Dossier “Cytokines”

Autocrine and paracrine functions of cytokines in malignant lymphomas

SM Hsu, PL Hsu

Department of Pathology, University of Arkansas for Medical Sciences, Arkansas Cancer Research Center, and John L. McClellan Veterans Memorial Hospital, 4300 West 7th Street, Little Rock, AR 72205-5411, USA

Summary – Cytokines play important roles in the pathogenesis of lymphomas via an autocrine or a paracrine mechanism, or both. The characteristic clinical and histopathological features of malignant lymphomas may be due in part to elevated serum or tissue levels of cytokines. Determination of the effects of cytokines on the growth or differentiation of lymphoma cells is often complicated by the fact that more than one cytokine is responsible, and by the failure of anti-cytokine antibodies or antisense oligonucleotides to block the proliferation in vitro of lymphoma cells. However, it appears that IL-6 and/or IL-9 may play a prominent role in the tumor cell proliferation of Hodgkin’s disease (HD), anaplastic large-cell lymphoma, or immunoblastic lymphoma. IL-6 may also be responsible for the plasmacytoid differentiation of lymphoma cells in polymorphic immunocytoma. The histopathological changes as a result of paracrine effects are most noticeable in HD. The malignant (H-RS) cells of HD have been shown to express IL-1, IL-5, IL-6, IL-9, TNF-α, M-CSF, TGF-β, and CD80, and, less frequently, IL-4 and G-CSF. These cytokines may be responsible for the increased cellular reaction and fibrosis observed in tissues involved by HD and for the immunosuppression found in patients with HD. In contrast to H-RS cells, most non-HD lymphoma cells do not produce cytokines in excess amounts and reveal only a minimal cellular reaction. Exceptions include T-cell-rich B-cell lymphoma, angiocentric T-cell lymphoma, and angio-immunoblastic lymphadenopathy (AILD)-like T-cell lymphoma. IL-4 is responsible for the T-cell reaction in AILD-like T-cell lymphoma, whereas IL-6 accounts for the plasma cell reaction in AILD-type T-cell lymphoma. The authors extensively review the role of cytokines in lymphomas because this may lead to major advances in the understanding of the molecular processes involved in the histopathogenesis of lymphomas.

cytokines / lymphomas / histopathogenesis

Introduction

The cytokines which play important roles in the hematopoietic and immune systems are generally classified as interleukins (ILs), interferons (IFNs), tumor necrosis factors (TNFs), colony stimulating factors (CSFs), transforming growth factors (table I). Their many regulatory functions include the control of cellular and humoral immune responses, inflammation, chemotaxis, tumor regression, hematopoiesis, fever, and acute-phase responses [1-3]. In view of the potent and profound biological effects of cytokines, it is not surprising that their activities are tightly regulated. In normal or reactive conditions, the production of cytokines by cells of the immune system is balanced; cytokines are synthesized by cells only when needed [3-6]. In lymphoma cells, such tightly controlled regulatory mechanisms may be defective, and certain cytokines may be secreted in large quantities. This altered regulation of cytokines may provide a growth advantage for the malignant cells via an autocrine mechanism, or lead to characteristic clinical and histopathological alterations [7,8]. Various forms of lymphomas of similar cellular origin may be attributed to the heterogeneity of cytokine production in lymphoma cells.

Functions of cytokines in lymphomas

The functions of cytokines in lymphoma are usually described as either autocrine or paracrine. Cytokines, however, often display multiple activities of both autocrine and paracrine nature simultaneously.
Table I. Human cytokines, classification, synonym and other characteristics.

