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RAPID COMMUNICATION

# *Expression of Prostaglandin H Synthase (Cyclooxygenase) in Hodgkin's Mononuclear and Reed-Sternberg Cells*

## *Functional Resemblance Between H-RS Cells and Histiocytes or Interdigitating Reticulum Cells*

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**The synthesis of prostaglandin, prostacyclin, and thromboxane, which requires the enzyme prostaglandin H (PGH) synthase (cyclooxygenase), is a general property of histiocytes, monocytes, and Langerhans cells. Previously the authors reported the production of prostaglandin $E_2$ in a Hodgkin's cell line, KM-H2, and suggested that these cells therefore have a functional similarity to histiocyte-related cells. The present study confirms that the Hodgkin's neoplastic (Reed-Sternberg, H-RS) cells in tissue are also capable of producing prostaglandins by demonstrating the presence of PGH synthase in these cells in B5-fixed, paraffin-embedded tissue sections. It was found that the H-RS cells from seven of ten patients with Hodgkin's disease were stained variously with anti-PGH synthase antibodies. In normal and reactive lymphoid tissues, anti-PGH synthase staining was restricted to histiocytes, endothelial cells, and interdigitating reticulum cells. Thus, this study provides further evidence for a possible relationship between H-RS cells and histiocytes or interdigitating reticulum cells; this relationship has also been supported by information obtained in extensive immunologic, biochemical, and cell-differentiation studies. The secretion of PGH-synthase products, especially prostaglandin $E_2$, in H-RS cells may play a major role in the regulation of cellular immunity in patients with Hodgkin's disease. (Am J Pathol 1988, 133:5–12)**

ARACHIDONIC ACID (AA) metabolism is active in several types of cells, including macrophages, monocytes, endothelial cells, and platelets. Among these, macrophages (histiocytes) have been recognized as the most active cellular source of prostaglandins and leukotrienes, a property at least partially attributable to the high AA content of the fatty acids in the cell membrane.[1] Stimulation of macrophages by soluble and particulate stimuli induces phospholipase activation, release of AA from fatty acids, and subsequent metabolism of AA by cell-associated cyclooxygenase (PGH synthase) and lipoxygenase.[1] The AA metabolites have many important functions, such as the modulation of cytokine activity and the regulation of cellular immunity.[2–17]

The authors wanted to study AA metabolism in the neoplastic (Reed-Sternberg, H-RS) cells in Hodgkin's disease (HD). This interest arose from the fact that immunosuppression is frequently observed in patients

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with HD even though abundant reactive T cells are present in lympoid tissues affected by HD.[18-20] Furthermore, it had been previously proposed that H-RS cells are probably related to interdigitating reticulum (IR) cells or cells in the histiocyte lineage.[20-29] The IR cells are antigen-presenting cells located in the T cell zone, and their lineage and immunophenotype are similar to those of tissue histiocytes.[20,30,31] Thus, it was suspected that, like histiocytes, both IR cells and their neoplastic counterpart, H-RS cells, may be actively committed to AA metabolism, a process in which several immunoregulatory substances can be generated. Indeed, it has been confirmed that KM-H2 cells (an H-RS cell line) can produce $PGE_2$.[32-34] To document further that the H-RS cells in tissues are involved in AA metabolism, the authors performed an immunohistochemical study in which the presence of PGH synthase in H-RS cells was demonstrated.

## Materials and Methods

### Production of Anti-PGH Synthase Antibody

Purified PGH synthase from ram seminal vesicles was obtained from Oxford Biomedical Research, Inc. (Oxford, MI).[35,36] The purity of this enzyme was greater than 90%, as measured by electrophoresis. To produce anti-PGH synthase, a rabbit was immunized with this enzyme and the antibody purified by affinity column chromatography. Briefly, the rabbit was immunized with 1.5 mg of PGH synthase in an equal volume of complete Freund's adjuvant containing heat-killed *Mycobacterium tuberculosis* H37RV (1 mg/ml). The emulsion (2 ml) was injected subcutaneously into four separate sites, in the back and footpads of the rabbit. Booster injections of the same amount of antigen in incomplete Freund's adjuvant were given in the same way at 3-, 5-, and 7-week intervals after the primary injection.

The antiserum was collected and purified by affinity chromatography. Briefly, PGH synthase was coupled to cyanogen bromide-activated Sepharose CL 4B (2.3 μmol/ml of wet gel). Immunoglobulin from rabbit antiserum was precipitated with ammonium sulfate at 50% saturation and applied to the column at room temperature. The column was washed with BBS (0.1 M borate buffer, 0.15 M NaCl, pH 8.0), and then antibodies were eluted with 6 M guanidine hydrochloride.

The specificity of the antibody was confirmed by gel electrophoresis, Western blotting, and immunostaining. For immunoblots, the purifed PGH synthase (approximately 5 μg per slot) was electrophoresed on 8% SDS polyacrylamide gel and then electroblotted onto BA83 nitrocellulose (0.2 μ thick, Schleicher and Schull, Keene, NH). The filter was blocked with normal goat serum (1:200 dilution) and 3% nonfat dry milk (prepared in TS buffer; 20 mM Tris-HCl, pH 7.6, 0.15 N NaCl, 0.1 mM PMSF) for 30 minutes and then reacted sequentially with rabbit anti-PGH synthase (3 hours), biotin-labeled goat anti-rabbit Ig (30 minutes), and avidin-biotin-peroxidase complex (ABC) (30 minutes).[23,37] The filters were washed extensively with TS and 0.1% Triton X-100 between steps and finally were developed in 4-chloro-1-naphthol (0.6 mg/ml) in TS with 20% methanol and 0.03% $H_2O_2$.

