Interleukin-6, But Not Interleukin-4, Is Expressed by Reed–Sternberg Cells in Hodgkin's Disease with or without Histologic Features of Castleman's Disease

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Hodgkin's disease (HD) is a neoplastic disease that is characterized by unbalanced and/or unregulated cytokine production. Information accumulated in our own and other laboratories indicates that the cytokines interleukin-1 (IL-1), IL-5, IL-9, tumor necrosis factor-α (TNF-α), granulocyte colony-stimulating factor (G-CSF), macrophage CSF (M-CSF), and transforming growth factor-β (TGF-β) are secreted by Hodgkin's and Reed–Sternberg (H-RS) cells. These and perhaps additional cytokines are likely to be responsible for the unique histopathologic and clinical alterations seen in patients with HD. In this study, we confirmed that IL-6 is produced by cultured H-RS cells as well as by H-RS cells in tissues. By using an enzyme-linked immunosorbent assay, we found that approximately 2 to 10 ng/ml of IL-6 was secreted by cultured H-RS cells (10⁶ cells/ml). In tissues, we were able to immunolocalize IL-6 in the cytoplasm in 10 to 30% of H-RS cells by using rabbit polyclonal and mouse monoclonal anti-IL-6 antibodies. There was no correlation among the IL-6 staining intensity, number of H-RS cells stained, and the degree of plasma cell infiltration. However, in 3 of 17 cases studied, a large number (60%) of H-RS cells were positive for IL-6, and in these patients, abundant plasma cells were present. In one patient, the involved lymph node also showed histologic features similar to those of Castleman's disease. In this patient, we noted abundant IL-6 expression not only in H-RS cells, but also in most reactive histiocytes. The cultured H-RS cells did not express functional receptors for IL-6, and exogenously added IL-6 did not induce proliferation of these cells. We also conducted studies with specific anti-IL-4 antibodies, which did not show IL-4 production by H-RS cells in both cultures and tissues. In tissues, only rare IL-4 positive lymphoid cells or dendritic cells were identified. Thus, the study demonstrated that adequate amounts of IL-6 are required for an abundant plasma cell reaction, and that an additional source of IL-6 from histiocytes is essential for the formation of Castleman's disease-like changes in lymph nodes involved by HD. Furthermore, IL-4 is not likely to be responsible for the T-lymphocyte reaction in tissues, by a mechanism distinct from that in T-cell–rich B-cell lymphomas. (Am J Pathol 1992, 141:129–138)

Interleukin-6 (IL-6) is a pleiotropic cytokine with a variable molecular mass of 26 to 30 kd. It has many biological effects, including both growth- and differentiation-inducing activities.[1,2] IL-6 can induce B-cell differentiation, augment plasmacytoma growth, and induce proliferation of cytotoxic T cells as well as early precursors of the hematopoietic compartment.[3,4] IL-6 also was shown to be an endogenous pyrogen and to enhance the synthesis of acute-phase proteins by hepatocytes.[5,6] This cytokine is produced by monocytes, macrophages, granulocytes, fibroblasts, endothelial cells, epidermal cells, lymphocytes activated *in vitro*, and various tumor cells.[7–18] Elevated levels of IL-6 are observed in human body fluids during acute and chronic infections, neoplasia, and autoimmune disease. IL-6 plays a key role in activating a variety of local and systemic host defense

mechanisms that are aimed at limiting tissue injury. Excess production of IL-6 may be involved in the pathogenesis of several diseases, including Castleman's disease and Kaposi's sarcoma.[19-21]

IL-4 is an 18-19 kd glycoprotein secreted by activated T lymphocytes or undetermined non-T/non-B cells.[22-25] It was first described in mice in terms of its capacity to promote B-cell proliferation by costimulation with anti-IgM antibodies. IL-4 is now known to have a number of other effects as well. IL-4 enhances the colony formation of a variety of hematopoietic progenitor cells and stimulates the growth of normal T cells, certain T-cell lines, and mast cell lines.[22] It has also been shown to enhance the expression of class II major-histocompatibility-complex antigens on resting B cells and macrophages.[26] IL-4 activates macrophages for increased tumoricidal activity and enhances the antigen-presenting capability of macrophages and related cells.[26-28]

In this study, we examined the production of IL-4 and IL-6 in Hodgkin's disease (HD). We selected these two cytokines because of their capacity to promote the proliferation of lymphocytes (IL-4 and IL-6), enhance the MHC antigen expression and the antigen-presenting capability of macrophages (IL-4 and IL-6), and induce the synthesis of acute-phase reactants (IL-6); all are features often associated with HD.[29-31] We noted the expression of IL-6, but not of IL-4, in cultured H-RS cells as well as in H-RS cells in tissues. Previously, we and others had shown that H-RS cells can secrete cytokines IL-1, IL-5, tumor necrosis factor (TNF-α), granulocyte colony-stimulating factor (G-CSF), macrophage-CSF (M-CSF), and transforming growth factor-β (TGF-β).[32-43] The discovery of IL-6 secretion by H-RS cells adds complexity to an already complicated cytokine network in HD.