<table>
<thead>
<tr>
<th>Interleukins (IL)</th>
<th>Synonym</th>
<th>Chromosomal location</th>
<th>Type of receptor</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-1</td>
<td>Endogenous pyrogen lymphocyte-activating factor</td>
<td>2q12(IL-1α) 2q13-2q21 (IL-1β)</td>
<td>Immunoglobulin (Ig) superfamily (CD121)</td>
</tr>
<tr>
<td>IL-2</td>
<td>T-cell growth factor</td>
<td>4q26-27</td>
<td>Cytokine receptor family (CD25 and CD122)</td>
</tr>
<tr>
<td>IL-3</td>
<td>Multipotential colony stimulating factor Mast-cell growth factor</td>
<td>5q23-q32</td>
<td>Cytokine receptor family (CD123)</td>
</tr>
<tr>
<td>IL-4</td>
<td>B-cell stimulatory factor I T-cell growth factor II Mast-cell growth factor II</td>
<td>5q23-q31</td>
<td>Cytokine receptor family (CD124)</td>
</tr>
<tr>
<td>IL-5</td>
<td>B-cell growth factor II Eosinophil differentiation factor</td>
<td>5q23.3-q31</td>
<td>Cytokine receptor family (CD125)</td>
</tr>
<tr>
<td>IL-6</td>
<td>IFN-β2</td>
<td>7p21</td>
<td>Cytokine receptor family (with one Ig superfamily domain) (CD126)</td>
</tr>
<tr>
<td>IL-7</td>
<td>Pre-B cell growth factor</td>
<td>8q12-13</td>
<td>Cytokine receptor family</td>
</tr>
<tr>
<td>IL-8</td>
<td>Neutrophil-activating peptide</td>
<td>4q12-21</td>
<td></td>
</tr>
<tr>
<td>IL-9</td>
<td>T-cell growth factor III megakaryocyte growth factor</td>
<td>5q23-31</td>
<td>Cytokine receptor family</td>
</tr>
<tr>
<td>IL-10</td>
<td>Cytokine synthesis inhibitory factor I</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-11</td>
<td></td>
<td>19q13.3-13.4</td>
<td></td>
</tr>
<tr>
<td>IL-12</td>
<td>Cytotoxic lymphocyte maturation factor</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tumor necrosis factors (TNF) and related cytokines</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TNF-α</td>
<td>Cachectin differentiation-inducing factor</td>
<td>6p23-6p12</td>
<td>TNF/NGF receptor family</td>
</tr>
<tr>
<td>TNF-β</td>
<td>Lymphotoxin</td>
<td></td>
<td>TNF/NGF receptor family</td>
</tr>
<tr>
<td>CD30L</td>
<td>CD30 ligand</td>
<td>9q32-34</td>
<td>TNF/NGF receptor family (CD30)</td>
</tr>
<tr>
<td>CD40L</td>
<td>CD40 ligand</td>
<td></td>
<td>TNF/NGF receptor family (CD40)</td>
</tr>
<tr>
<td>CD95L</td>
<td>CD95 ligand</td>
<td></td>
<td>TNF/NGF receptor family (CD95, or Fas/APO-1)</td>
</tr>
<tr>
<td>Colony stimulating factors (CSF)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GM-CSF</td>
<td>CSF-α</td>
<td>5q23-q31</td>
<td>Cytokine receptor family</td>
</tr>
<tr>
<td>G-CSF</td>
<td>CSF-β</td>
<td>17q21-22</td>
<td>Cytokine receptor family (with one Ig superfamily domain)</td>
</tr>
</tbody>
</table>
Cytokines as autocrine growth factors
The production of cytokines in lymphoma cells provides a growth advantage. Examples of an autocrine growth effect are seen in the action of IL-2 or IL-9 in some T-cell lymphomas [7, 9] and IL-6 in some forms of Hodgkin’s disease (HD) [10].

Cytokines as autocrine differentiation factors
Cytokines may also play an important role in the induction of maturation or differentiation of cells. An example of this autocrine differentiation effect is seen in the plasmacytoid lymphoma cells of polymorphic immunocytoma (lymphoplasmacytic lymphoma) due to effects of IL-6 [11].