Rabbit anti-ram PGH synthase has been reported to react with PGH synthase from many other species, including humans.[38,39] To document that the anti-PGH synthase prepared in this laboratory also reacts with human enzyme, the authors examined whether the antibody could react with PGH synthase from human endothelial cells. The endothelial cells were cultured as reported previously,[40] and gel electrophoresis and Western blotting were performed as described above.

### Tissues and Anti-PGH Synthase Staining

The avidin-biotin-peroxidase technique was used to examine the expression of PGH synthase in H-RS cells in 10 B5-fixed and paraffin-embedded tissue sections. These included seven sections of nodular sclerosis (NS) and three of the mixed cellularity (MC) form of HD. In addition, five tonsils and five lymph nodes from patients with reactive hyperplasia, sinus histiocytosis, or dermatopathic lymphadentitis were included for evaluation of PGH synthase-containing cells in nonneoplastic tissues. The dermatopathic nodes were included because these tissues contain numerous IR cells. The nature of IR cells is confirmed by their characteristic enzyme and immunohistochemical staining patterns with acid phosphatase and anti-Leu M1, which are distinct from those of histiocytes.[20,21,30]

The paraffin sections were dewaxed and rehydrated routinely. After being washed in Tris-buffered saline (TBS), 0.05 M, pH 7.6, the sections were incubated with rabbit antisheep PGH synthase at a concentration of 1 μg/ml, followed by sequential incubation with biotin-labeled goat anti-rabbit Ig (1:200) and ABC. Each step lasted 30 minutes, with an interval of 5 minutes for washing with TBS. The slides were developed in a DAB-hydrogen peroxide-nickel chloride solution.[41,42]

Control tests for staining specificity were performed by omitting the primary antibody, replacing the pri-

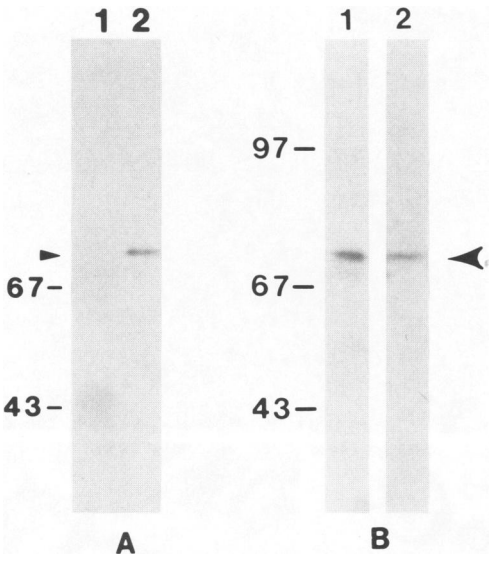


**Figure 1A**—Rabbit antiserum binds to purified sheep PGH synthase, an antigen of 70 kd molecular weight. Lane 1, control markers; lane 2, sheep PGH synthase. **B**—Rabbit antiserum binds to human PGH synthase prepared from endothelial cells (lane 2). In lane 1, sheep PGH synthase was used as control. Staining was carried out on nitrocellulose paper after gel electrophoresis and Western blotting.

mary antibody with nonimmune serum, or absorbing the primary antibody with purified PGH synthase.

### Culture of H-RS Cells

To study the expression of PGH synthase in cultured H-RS cells, the authors stained the cytospin smears as described above. Two H-RS cell lines, HDLM-1 and KM-H2, were grown at $4 \times 10^5$ to $2 \times 10^6$ cells/ml in RPMI 1640 medium (Gibco) supplemented with 10% fetal calf serum, 2 mM glutamine, 50 μg/ml 2-mercaptoethanol, and 50 μg/ml gentamycin at 37 C in a humidified, 5% $CO_2$ atmosphere.[43–45] For detection of PGH synthase, the cells were spun down onto slides, dried, fixed with acetone, and immunostained.

## Results

On gel electrophoresis and Western blotting, the antibody described above reacted specifically with the 70 kd molecular weight PGH synthase. The antibody, although raised against sheep PGH synthase, cross-reacted with human antigen (Figure 1). This was confirmed in studies performed on human endothelial cells in which a single 70 kd protein was precipitated by the same antibody. In tissue staining, the binding of PGH synthase to histiocytes, IR cells, and H-RS cells was inhibited completely because of absorption of antibody by purified antigen. Thus, the results confirm the specificity of this anti-PGH-synthase staining.