Materials and Methods

Cell Lines and Culture

We used the H-RS cell lines KM-H2, HDLM-1, and HDLM-1d and their single-cell clones[44,45] for studies on the production of IL-4 or IL-6. The HDLM cells were established from the pleural effusion of a 74-year-old male patient with the nodular sclerosing subtype of HD.[45] The KM-H2 cells were established from the pleural effusion of a 32-year-old male patient with the mixed cellularity subtype of HD.[44] The phenotypes and properties of these cells have been described in detail previously.[46-48] The cells were grown at 1×10^6 cells/ml in RPMI 1640 medium supplemented with 2 μmol/l glutamine, 50 μmol/l 2-mercaptoethanol, and 50 μg/ml gentamicin with 10% fetal calf serum or 1% Nutridoma (serumfree, Boehringer

Mannheim, Indianapolis, IN) at 37°C in a humidified, 5% CO_2 atmosphere.

Because phorbol ester (tetradecanoyl phorbol-13-acetate, TPA) had been shown previously to enhance the production of cytokines, we wanted to examine whether IL-4 or IL-6 production is affected when H-RS cells are treated with TPA. The protocol that we used for TPA treatment was the same as reported previously.[48-50] Briefly, TPA (dissolved in dimethylsulfoxide, 14 μg/ml) (Sigma, St. Louis, MO) was added at a final concentration of 2 ng/ml to cultures of H-RS cells for 2 days. The culture supernatant was examined for the presence of IL-4 or IL-6.

Enzyme-linked Immunosorbent Assay (ELISA)

InterTest-4 and InterTest-6X ELISA kits (Genzyme Co., Boston, MA) were used for the quantitative determination of IL-4 or IL-6 in cell culture supernatant and in serum from seven patients with HD. The lower limit of detection for this assay was 45 pg/ml and 150 pg/ml, respectively. This assay specifically measures native or recombinant IL-4 or IL-6, without any detectable crossreaction with human IL-1, IL-2, IL-3, G-CSF, M-CSF, GM-CSF, or IFN-γ. In all assays, wells were run in triplicate, and standards were run in duplicate. Serum IL-4 or IL-6 levels obtained from 50 healthy individuals were used as normal controls. Highly elevated IL-6 levels were often detected in patients with infection, autoimmune diseases, and Castleman's disease (plasma cell variant, CD-P), and in a subset of myeloma patients.

Expression of IL-4 or IL-6 in Cultured H-RS Cells

Expression of IL-4 or IL-6 in H-RS cells was determined by staining of cells with monoclonal anti-IL-4 or -IL-6 antibodies. Murine MAbs to human IL-4 and IL-6 were obtained from Genzyme (Boston, MA). The staining intensity was determined by the avidinbiotin-peroxidase (ABC) method.[51,52] Cytospin smears were fixed in acetone at room temperature for 5 minutes. The antibodies were added at 2 μg/ml; this was followed by addition of biotin-labeled horse anti-mouse Ig (1:400). After extensive washing, the sections were incubated with ABC and then developed in DAB-Ni-H_2O_2 solution.[52] The smears were counterstained with methyl green, dehydrated, and cleared as in routine processing.

As a control for staining specificities, we replaced monoclonal anti-IL-4 or IL-6 with mouse nonimmune as-

cites at equivalent concentrations, or we used antibodies pre-absorbed with recombinant cytokines (10 ng/ml).

Expression of IL-4 and IL-6 in Lymphoid Tissues Involved by HD and NHL

We used the ABC technique as described earlier to examine the expression of IL-6 in H-RS cells in frozen sections of lymph nodes from 20 patients with HD. These included 12 sections from patients with the nodular sclerosis (NS) and 8 sections from patients with the mixed cellularity (MC) form of HD. In one patient (NS type), the lymph node also revealed histologic features of CD-P. The diagnosis of HD in each of these cases was confirmed by the expression of CD30, but not of other T- or B-cell markers. Control for staining specificities was performed as describe earlier.