Cytokines as paracrine growth or differentiation factors
The paracrine effect of cytokines associated with lymphoma is broadly defined as having two components: i) growth promotion, inhibition, or differentiating activity on lymphoma cells by cytokines released from non-neoplastic (reactive) cells, and ii) histopathological alterations or clinical syndromes induced by cytokines released from lymphoma cells or reactive cells. Examples of paracrine effects include the effects of TGF-β in anaplastic large-cell (Ki-1) lymphoma (as a growth inhibition factor) [12], and the effects of multiple cytokines in HD (as modulators of histopathological alteration) [7, 8, 13-26].
Hodgkin’s disease

Hodgkin’s disease is characterized by the presence of mononuclear tumor cells (Hodgkin’s (H) cells) as well as binucleated or multinucleated tumor cells (Reed-Sternberg (RS) cells) in an environment of variable, often abundant populations of reactive cells [27]. The types of reaction may differ among various lymphoid tissues, and may change during the course of the disease or during treatment. In addition, the tissues involved by HD usually show varying degrees of fibrosis. The diversity of reactive non-neoplastic cells and the varying degrees of fibrosis result in several distinct histological subtypes of HD, namely, nodular sclerosing (NS), mixed cellularity, and lymphocyte depletion forms.

H-RS cells, both in culture and in tissues, have shown variable expression of the cytokines IL-1, IL-4, IL-5, IL-6, IL-9, TNF-α, G-CSF, M-CSF, TGF-β, and CD80 (B7/BB1, a 44/54 kD growth factor of the Ig gene superfamily) [7, 8, 13-26, 28]. In addition, H-RS cells are known to express CD30, CD40, and c-kit (all are cytokine receptors) as well as receptors for IL-2 (CD2/Wp55 and/or CD122/p75), IL-6 (CD126), and M-CSF (c-fms) [10, 19, 20, 29-31]. These cytokines and the secondary cytokines secreted by reactive cells are likely to be responsible for the neoplastic proliferation as well as for the histopathological and clinical alterations seen in patients with HD.

Histopathological effects of cytokines in HD

Cytokines released from H-RS cells and from reactive cells both contribute to the profound cellular reaction process which occurs in tissues involved by HD. Substances other than cytokines, such as arachidonic acid metabolites or as yet unidentified tumor antigens, may also contribute to the causation of cellular reactions.

The increased T-cell reaction or proliferation in HD is due in part to the expression of CD80 (B7/BB1) and secretion of IL-1, IL-6, IL-9, and TNF-α by H-RS cells and, perhaps, to the subsequent release of IL-2, IL-4, IL-9, and other cytokines by reactive T lymphocytes and of IL-1, IL-6, IL-8, and TNF-α by histiocytes. The profound T-cell reaction in HD, however, cannot be explained exclusively by the presence of these cytokines. For example, three cytokines (IL-1, IL-6, and IL-9) may also be detected in lymphomas (ie, some anaplastic large-cell lymphomas and immunoblastic lymphomas) and IL-1, IL-6, TNF-α, and CD80 are detected in histiocytes in granulomas. In both conditions, only a minimal lymphoid reaction is observed [28, 32] and it thus appears that the amplified cellular reaction of HD may require additional factors or the presence of accessory (antigen-presenting) cells.

The cause of a histiocytic reaction in lymphomas including HD is not well understood. It may result from diverse mechanisms, because an increased histiocytic reaction can be observed in numerous lymphomas of different lineage and biological complexity. In HD, several cytokines, eg, M-CSF, IFN-γ, and TGF-β, may be responsible, because these cytokines are extremely potent chemoattractants for monocytes and macrophages and/or are known to activate histiocytes [33-35].

A granulocytic reaction, especially of eosinophils, is very common in tissues involved by HD. IL-5 is probably responsible for this reaction [17]. The mechanism of neutrophilia in some HD cases is not known. In some cases, the reaction may be attributed to G-CSF or IL-8 secreted by reactive histiocytes [36, 37]. The expression of IL-8 by H RS cells has yet to be confirmed.