In tissue from seven lymph nodes that were involved by HD, approximately 15–40% of the H-RS cells were positive for PGH synthase with various staining intensities (Figure 2). In these tissues, a few normal or reactive histiocytes as well as endothelial cells were also stained. In the other three tissue samples (2 NS and 1 MC HD), the staining of H-RS cells was minimal or undetectable. In these three specimens, there was also an extremely weak reaction in histiocytes and endothelial cells. The staining was the same in mononuclear and binuclear or multinuclear cells, and in the NS and MC types of HD. In the cytospin smears of cultured H-RS cells, PGH synthase was detected in 15–20% of KM-H2 cells, but not in HDLM-1 cells.

In normal tonsils and in reactive lymphoid tissues, the expression of PGH synthase was also highly variable. In the normal tonsils, the PGH-synthase positivity was restricted to free histiocytes in the peritonsillar connective tissue or in the capsule (Figure 3). The fixed histiocytes, including histiocytes in germinal centers, as well as the IR cells in the T cell zone were not stained or stained minimally.

In lymph nodes, the distribution of PGH-synthase-positive cells was similar to that in tonsils, except for the fact that there was a significant increase in PGH-synthase-positive cells in the T cell zone (Figure 4). The positively stained cells had an elongated and irregularly shaped nucleus, as is characteristic for IR cells. The IR-cell nature of these PGH-synthase-positive cells was further suggested by the staining pattern in dermatopathic lymph nodes. In all lymph nodes, only a few (10%) sinus histiocytes were positive for PGH synthase. No staining of T or B lymphocytes, plasma cells, immunoblasts, follicular dendritic cells, or fibroblastic reticulum cells was observed.

## Discussion

PGH synthase was detected in H-RS cells, IR cells, histiocytes, and endothelial cells. The positive staining of the last two types of cells confirms the well-known active production of PGs in these cells. The detection of PGH synthase in IR cells has also been predicted, however, because these cells have functional properties and immunophenotypes similar to those of histiocytes and Langerhans cells.[30,46] Langerhans cells and IR cells are antigen-presenting dendritic cells located in skin and lymphoid tissue, respectively. Although they have not been studied as exten-

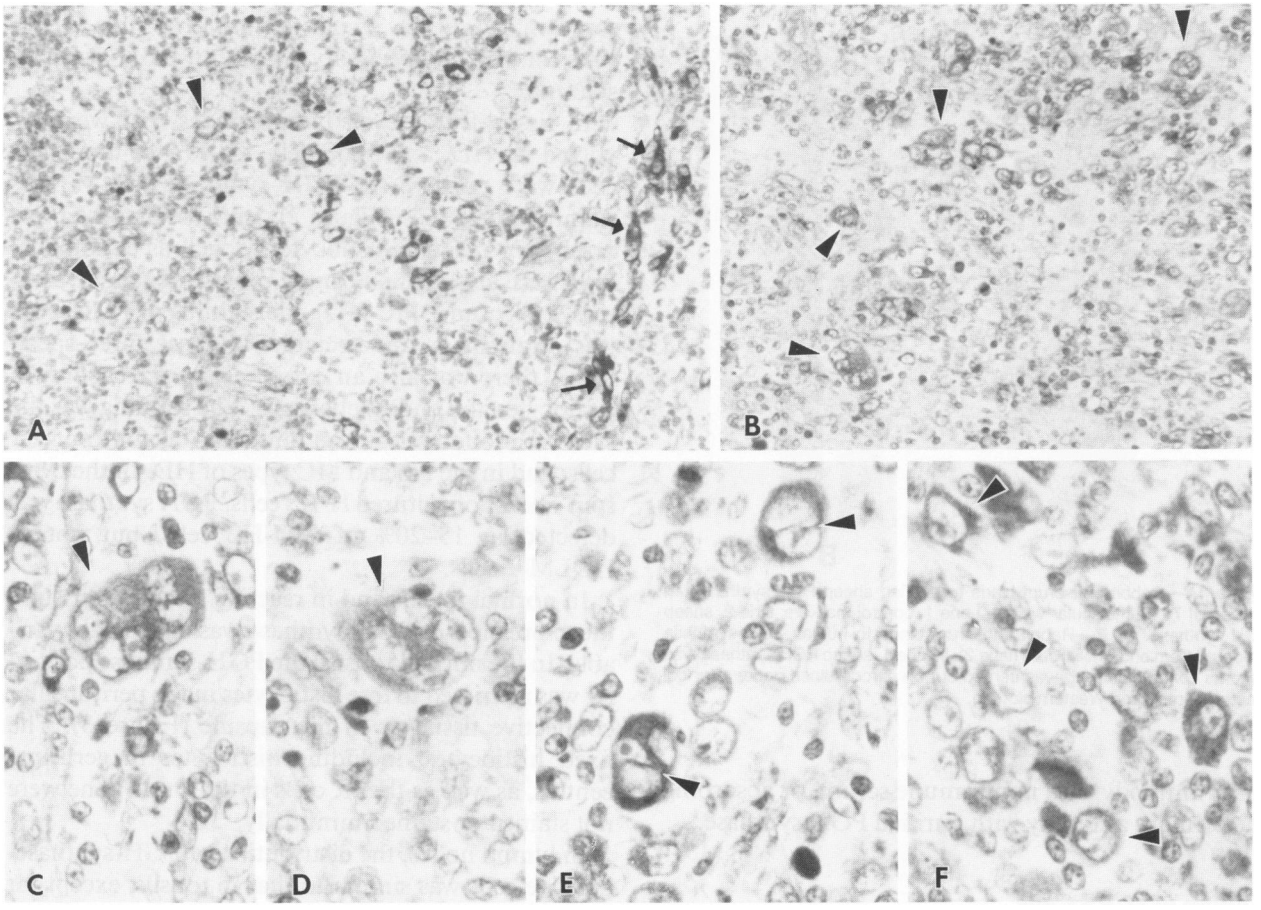


**Figure 2A–F**—PGH synthase distribution in tissue affected by HD. Note the positive staining in H-RS cells (arrowheads in A–F) and in endothelial cells (arrows in A). The staining in H-RS cells was highly variable. C, D, and E are higher magnifications of A and B.