In addition, B5-fixed, paraffin-embedded sections from the same patients were stained with rabbit anti-IL-4 or anti-IL-6 antibodies obtained from Genzyme. The staining results in paraffin sections allow a better evaluation of the stained cells than can be achieved with frozen sections. The staining procedure was similar to that described for monoclonal antibodies, except that a biotin-labeled goat anti-rabbit antibody and a modification of the antibody absorption test were used. The absorption was carried out with an IL-4 or IL-6 ELISA plate that was precoated with monoclonal anti-IL-4 or anti-IL-6 (InterTest 4 and InterTest 6X ELISA kits, Genzyme). Before absorption, recombinant IL-4 or IL-6 (1 ng/ml) was added to the wells for 2 hours. The supernatant was removed, and rabbit anti-IL-4 or anti-IL-6 (1:200 dilution) was then added to the wells for 1 hour. The absorbed rabbit antibody was recovered and used for immunostaining.

To rule out that the staining of IL-4 or IL-6 was a result of nonspecific uptake of serum protein by H-RS cells, we also performed staining with anti-albumin and anti-cytokine antibodies in consecutive sections. The distribution of albumin-containing cells was compared with that of IL-6- or IL-4-containing cells.

Effects of IL-6 and Anti-IL-6 on Growth of Cultured H-RS Cells

We added recombinant IL-6 or anti-IL-6 to cultures to determine the effects of these reagents on the proliferation of H-RS cells. These cells were cultured in 96-well flat-bottom microtiter plates (Costar, Cambridge, MA) at 1×10^4 or 1×10^6 cells/well in 0.2 ml RPMI 1640 medium containing 10% FCS or 1% Nutridoma (serumfree, Boehringer Mannheim, Indianapolis, IN). Recombinant IL-6 was added at concentrations of 0.1, 1, 10, 50, and 100

U/ml, and the rabbit anti-IL-6 antibody was added at 1:10, 1:100, or 1:200 dilution/10^6 cells. Each treatment was carried out for 6 days.

We determined the effects of IL-6 and anti-IL-6 on the proliferation of H-RS cells by measuring the capacity of these cells to take up isotope-labeled deoxyuridine and by noting the increase in the numbers of cells in the S and G_2/M phases of the cell cycle.[47,53] To measure the nucleic-acid uptake, we pulsed cells with [^{125}I]-deoxyuridine for 4 hours. The cells were harvested, and their activity was counted. For DNA cycle analysis, we examined the nuclear staining by using propidium iodide (50 μg/ml) and flow cytometry, as previously reported.[53] The numbers of cells in control and IL-6- or antibody-treated cultures were monitored with a Coulter counter (Coulter Electronics, Inc., Hialeah, FL).

We evaluated the differentiation and/or growth inhibition of cells by looking for cytologic, phenotypic, and cell-cycle changes that have been described previously.[48,53,54] Cytologically, differentiated H-RS cells were characterized by the presence of blunt cytoplasmic projections, a decreased nuclear/cytoplasmic ratio, and an increased number of binucleated or multinucleated cells. Phenotypically, the expression of CD30 and 2H9 was downmodulated, whereas the expression of IRac and Tac could be either increased or decreased in differentiated cells.[48,53,55] The proliferation rate of differentiated H-RS cells was decreased.

Controls for the effects of IL-6 and anti-IL-6 on cell growth were also performed on a long-term myeloma cell line, AR-P1, established in our laboratory. AR-P1 is sensitive to steroid treatment. Recombinant IL-6 can block the steroid-induced cytotoxicity of AR-P1 cells. Concomitant addition of anti-IL-6 abolished the effects of recombinant IL-6.

Test of Expression of IL-6 Receptor (IL-6R) by H-RS Cells

Cultured H-RS cells were washed twice in RPMI medium or RDF1 buffer (R & D Systems, Minneapolis, MN) and resuspended in RDF1 to a final concentration of 4×10^6 cells/ml. To identify the presence of IL-6R, we incubated cells with biotin-labeled IL-6 at a concentration of 4 μg/ml for 60 minutes at 4°C. After this incubation, cells were washed twice and treated with avidin-FITC (1 μg/ml) or ABC. The binding of IL-6 to cells was analyzed by flow cytometry or by the peroxidase reaction. As a control for specificity, cells were pretreated with a 100-fold molar excess of unlabeled IL-6 (Genzyme) before incubation with biotinylated IL-6. Additional controls were the cultured myeloma cell line U266 and freshly isolated monocytes. Both U266 cells and unstimulated monocytes are

known to express IL-6R, but these receptors were found to decrease in number when the monocytes were treated with mitogens.[56]