The cytokines IL-1, IL-6, TNF-α, TGF-β and arachidonic acid metabolites derived from either H-RS cells or reactive cells in HD can stimulate fibroblast proliferation as well as synthesis of collagen and extracellular matrix. These substances may account for the sclerosing change which occurs in lymph nodes involved by HD [38-40].

Clinical alterations – immunosuppression

Despite the increased number of T cells in tissues of patients with HD, these patients often have significant impairment of the cellular immune response [41, 42]. The mechanism of immunosuppression observed in HD may be due to the secretion of TGF-β by H-RS cells and/or reactive cells [23-24]. TGF-β exerts a potent immunosuppressive effect and inhibits the proliferation of T and B lymphocytes, thermocytes, and immature hematopoietic cells [43-45]. It also inhibits B-cell maturation, antibody secretion, natural killer cell function, and macrophage activation [46].

TGF-β is not deficient in tissues involved by HD; a considerable portion of reactive T lymphocytes (> 30%) and/or eosinophils can secrete TGF-β [24, 47]. Only eosinophils of NS-HD express TGF-β, but not by eosinophils of other
histological subtypes of HD. The reason for this selective eosinophilic production of TGF-β is unknown. On the other hand, the increased TGF-β expression in reactive T cells in HD may be explained by a cytokine feedback mechanism [48]. The H-RS cells can secrete several types of cytokines, which are responsible for the increased number of T lymphocytes in tissues involved by HD. At the same time, T lymphocytes may respond to the excess IL-1 or IL-6 secreted by H-RS cells by increasing the production of TGF-β [48, 49]. TGF-β may then serve as a negative-feedback regulator (inhibitor) to prevent overzealous T- and B-cell activity. The secretion of TGF-β and, perhaps, other immunosuppressive agents in HD may provide a potent mechanism for suppressing the host’s immune systems and thereby favor the unrestricted growth of tumor cells.

**Roles of cytokines in the proliferation of H-RS cells**

The multiple cytokines produced by H-RS cells as well as by the associated reactive cells strongly argues for their importance in the growth regulation of H-RS cells. This is supported by the fact that H-RS cells express numerous cytokine receptors (eg. IL-2R, IL-6R, c-fms/M-CSF-R, c-kit) and that the H-RS cells proliferate in vitro in response to IL-2 and IL-6 [10, 19, 20, 29, 31, 50]. Most recently, the two antigens CD30 and CD40, commonly associated with H-RS cells have been identified as members of the TNF receptor family [51, 52]. Thus, CD30 ligand (CD30L) or CD40L (as cytokines) expressed or secreted by activated T cells could affect the proliferation of H-RS cells [53, 54].

CD30 was initially reported to be specific for H-RS cells, but is now being considered to be an activation marker for lymphoreticular cells. CD30L exhibits classic pleiotropic cytokine activity on various types of CD30+ cells [53]. CD30L has been shown to induce proliferation of selected H-RS cells in cultures [53]. CD40 was originally thought to be restricted to B lymphocytes, as well as to certain solid carcinomas however, it has since been detected on macrophages, follicular dendritic cells, Langerhans cells, interdigitating reticulum cells, and H-RS cells [52, 55, 56]. Anti-CD40 (functions as CD40L) has been shown to promote H-RS cell in vitro growth (unpublished data). In addition, anti-CD40 or CD40L can upgrade BCL-2 expression and prevent apoptosis in germinal-center B-cells [55]. It is likely that CD40L, in addition to having a proliferative function, plays an important role in the survival of H-RS cells.

The significance of c-kit receptor expression in H-RS cells is not known [31]. The c-kit ligand (stem-cell growth factor) is usually absent from normal or activated lymphoid cells, but is expressed by fibroblasts and bone marrow stromal cells. It is possible that a fibroblastic reaction often presents in tissues involved by HD providing a growth advantage for H-RS cells.