sively as histiocytes, Langerhans cells have been shown to engage in PGH-synthase metabolism. The Langerhans cells of guinea pigs can transform AA, predominantly to $PGD_2$.[47] In humans, histiocytosis X, a Langerhans cell neoplasm, has been reported to produce $PGE_2$.[48] Thus, one can expect an analogy between Langerhans cells and IR cells and their neoplastic counterparts as to their capacity to metabolize AA.

Expression of PGH synthase in lymphoid tissues appears to be restricted to histiocytes or to cells related to them (ie, IR cells), and not to occur in B or T lymphocytes. This was the finding in previous studies on subpopulations of highly purified human lymphocytes (<1% monocytes), in which both T cells and B cells failed to synthesize detectable levels of $PGE_2$.[1] Thus, the expression of PGH synthase and the production of $PGE_2$ by H-RS cells indicate a functional similarity between H-RS cells and IR cells/histiocytes. This proposal for the cell lineage of H-RS cells is also supported by extensive studies of their immunophenotype, their biochemical and functional characteristics (cytokine production), and their pattern of differentiation induced by phorbol ester, extracellular matrix, and retinoic acid.[20–34,49,50]

The presence of PGH synthase in cells enables them to convert AA to $PGE_2$, thromboxane, prostacyclin, or other PG metabolites. The metabolites present in PGH-synthase-positive cells depends on the cell type, the environment from which the cells were obtained, the particular subpopulation of cells under study, and the nature of the stimuli that the cells encounter in their pericellular environment.[1] Numerous studies have illustrated the complexity of AA metabolism in cells. For example, human monocytes can be separated into different subpopulations on the basis of their production of interleukin-1 (IL-1) and $PGE_2$; the density of $PGE_2$-producing monocytes is lower than that of IL-1-producing monocytes.[51] Secretion of the various AA metabolites has been detected in mouse macrophage cell lines, perhaps because these cells had been frozen at certain stages of maturation or differentiation.[52] Such complexity may occur in H-RS cells as well.