Results

Production of IL-4 and IL-6 in Cultured H-RS Cells

Staining of IL-6 with anti-IL-6 antibody was detected in both HDLM-1 and HDLM-1d as well as their several subclones (Figure 1A, B). The staining was granular and appeared to be confined to the paranuclear region; no membranous staining was detected. The staining intensity was increased when cells were treated with TPA. IL-6 was expressed weakly by KM-H2 cells. Anti-IL-4 did not stain any of the cultured H-RS cells.

By ELISA, the culture supernatants of the various types of HDLM cells contained approximately 2 to 10 ng/ml of IL-6, whereas only 0.5 ng/ml of IL-6 was detected in KM-H2 culture supernatant. The amounts of IL-6 in culture supernatant increased twofold in both types of cells when they were treated with TPA for 2 days. IL-4 was not detectable in HDLM cells or KM-H2 cells, irrespective of TPA treatment.

Expression of IL-4 and IL-6 in Lymphoid Tissues Involved by HD and NHL

Staining of IL-6 was detected in frozen sections of 17 HD specimens. In 14 cases (9 of the NS and 5 of the MC subtype), the staining was variable and was confined to the cytoplasm of 10 to 30% of the H-RS cells and of a small number (10 to 20%) of scattered histiocytes. In these cases, a considerable, but variable number of plasma cells was present, but the distribution was uneven. There was no correlation among the IL-6 staining intensity, the number of H-RS cells stained, and the degree of plasma cell infiltration.

In the other three cases (2 of the NS and 1 of the MC subtype), a large number (60%) of H-RS cells were positive for IL-6 (Figure 1C, D), and an abundant plasma cell infiltration was present throughout the tissue. In one patient (NS subtype), the lymph node also revealed histologic changes resembling those observed in CD-P. Unlike the finding in other patients studied, most reactive histiocytes adjacent to H-RS cells in this patient were also stained positively for IL-6. The follicles in this patient with CD-like change did not react with anti-IL-6.

In paraffin sections, anti-IL-6 staining was confirmed in 10 of these 17 cases. IL-6 was present in some H-RS cells, histiocytes, and endothelial cells (Figure 1E). In

seven cases, there was no IL-6 staining in H-RS cells or in reactive histiocytes, presumably because the antigen was destroyed by fixation. The abundant expression of IL-6 in histiocytes was readily identifiable in the paraffin sections of lymph nodes that also revealed histologic features of CD-P (plasma cell variant) (Figure 2).

Anti-IL-4 stained 2 to 5% of small to medium-sized lymphoid cells (? activated T cells) and a small number of elongated dendritic-like cells in all tissues involved by HD. No staining was observed in H-RS cells.

The staining of anti-IL-6 or -IL-4 in cytospin smears or tissue sections was blocked completely by absorption of antibodies with recombinant cytokine. The expression of IL-6 in H-RS cells is not likely to be a result of uptake of serum IL-6 by H-RS cells because, thus far, there has been no evidence that cytokines can be taken up and accumulated in cells to an extent that is visible by immunohistochemical staining. Furthermore, the distribution of weak and variable albumin-containing H-RS cells was different from that of IL-6-containing H-RS cells.

Serum IL-6 Levels in Patients with HD

Serum from seven HD patients, including the patient with histologic features of Castleman's disease, was examined for the presence of IL-6. These patients did not have infections, but had only enlarged lymph nodes. In all seven patients, the serum IL-6 level was below the limit of detection.

Effects of IL-6 and Anti-IL-6 on the Growth of Cultured H-RS Cells

The addition of recombinant IL-6 or anti-IL-6 to cultures of H-RS cells did not elicit any significant changes in terms of cell proliferation or differentiation. The cytologic appearance, phenotype, cell number, growth pattern, cell doubling time, cell-cycle distribution, and nucleic-acid uptake of treated cells remained the same as those of control cells.

