-5 is a selective activator of human eosinophil function. J Exp Med 1988, 167:219–224
24. Ben-Ezra J, Sheibani K, Swartz W, Stroup R, Traweek ST, Kezirian J, Rappaport H: Relationship between eosinophil density and T-cell activation markers in lymph nodes of patients with Hodgkin's disease. Hum Pathol 1989, 20:1181–1185
25. Rand TH, Silberstein DS, Kornfeld H, Weller PF: Human eosinophils express functional interleukin-2 receptors. J Clin Invest 1991, 88:825–832
26. Enokifara H, Furusawa S, Nakakubo H, Kajitani H, Nagashima S, Saito K, Shishido H, Hitosi Y, Takatsu K, Noma T, Shimuzu A, Honjo T: T cells from eosinophilic patients produce interleukin-2 stimulation. Blood 73:1809–1813
27. Butterfield JH, Hephart GM, Banks PM, Gleich GJ: 1986. Extracellular deposition of eosinophil granule major basic protein in lymph nodes of patients with Hodgkin's disease. Blood 1971, 68:1250–1256
28. Samoszuk M, Sholly S, Epstein A: Eosinophil peroxidase is detectable with a monoclonal antibody in collagen bands of nodular sclerosis Hodgkin's disease. Lab Invest 1987, 56:394–400
29. Newcom SR, Kadin ME, Ansari AA, Diehl V: The L-428 nodular sclerosing Hodgkin's cell secretes a unique TGF-β active at physiologic pH. J Clin Invest 1988, 82:1915–1921
30. Danielpour D, Sporn MB: Differential inhibition of TGF-β_1 and β2 activity by alpha 2-macroglobulin. J Biol Chem 1990, 265:6973–6977
31. Keller AR, Kaplan HS, Lukes RJ, Rappaport H: Correlation of histopathology with other prognostic indicators in Hodgkin's disease. Cancer 1968, 22:487–499
32. Burn C, Davies JNP, Dodge OG, Nias BC: Hodgkin's disease in English and African children. JNCI 1971, 46:37–41
33. Ree J, Strauchen JA, Khan A, Gold JE, Crowley JP, Zalusky R: Hodgkin's disease in HIV-infected patients. Clinicopathologic studies of 24 cases: Preponderance of mixed cellularity type. Lab Invest 1990, 82 (abst)
34. Rosas-Uribe A, Rappaport H: Malignant lymphoma, histiocytic type with sclerosis (sclerosing reticulum cell sarcoma). Cancer 1972, 29:946–953