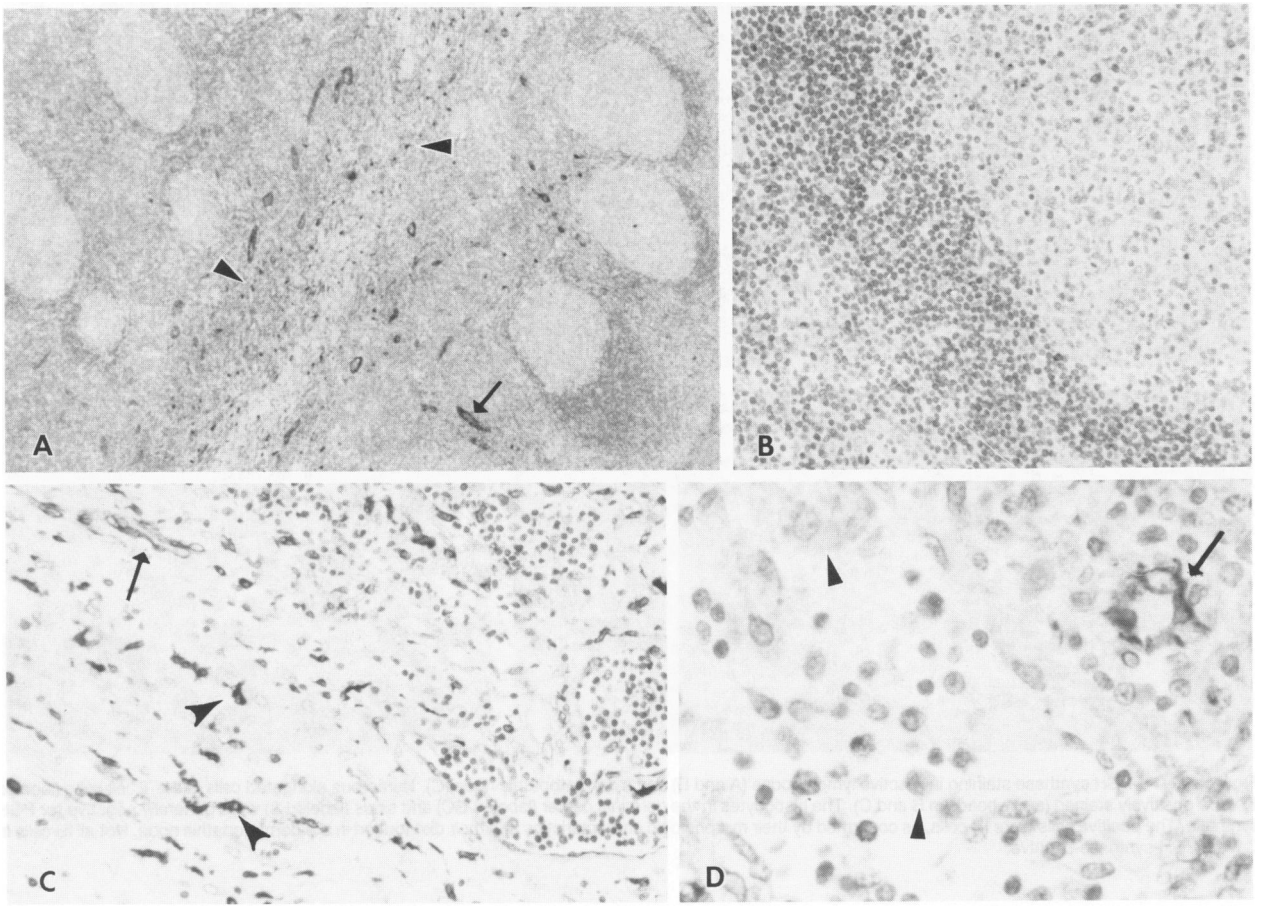


**Figure 3A–D**—PGH synthase staining of tissue from human tonsils (A–C) and a reactive lymph node (D). The staining was generally restricted to endothelial cells (arrows in A, C, and D) and to free histiocytes in subepithelial and peritonsillar connective tissue (arrowheads in A and C). The fixed histiocytes in germinal centers (B) and sinuses (D) were negative or weakly positive for PGH synthase.

It is difficult to assay the various AA metabolites in H-RS cells in tissue. The metabolites may vary from one cell to another because of differences in their maturation or microenvironment. The study of pooled H-RS cells may not reveal such heterogeneity. In studying cultured H-RS cell lines, the authors had noted previously that the major AA metabolite is $PGE_2$ in KM-H2 cells, whereas it is 15-HETE (a lipoxygenase metabolite) in HDLM-1 cells.[32–34] The types of AA metabolites produced appear to be related to the expression of PGH synthase in these cells. PGH synthase was detected in 20% of KM-H2 cells, but none was found in HDLM-1 cells.

The presence of PGH synthase in H-RS cells may explain, in part, the impaired T cell immunity associated with HD. The lymphoid tissues affected by HD usually have a scant infiltrate of neoplastic cells (less than 2% of the cells), but an abundant T lymphocyte reaction.[20] Despite the increased number of T cells in tissues, patients with HD often have significant impairment of their cellular immune response.[18,19] This depressed cellular immunity has been attributed to an excess of suppressive activity by monocytes or T lymphocytes.[18,19] The detetion of PGH synthase and the release of $PGE_2$ and 15-HETE from H-RS cells indicate that H-RS cells may have a direct role in regulating cellular immunity in patients with HD, however. $PGE_2$ and 15-HETE are well known for their T cell-suppressing activity, including inhibition of antigen- and mitogen-induced blastogenesis and of lymphokine secretion, cytotoxicity, lymphocyte proliferation, and IL-2 production.[1,14,16]

In conclusion, it has been demonstrated that H-RS cells express PGH synthase and that these cells are likely to be engaged in the production of PGs. This unique biochemical property of H-RS cells suggests that they are related to cells in the histiocyte lineage. The various degrees of staining of PGH synthase in H-RS cells in tissue and the difference in AA metabolites in cultured H-RS cells reflect a high degree of functional heterogeneity of these cells. It is possible that the production of AA metabolites and of immu-