Expression of IL-6R by H-RS Cells

HDLM-1 and KM-H2 cells did not express IL-6R, or expressed them in small amounts beyond the sensitivity of the biotin-IL-6 binding assay. No receptors were detected when cells were treated with TPA. The use of biotin-labeled IL-6, however, successfully demonstrated the presence of IL-6 receptors in U266 cells and monocytes. No membranous form or membrane-bound IL-6
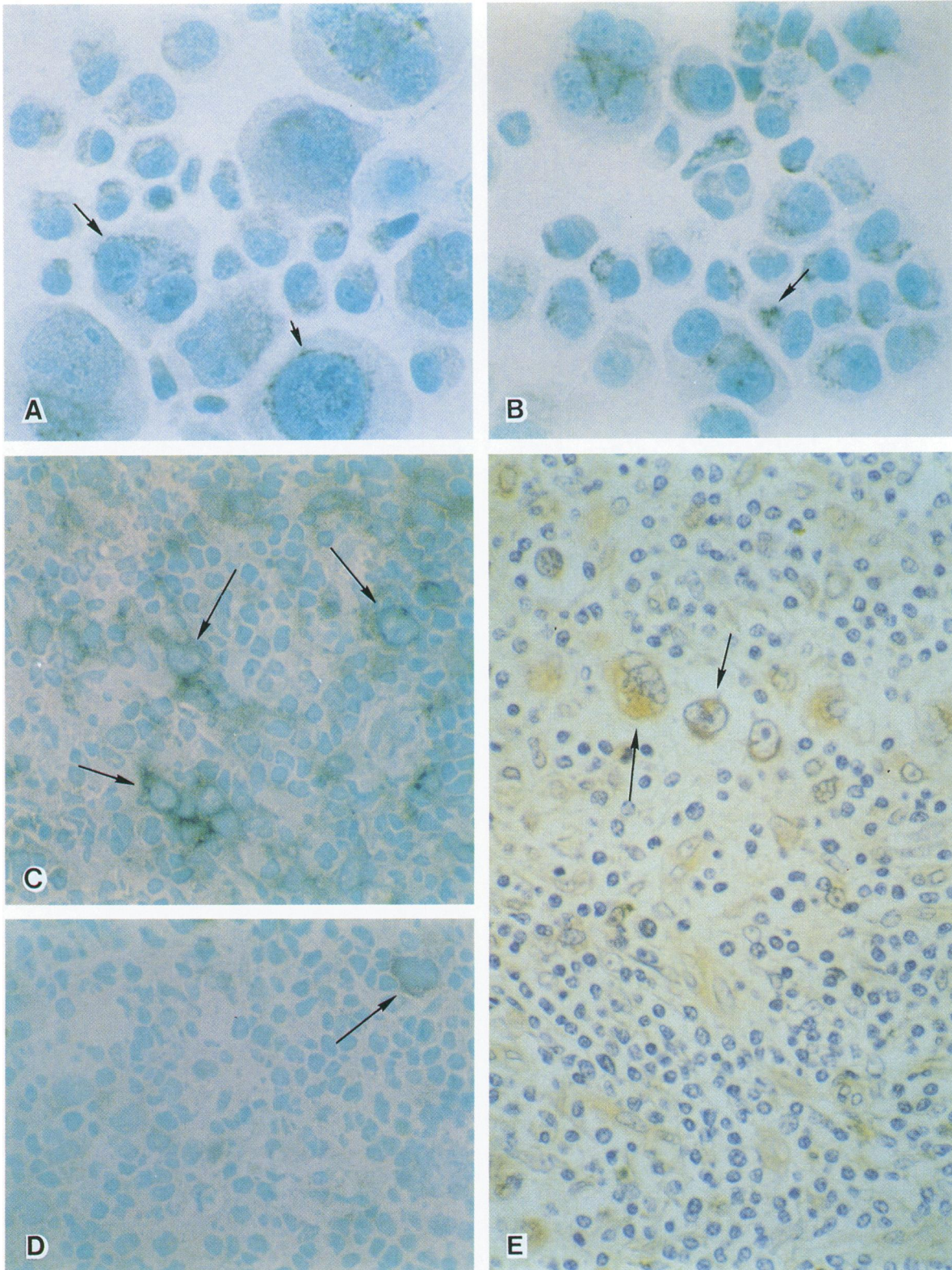
Figure 1. *Expression of IL-6 in cultured H-RS cells HDLM-1d* (**A**) *and HDLM-1* (**B**) *and in H-RS cells in tissues* (**C–E**). *The staining was granular and was located in the paranuclear region in cultured cells. In tissues, the number of H-RS cells in tissues positive for IL-6 varied from more than 60%* (**C, E**) *to less than 10%* (**D**). *A monoclonal anti-IL-6 antibody was used for staining of cell lines* (**A, B**) *and frozen