TGF-β may be one of a few cytokines which may play an important role in the growth inhibition of H-RS cells. H-RS cells are known to have variable expression of TGF-β or TGF-β receptors, both in vitro and in vivo [22-24]. In long-term culture, the growth of H-RS cells is generally not affected by exogenous TGF-β. This lack of response is attributed to the absence of TGF-β receptors and/or excessive endogenous production of TGF-β by cultured H-RS cells. In vivo tissue study reveals the presence of TGF-β receptors on H-RS cells, and many H-RS cells do not express TGF-β [23]. Perhaps the binding of TGF-β to H-RS cells leads to growth inhibition and, eventually, to the death of these cells, a condition similar to that seen in a subtype of anaplastic large-cell lymphoma [47-57].

**B-cell lymphomas**

**Small lymphocytic lymphoma**

Small lymphocytic lymphoma (SLL, or well-differentiated lymphocytic lymphoma) is characterized by a diffuse infiltration of lymphoid tissues by small lymphoid cells. Three types of lymphoma cells can be recognized: i) small lymphoid cells resembling normal lymphocytes, ii) polymorphocytes and blasts which frequently concentrate in proliferation centers (pseudogerminal centers), and iii) cells with varying degrees of plasmacytoid differentiation (lymphoplasmacytoid or lymphoplasmacytic cells).

Small quantities of IL-1 and IL-6 are detected only in the proliferation-center cells, suggesting an autocrine role for these cytokines [11, 32]. No expression of TNF-α was detected in SLL cells, although this cytokine is implicated as an autocrine growth factor in a number of cells in a closely-related malignancy, chronic lymphocytic leukemia (CLL, B-cell type) [58]. Three other cytokines, IL-2, IFN-α, TNF-α and anti-CD40 (as paracrine growth factors) have been shown to in-
duce proliferation of CLL cells in vitro [58-61]. Possibly, the tissue microenvironment may contain these cytokines at concentrations sufficient to promote the growth of proliferation-center cells.

In some SLLs (especially in the lymphoplasmacytoid form), various lymphoma cells can differentiate to become lymphoplasmacytoid cells. The formation of these cells appears not to be mediated by an autocrine IL-6 mechanism, but may require exogenous IL-6 (via a paracrine mechanism) [11].

Two cytokines TGF-β and IL-4 may be involved in the growth inhibition in SLL or CLL. TGF-β appears to function as an autocrine and paracrine growth inhibitor in CLL [62, 63]. In some cases of CLL, TGF-β neutralizing antibodies caused increased proliferation, suggesting that these CLL B cells are sufficiently activated to enter the S-phase and that this is prevented by endogenous TGF-β. Furthermore, exogenous TGF-β completely inhibited CLL B cell proliferation induced by anti-μ [63]. IL-4 inhibits the spontaneous proliferation of B-CLL cells by blocking the progression of the B-CLL cells into the G1 stage of the cell cycle [64-66]. IL-4 also inhibits TNF-α, IFN-α, and IL-2-induced proliferation in CLL [65, 66]. The anti-proliferative effects of IL-4 suggest that this lymphokine may have important therapeutic implications for some B-CLL patients, but any benefit of treatment with IL-4 must be weighed against a possible IL-4-induced prolongation of survival of B-CLL cells [65]. IL-4 has been shown to prevent B-CLL cells from apoptosis [65].

Polymorphic immunocytoma

Polymorphic immunocytoma (lymphoplasmacytic L.P) lymphoma is characterized by the presence of lymphocytes, plasmacytoid cells, and numerous cells of moderate or large size [67]. The moderate or large lymphoma cells express Ki-67 or PCNA, representing the lymphoma proliferative pool [11]. In contrast, the plasmacytoid cells are positive for IL-6, but are completely devoid of Ki-67 or PCNA. The restricted expression of IL-6 by plasmacytoid cells, but not by proliferating cells, suggests that IL-6 is an autocrine factor which is responsible for the differentiation of some LP lymphoma cells. It should be noted, however, that the mechanism responsible for the formation of plasmacytoid cells in LP lymphoma is probably different from that for lymphoplasmacytoid cells in SLL. The former express IL-6 and glutathione-S-transferase (GST)-π, whereas the latter do not [68].