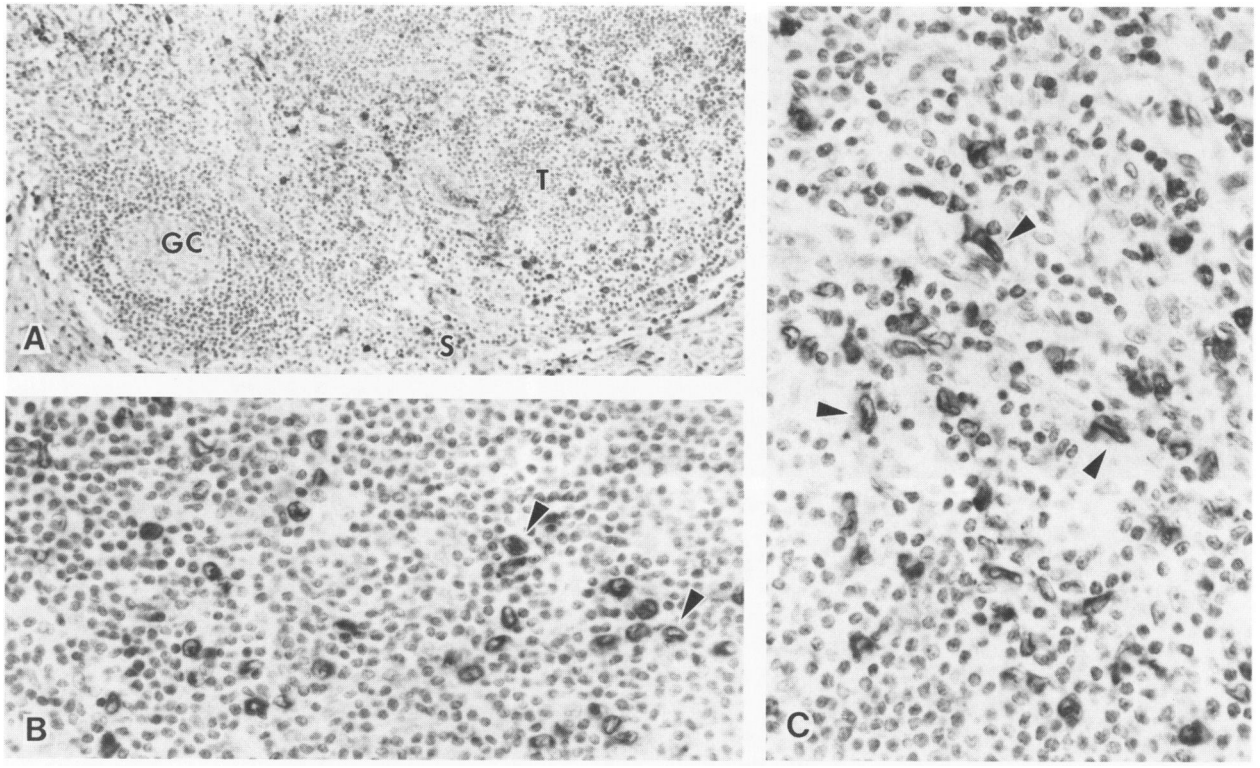


**Figure 4A–C**—PGH synthase staining in reactive lymph nodes (A and B) and a dermatopathic node (C). Numerous elongated cells in the T cell zone (labeled T) were positively stained (arrowheads in B and C). The histiocytes in the germinal center (labeled GC) and sinus (labeled S) were generally negative for PGH synthase. The positive cells were IR cells, as confirmed by their morphologic characteristics and their distribution in the dermatopathic node. Not all IR cells in C were PGH-synthase-positive.

noregulatory cytokines, whose functions may be antagonistic or synergic, in IR cells and histiocytes is harmonious and well balanced. Such a delicate balance may not be preserved in neoplastic H-RS cells. H-RS cells are known to secrete IL-1 ($\alpha$ or $\beta$), granulocyte colony stimulating factor, tumor necrosis factor (TNF-$\alpha$), and perhaps many other cytokines (ie, transforming growth factor-$\beta$).[24,53,54] Future studies of AA metabolism and its interaction with various cytokines in H-RS cells could lead to a better understanding of pathophysiology of HD.

## References

1. Schultz RM: The role of macrophage-derived arachidonic acid oxygenation products in the modulation of macrophage and lymphocyte function, The Reticuloendothelial System: A Comprehensive Treatise. Vol 8, Pharmacology. Edited by JW Hadden, A Szentivanyi. New York, Plenum Press, 1985, pp 129–153
2. Yoshida T, Suko M, Cohen S: Antigen-specific and nonspecific regulation of lymphokine production, Interleukins, Lymphokines, and Cytokines: Proceedings of the Third International Lymphokine Workshop, Edited by JJ Oppenheim, S Cohen. New York, Academic Press, 1983, pp 391–396
3. Salo RJ, Maddux NL, Bleam DK: Effect of prostaglandins on interferon synthesis in murine macrophage-like cell lines. Immunobiology 1986, 171:155–163
4. Kunkel SL, Wiggins RC, Chensue SW, Larrick J: Regulation of macrophage tumor necrosis factor production by prostaglandin $E_2$. Biochem Biophy Res Comm 1986, 137:404–410
5. Roth A, Kaufmann M-T, Cruchaud A, Dayer J-M: Human lymphocytes induce a differential release of prostaglandin $E_2$ and interleukin 1-like mononuclear cell factor from normal blood monocytes. Eur J Immunol 1985, 15:960–963
6. Stobo JD, Kennedy MS, Goldyne ME: Prostaglandin E modulation of the mitogenic response of human T cells: Differential response of T-cell subpopulations. J Clin Invest 1979, 64:1188–1195
7. Hayari Y, Kukulansky T, Globerson A: Regulation of thymocyte proliferative response by macrophage-derived prostaglandin $E_2$ and interleukin 1. Eur J Immunol 1985, 15:43–47
8. Knudsen PJ, Dinarello CA, Srom TB: Prostaglandins posttranscriptionally inhibit monocyte expression of interleukin 1 activity by increasing intracellular cyclic adenosine monophosphate. J Immunol 1986, 137:3189–3194
9. Farrar WL, Humes JL: The role of arachidonic acid metabolism in the activities of interleukin 1 and 2. J Immunol 1985, 135:1153–1159
10. Lu L, Welte K, Gabrilove JL, Hangoc G, Bruno E, Hoffman R, Broxmeyer HE: Effects of recombinant hu-