sections* (**C, D**). *A rabbit polyclonal anti-IL-6 antibody was used for staining of paraffin sections* (**E**). *In addition to H-RS cells (arrows), numerous histiocytes and endothelial cells were positively stained by anti-IL-6* (**E**). *Counterstains, methyl green* (**A–D**) *and hematoxylin* (**E**). *Original magnification,* ×400.
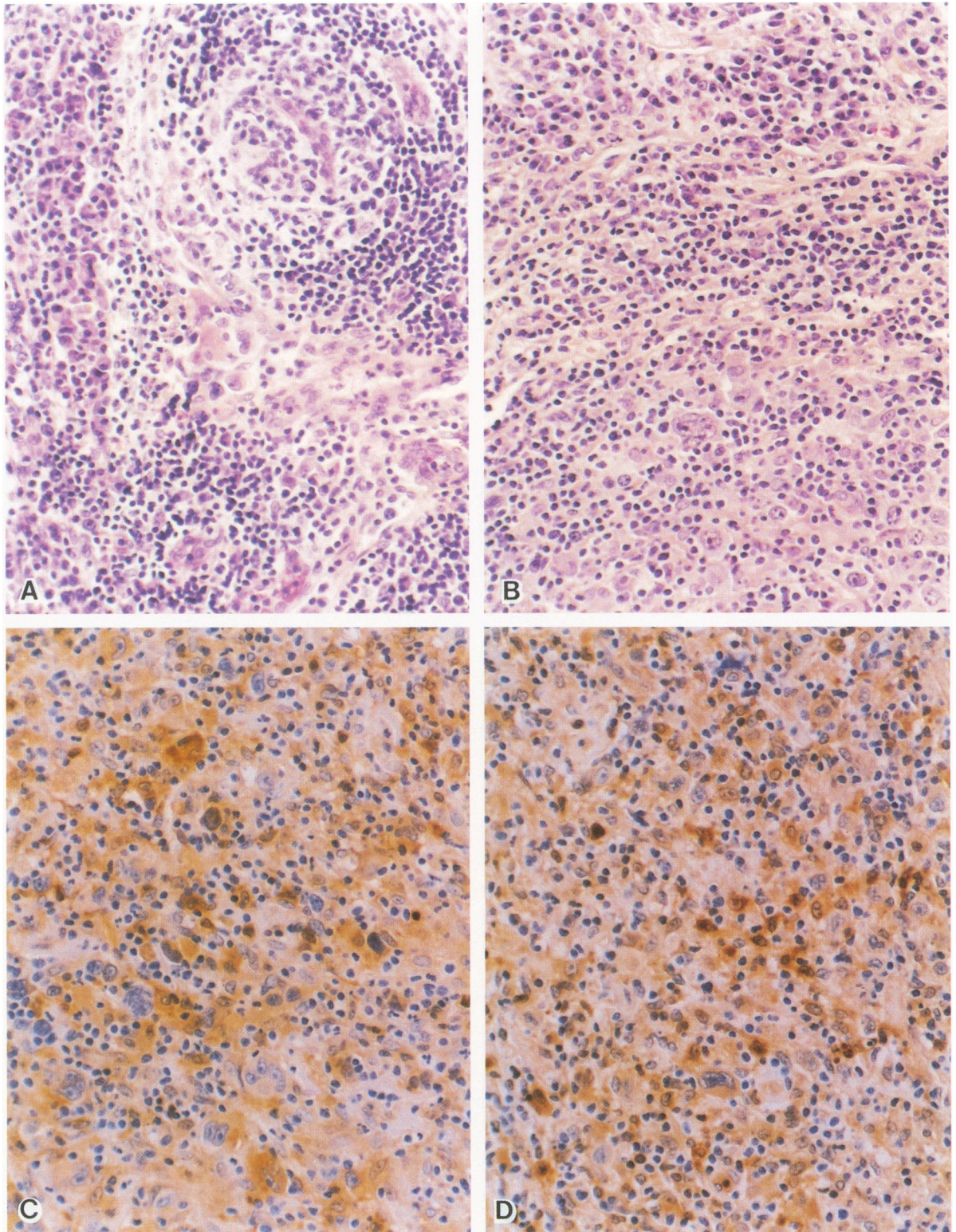
Figure 2. *IL-6 expression in a patent with HD and histologic features of Castleman's disease* (**A, B**). *Expression of IL-6 was observed not only in H-RS cells, but also in reactive histiocytes* (**C, D**). *A rabbit polyclonal anti-IL-6 antibody was used for staining of this paraffin section. It seems that secretion of IL-6 by both H-RS cells and histiocytes is required for the generation of histologic features of Castleman's disease in HD.*

was detected when cells were stained with anti-IL-6 antibody.