Follicular lymphoma

Follicular lymphoma cells are usually found in close association with follicular T lymphocytes and dendritic cells, suggesting that the surrounding cells may play a supportive role. Clayberger et al [69] have shown that supernatants from activated human peripheral blood lymphocytes can promote the proliferation in vitro of follicular lymphoma cells. This effect is due to IL-3, but not IL-1, IL-2, IL-4, IL-5 or IL-6. Interestingly, IL-3 receptors are detected on fresh isolates of follicular lymphoma cells.

The response of follicular lymphoma cells to IL-3 is not totally unexpected. Both normal and leukemic human B cell precursors proliferate in response to IL-3 [69]. Although follicular lymphomas are considered to be tumors of mature B cells, the presence of the (14;18) translocation in these lymphomas indicates that they may retain some specific characteristics of pre-B cells, such as the capacity to respond to IL-3 [69].

Immunoblastic lymphoma

Immunoblastic lymphoma (IBL) cells are characterized by large, round, vesicular nuclei which contain a large central nucleolus. A variable, but often considerable number of lymphoma cells in some IBLs (B-cell type) may show plasmacytoid differentiation. Other IBLs may contain only immunoblastic cells without discernible plasmacytoid differentiation.

In IBL without plasmacytoid differentiation, cytokines such as IL-1 and IL-6 are usually present in most tumor cells which have a high proliferative activity (Ki-67+/PCNA+) [11]. Perhaps they act as growth factors in this tumor. In IBL with plasmacytoid differentiation (IBL-P), IL-6 was restricted to plasmacytoid lymphoma cells (Ki-67+/PCNA+) and was not present in other immunoblastic tumor cells [11]. It appears that an autocrine IL-6 loop could also play an important role in plasmacytoid differentiation in some IBLs (ie, IBL-P).

Despite the abundant expression of IL-1 and IL-6 in IBL cells, the cellular reaction is often minimal [11]. Perhaps the synthesized cytokines are largely consumed by lymphoma cells, and free cytokines in tissue are not capable of provoking
the cellular reaction. Alternatively, a cellular reaction may require secretion of additional factors by reactive T cells or macrophages. The latter possibility is supported by the observation of a lack of plasmacytosis in nude mice (which are deficient in T-cell function) after they receive IL-6-gene-transfected Chinese hamster ovarian tumor cells [70]. However, plasmacytosis can be observed in IL-6 transgenic mice in which T-cell function is intact [71].

T-cell-rich B-cell (TriB) lymphoma

TriB lymphoma is an unusual malignancy characterized by a small number (< 10%) of immunoblastoid lymphoma cells, or by lymphoma cells resembling Hodgkin's mononuclear cells, in an abundant reactive T-cell environment [72]. We have detected IL-4 expression in lymphoma cells as well as in reactive histiocytes and dendritic cells [73]. Other cytokines such as IL-1, IL-6, and TNFα are generally absent. The abundant production of IL-4 may thus be responsible for the increased T-cell reaction in TriB lymphoma. The mechanisms of the T-cell reaction in TriB lymphoma and HD are thus quite different from one another. Whether IL-4 can inhibit the growth of tumor cells in TriB lymphoma is still unknown.

Hairy cell leukemia (HCL)

Lindenmann et al. [74] showed high levels of TNF-α in the bone marrow and serum of patients with hairy cell leukemia (HCL). In addition, anti-TNF-α-neutralizing antibodies have been shown to enhance in vitro hematopoiesis using bone marrow specimens obtained from patients with HCL. These findings were used to explain a TNF-α-related pancytopenia frequently associated HCL. Another study, however, indicated that an elevated serum TNF-α level is not a common feature in patients with HCL, nor in those with other types of B-cell lymphomas [75]. In culture, TNF-α is able to trigger the proliferation of HCL cells which are known to express high-affinity, but not the low-affinity receptors [76]. Other growth factors which could regulate the in vitro growth of HCL cells may include IL-4, IL-5, IL-6 (proliferation), and IFN-α (inhibition), but not IL-2, despite the abundant expression of the IL-2 receptor in HCL cells.