man tumor necrosis factor α, recombinant γ-interferon, and prostaglandin E on colony formation of human hematopoietic progenitor cells stimulated by natural human pluripotent colony-stimulating factor, pluripoietin α, and recombinant erythropoietin in serum-free cultures. Cancer Res 1986, 46:4357–4361
11. Piacibello W, Rubin BY, Broxmeyer HE: Prostaglandin E counteracts the γ-interferon induction of major histocompatibility complex class-II antigens on U937 cells and induction of responsiveness of U937 colony-forming cells to suppression by lactoferrin, transferrin, acidic isoferrins, and prostaglandin E. Exp Hematol 1986, 14:44–50
12. Dayer J-M, de Rochemonteix B, Burns B, Demczuk S, Dinarello CA: Human recombinant interleukin 1 stimulates collagenase and prostaglandin $E_2$ production by human synovial cells. J Clin Invest 1986, 77:645–648
13. Spear GT, Marshall P, Teodorescu M: Increase in proliferation and cytotoxic cell development in human mixed lymphocyte cultures in the presence of very low concentrations of LPS: Role of IL-1 and prostaglandin $E_2$. Clin Immunol Immunopathol 1986, 38:32–46
14. Bailey JM, Bryant RW, Low CE, Pupillo MB, Vanderhoek JY: Regulation of T-lymphocyte mitogenesis by the leukocyte product 15-hydroxy-eicosatetraenoic acid (15-HETE). Cell Immunol 1982, 67:112–120
15. Dinarello CA, Marnoy SO, Rosenwasser LJ: Role of arachidonate metabolism in the immunoregulatory function of human leukocytic pyrogen/lymphocyte-activating factor/interleukin 1. J Immunol 1983, 130:890–895
16. Browning JL, Ribolini A: Interferon blocks interleukin 1-induced prostaglandin release from peripheral monocytes. J Immunol 1987, 2857–2863
17. Moore RN, Pitruzzello FJ, Larsen HS, Rouse BT: Feedback regulation of colony-stimulating factor (CSF-1)-induced macrophage proliferation by endogenous E prostaglandins and interferon-α/β. J Immunol 1984, 133:541–543
18. Fisher RI: Implications of persistent T cell abnormalities for the etiology of Hodgkin's disease. Cancer Treat Rep 1982, 66:681–687
19. Hellinger SM, Herzig GP: Impaired cell-mediated immunity in Hodgkin's disease mediated by suppressor lymphocytes and monocytes. J Clin Invest 1978, 61: 1620–1627
20. Hsu SM, Yang K, Jaffe ES: Phenotypic expression of Hodgkin's and Reed-Sternberg cells in Hodgkin's disease. Am J Pathol 1985, 118:209–217
21. Hsu SM, Jaffe ES: Leu M1 and peanut agglutinin stain the neoplastic cells of Hodgkin's disease. Am J Clin Pathol 1984, 82:29–32
22. Hsu SM, Ho YS, Hsu PL: Expression of activated interdigitating reticulum cell antigen (IRac) by H-RS cells (abstr). Lab Invest 1986, 54:27A
23. Hsu SM, Pescovitz M, Hsu PL: Monoclonal antibodies against SU-DHL-1 cells stain the neoplastic cells in true histiocytic lymphoma, malignant histiocytosis, and Hodgkin's disease. Blood 1986, 68:213–219
24. Hsu SM, Zhao X: Expression of interleukin 1 in H-RS cells and neoplastic cells from true histiocytic lymphomas. Am J Pathol 1986, 125:221–226
25. Hsu SM, Hsu PL: Phenotypes and phorbol ester-induced differentiation of human histiocytic lymphoma cell lines (U-937 and SU-DHL-1) and Reed-Sternberg cells. Am J Pathol 1986, 122:223–230
26. Hsu SM, Zhao X, Hsu PL, Lok MS: Extracellular matrix does not induce the proliferation, but promotes the differentiation of Hodgkin's cell line HDLM-1. Am J Pathol 1987, 127:9–14
27. Hsu SM, Ho YS, Monheit J, Li PJ, Ree HJ, Sheibani K, Winberg CD: L & H variants of Reed-Sternberg cells express sialylated Leu M1 antigen. Am J Pathol 1986, 122:199–204
28. Krupen K, Zhao X, Brown L, Lachman L, Hsu SM: Expression of interleukin-1 in Reed-Sternberg cells (abstr). Clin Res 1987, 35:609A
29. Hsu SM, Zhao X: The H-RS-like cells in infectious mononucleosis and other nonspecific reactive lymphoid tissues are transformed interdigitating reticulum cells. Am J Pathol 1987, 127:403–408
30. Hsu SM: Phenotypic expression of histiocytes, reticulum and dendritic cells. Hematol Pathol 1987, 1:45–56
31. Hsu SM, Pescovitz M, Hsu PL: Monoclonal antibodies for histiocyte/interdigitating reticulum cell-related lymphomas, Monoclonal Antibodies and Cancer Therapy: UCLA Symposium on Molecular and Cellular Biology, Vol. 27. Edited by RA Reisfeld, S Sell. New York, Alan R. Liss Inc., 1985, pp 53–62
32. Sanduja SK, Hsu SM, Hatzakis H, Wu KK: Stimulation by phorbol ester of 15-HETE synthesis in Hodgkin cell line (KM-H2) is related to cell differentiation (abstr). FASEB J 1988, 2:A1262
33. Hsu SM, Sanduja SK, Wu K: Arachidonic acid metabolism in cultured Hodgkin cells: Phorbol ester induces selective synthesis of 15-hydroxyeicosatetraenoic acid. Biochim Biophys Acta, in press
34. Sanduja SK, Hsu SM, Zhao X, Hatzakis H, Wu KK: Phorbol ester selectively stimulates 15-hydroxy-eicosatetraenoic acid (15-HETE) production by cultured Reed-Sternberg cells (abstr). Blood 1987, 70:267a
35. Melmer M, Lands WEM: Purification of the cyclooxygenase that forms prostaglandins: Demonstration of two forms of iron in the holoenzyme. J Biol Chem 1976, 251:5575–5579
36. Rollins TE, Smith WL: Subcellular localization of prostaglandin-forming cyclooxygenase in Swiss mouse 3T3 fibroblasts by electron microscopic immunohistochemistry. J Biol Chem 1980, 255:4872–4875
37. Hsu SM, Hsu PL, Mulshine J, Ho YS, Ge ZH, Huang L: Ultrastructural and biochemical studies of Leu M1 antigens in granulocytes and H-RS cells in Hodgkin's disease. J Natl Cancer Inst 1986, 77:363–370
38. Smith WL, Rollins TE: Characteristics of rabbit anti-PGH synthase antibodies and use in immunochemistry. Meth Enzymol 1982, 86:213–228
39. DeWitt DL, Dat JS, Gauger JA, Smith WL: Monoclonal antibodies against PGH synthase: An immunoradiometric assay for quantitating the enzyme. Meth Enzymol 1982, 86:229–240
40. Freshney RI: Culture of Animal Cells. A Manual of Basic Technique. New York, Alan R. Liss, Inc., 1987, pp 275–277
41. Hsu SM, Raine L, Fanger H: Use of avidin-biotin-peroxidase complex (ABC) in immunoperoxidase techniques: A comparison between ABC and unlabeled an-