Discussion

This study demonstrates that IL-6, but not IL-4, can be produced by cultured H-RS cells, as well as by H-RS cells in tissues. The absence of IL-4 from H-RS cells and from most reactive lymphoid cells in tissue suggests that IL-4 does not serve as a growth factor for these tumor cells, nor as a primary factor that induces a T-cell reaction in most cases of HD. A previous report by Necome et al[57] had shown that L428 cells (an H-RS cell line) can produce IL-4. The variation in IL-4 expression confirms a functional heterogeneity among H-RS cell lines. However, discrepancies in cytokine expression between H-RS cells *in vitro* and those *in vivo* are frequently observed. For example, the HDLM-1 cells expressed TNF-β (protein and mRNA)[35] and TGF-α (mRNA only, unpublished data), but the expression of TNF-β and TGF-α protein in H-RS cells in tissues has yet to be confirmed.

The fact that IL-6 was readily detectable in H-RS cells and reactive histiocytes in tissues strongly suggests that the secreted IL-6 could contribute to the histopathologic and clinical changes in patients with HD, such as hyperplastic reactions in lymph nodes or spleens, with increased plasma cell infiltration and elevated levels of acute-phase reactants in serum. We observed a marked plasma cell reaction in three patients whose lymph nodes showed abundant IL-6 expression in the majority of H-RS cells. In one of these patients, a histologic feature mimicking that of CD-P was noted.

The plasma cell variant form of CD is a hyperplastic lymphoproliferative disorder characterized by multicentric follicular hyperplasia and an abundant plasma cell proliferation.[44] The disease is known for its dysregulated IL-6 secretion in lymph nodes; abundant IL-6 expression can be detected in germinal center cells as well as in the large transformed and immunoblastoid B cells in the mantle zone and interfollicular areas.[44,58] A role of IL-6 in the induction of plasma cell proliferation has also been confirmed in mice receiving transplants of IL-6 retrovirus-infected bone marrow cells.[20] The production of IL-6 by the GC cells in CD-P is enormous; elevated serum IL-6 and systemic plasmacytosis (e.g., increased number of plasma cells in bone marrow) are often detected.

The difference in the type of cells that secrete IL-6 and in the quantity of IL-6 secreted may contribute to the difference in the degree of plasma cell reaction between HD and CD-P. In HD, the neoplastic cells typically make up less than 5% of the total cell population. Furthermore, in the majority of HD cases studied, we detected IL-6 only

in approximately 10–30% of H-RS cells. The quantity of IL-6 secreted by H-RS cells may not be as high as that secreted by abnormal germinal-center cells in CD-P. This may explain why the degree of plasma cell infiltration in HD is not as intense as that in CD-P and why the serum IL-6 level may not be detectable.

Other factors may affect or modify the degree of plasma cell reaction in HD, if the amount of IL-6 secreted by H-RS cells is not overwhelming. In the majority of patients examined, we did not detect a correlation between the numbers of plasma cells in tissue and the extent of IL-6 expression in H-RS cells. This lack of correlation may be due to the fact that the histopathologic alterations in HD are a consequence of the secretion of several cytokines, including IL-1, IL-5, IL-9, TNF-α, M-CSF, G-CSF, and TGF-β, by H-RS cells or reactive cells, and not only the result of IL-6 secretion.[32–43] The functional heterogeneity among H-RS cells may result in the secretion of cytokines in various combinations or quantities. These factors influence the overall outcome of the histopathologic reaction in tissues involved by H-RS cells. Furthermore, the degree of plasma cell infiltration in tissues, may depend on the rate of release of IL-6 by H-RS cells and on the stability of IL-6 in tissues, which may vary from one patient to another.

A histologic change similar to that in CD-P has been reported in some lymph nodes involved by HD.[59] Such a histologic change may occur only in patients with plentiful IL-6 production in tissue. In one such patient studied, we noted abundant IL-6 expression not only in H-RS cells, but also in most reactive histiocytes. It seems that secretion of IL-6 by H-RS cells per se is not sufficient to induce the histologic features of CD-P, and that an additional source from histiocytes is required. IL-6 was usually detectable in approximately 10 to 20% of reactive histiocytes in all types of reactive or neoplastic lymphoid tissues. The reason for the abundant IL-6 expression in most histiocytes in this patient has not yet been resolved, but it may be driven by undetermined cytokines secreted by H-RS cells.