tibody (PAP) procedures. J Histochem Cytochem 1981, 29:577–580
42. Hsu SM, Soban E: Color modification of diaminobenzidine (DAB) precipitation by metallic ions and its application for double immunohistochemistry. J Histochem Cytochem 1982, 30:1079–1082
43. Drexler HG, Gaedicke G, Lok MS, Diehl V, Minowada J: Hodgkin's disease derived cell lines HDLM-2 and L-428: Comparison of morphology, immunological and isoensyme profiles. Leukemia Res 1986, 10:487—500
44. Kamesaki H, Fukuhara S, Tatsumi E, Uchino H, Yamabe H, Miwa H, Shirakawa S, Hatanaka M, Honjo T: Cytochemical, immunologic, chromosomal, and molecular genetic analysis of a novel cell line derived from Hodgkin's disease. Blood 1986, 68:285–292
45. Hsu SM, Zhao X, Chakraborty S, Liu YF, Whang-Peng J, Lok MS, Fukuhara S: Reed-Sternberg cells in Hodgkin's cell lines HDLM, L-428, and KM-H2 are not actively replicating: Lack of bromodeoxyuridine (Brd-Urd) uptake in multinuclear cells in culture. Blood 1988, 71:1382–1389
46. Hsu SM: Tumors of macrophages, Lymphoproliferative Diseases. Edited by DB Jones, DH Wright. Lancaster, MTP Press Limited, 1988, in press
47. Ruzika T, Aubock J: Arachidonic acid metabolism in guinea pig Langerhans cells: Studies on cyclooxygenase and lipoxygenase pathways. J Immunol 1987, 138:539–543
48. Arenzana-Seisdedos F, Barbey S, Virelizier JL, Kornprobst M, Nezelof C: Histiocytosis X: Purified (T6+) cells from bone granuloma produce interleukin 1 and prostaglandin $E_2$ in culture. J Clin Invest 1986, 77:326–329
49. Fisher RI, Bostick-Bruton F, Sauder DN, Scala G, Diehl V: Neoplastic cells obtained from Hodgkin's disease are potent stimulators of human primary mixed lymphocyte cultures. J Immunol 1983, 130:2666–2670
50. Fisher RI, Bates SE, Bostick-Bruton F, Tuteja N, Diehl V: Neoplastic cells obtained from Hodgkin's disease function as accessory cell for mitogen-induced human T cell proliferative responses. J Immunol 1984, 132: 2672–2677
51. Khansari N, Chou YK, Fundenberg HH: Human monocyte heterogeneity: Interleukin 1 and prostaglandin $E_2$ production by separate subsets. Eur J Immunol 1985, 15:48–51
52. McGuire JC, Richard KA, Sun FF, Tracey DE: Production of prostaglandin $D_2$ by murine cell lines. Prostaglandins 1985, 30:949–967
53. Burrichter H, Heit W, Schaadt M, Kirchner H, Diehl V: Production of colony stimulating factors by Hodgkin's cell lines. Int J Cancer 1983, 31:269–274
54. Newcom SR, O'Rourke L: Potentiation of fibroblast growth by nodular sclerosing Hodgkin's disease cell cultures. Blood 1982, 60:228–237