The failure to detect an elevated serum level of IL-6 in this patient with CD-like features is somewhat unexpected. We have observed elevated serum IL-6 levels in patients with CD-P and in patients who had angioimmunoblastic lymphadenopathy (AILD)-type T-cell lymphoma with hypergammaglobulinemia.[58,60] In both of these, the serum IL-6 levels correlated well with the disease activity. We suspect that a low tumor burden, a rapid degradation of IL-6, and the sclerosing change in lymph nodes may affect the release of IL-6 into the circulation. Because, in the majority of HD patients, IL-6 was detected only in a small population of H-RS cells, and because many factors (e.g., infections, inflammation) are

likely to be involved in the elevated IL-6 level in serum, the examination of serum IL-6 levels may not serve as a sole indicator of tumor burden in the majority of patients with HD.

IL-6, as well as IL-4, is known to promote lymphocyte proliferation and maturation.[1,2,22-24] Whether IL-6 also plays a role in the induction of a lymphoid cell reaction in HD cannot be ascertained. The H-RS cells are also known to secrete IL-1, IL-9, and TNF-α. The secretion of IL-1, IL-6, and TNF-α per se may not account for the abundant lymphoid reaction in tissues, because expression of these cytokines was noted in epitheliod histiocytes in granulomas in which only a minimal lymphoid reaction was observed (unpublished data). Expression of IL-9, a T-cell growth factor, is unique for H-RS cells and for lymphoma cells in a subset of anaplastic large cell lymphoma.[43] The T-cell reaction in HD is likely to be a result of a combination of multiple factors, including IL-1, IL-6, IL-9, and TNF-α, together with the potent accessory function of H-RS cells.[29]

An abundant T-cell reaction is not unique to HD, but can be seen in a group of B-cell lymphomas known as T-cell–rich B-cell (TriB) lymphomas.[61] TriB lymphoma is characterized by a small number (< 10%) of immuno-blastoid lymphoma cells, or by lymphoma cells resembling Hodgkin's mononuclear cells in an abundant reactive T-cell environment. Unlike the finding in HD, a large number of IL-4 positive dendritic-like cells was observed in TriB lymphoma. IL-4 probably plays an important role in the induction of an abundant T-cell reaction in TriB lymphoma.[61]

IL-6 has been shown to be the growth factor for some myeloma or hepatoma cells.[56,62] Treatment of cultured H-RS cells with anti-IL-6 or recombinant IL-6 did not affect the rate of proliferation of the cells. Thus, cultured H-RS cells are apparently not dependent on IL-6 for their growth. The lack of response to IL-6 by H-RS cells may be due to the absence of receptors in these cells. Alternatively, cultured H-RS cells may express small numbers of IL-6R, and the binding of IL-6 to these receptors, if present, may not elicit significant effects on cell proliferation or differentiation. This situation has also been well documented for the IL-6R positive myeloma cell line U266.[56]

Expression of IL-6R, however, has been reported in H-RS cells in culture.[63] The discrepancy is attributed to the difference in the type of cultured cells, in various stages of cell passages and culture conditions. The amounts of fetal bovine serum albumin in culture medium, for example, can affect the expression of IL-6R, and IL-2R (p75), and M-CSF-R as well, in H-RS cells. The expression of cytokine receptors by H-RS cells in tissues could be under a complicated control because of a vast variation in the microenvironment of H-RS cells. The sig-

nificance of IL-6 and IL-6R expression in terms of their role for H-RS cell growth and differentiation has yet to be determined.

In summary, the secretion of IL-6 by H-RS cells may contribute in part to the hyperplastic lymphoid cell reaction, to an increase in the number of plasma cells in lymph nodes involved by HD, and to an increase in the level of acute-phase reactants in the serum of these patients. The histopathologic changes in lymph nodes in HD can be attributed to the overall activity of several different cytokines secreted by H-RS cells, as well as by reactive T lymphocytes and histiocytes. The histopathologic changes, however, can be modified by the dominant cytokine. An abundant plasma cell reaction or CD-like changes can be observed only if sufficient IL-6 is produced. The ready detection of cytokines in H-RS cells indicates that HD is not only a neoplastic disease, but also a disease characterized by unbalanced and unregulated cytokine production.

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