Nodular paragranuloma

Nodular paragranulomas (NPs) are considered to be variants of the lymphocytic predominance (LP) type of HD [77, 78]. The most prominent features of NP are a predominance of small lymphocytes and a nodular growth pattern. A special type of H-RS-like cell (L & H variant) which expresses B cell markers, is found in variable numbers in NP [77, 78]. The NP may eventually be reclassified as B-cell lymphomas, rather than as a subtype of HD.

Similar to TriB lymphoma, NP exhibits an abundant lymphocyte reaction. However, NP, in contrast to the finding in TriB lymphoma, IL-4 is not readily detectable. Instead, variable expression of IL-1 and IL-6 (and perhaps of many other cytokines) can be observed in the L & H cells. The mechanism of the lymphoid reaction is somewhat similar in NP and in HD.

T-cell lymphomas

T cells often require endogenous or exogenous cytokines for their growth in vitro. Frequently, only cells which produce IL-2, IL-4, or IL-9 become independent of exogenous cytokines [9, 80-82]. It is reasonable to assume that these cytokines, particularly IL-2, play a general and important role in the growth of T-cell lymphomas. Unfortunately, systematic studies of the production in vivo of cytokines by T-lymphoma cells have not yet been performed, and conflicting results for IL-4 and IL-6 expression in T-cell lymphomas have been reported [83, 84].

Lennert's lymphoma

Lennert's lymphoma is a special form of T-cell lymphoma. The lymph node lesion is characterized by complete effacement of the normal lymph node architecture by a massive proliferation of epithelioid cell (histiocyte) clusters intermingled with generally small lymphoma cells. This suggests a prominent cytokine production by the malignant and/or reactive cells.

A cell line established from a patient with Lennert's lymphoma showed IL-6-dependent growth [85]. The massive infiltration of histiocytes capable of producing IL-6 in lymphoma tissues suggests that IL-6 may provide a growth advantage for this lymphoma [85]. However, Merz et al. [82] reported that IL-6 mRNA was not readily detectable in a patient with Lennert's lymphoma. The role of IL-6 in the growth in vivo of Lennert's t-lymphoma cells awaits further confirmation.
The biological basis for the histiocytic reaction in Lennert’s lymphoma has not yet been firmly determined. Ohnishi et al [84] reported that IFN-γ was observed in seven out of 18 patients with T-cell lymphoma, and there was a positive correlation between the relative percentage of histiocytes and IFN-γ expression. However, in a study by Merz et al [82], expression of IFN-γ was variable among T-cell lymphomas, and, in fact, IFN-γ was absent from a patient with Lennert’s lymphoma. The histiocytic reaction may not be due only to IFN-γ, but rather to multiple cytokines.

**Mycosis fungoides (cutaneous T-cell lymphoma, CTCL)**

Cytokines such as IL-4 (as an autocrine factor), IL-2 (as a paracrine factor), and IL-7 (as either autocrine or paracrine factor) may play important roles in the pathogenesis of mycosis fungoides and the associated Sezary syndrome. Transgenic mice carrying IL-7 cDNA have been shown to develop a progressive cutaneous disorder with a polyclonal dermal lymphoid infiltrate [86]. Some of these mice eventually succumb to a full-blown T-cell lymphoma. The transgenic infiltrates are distinct from those seen in human CTCL in that they do not significantly invade the epidermis. On the other hand, the polyclonal expansion of cutaneous lymphocytes in IL-7-carrying transgenic mice is similar to certain benign human disorders (such as lymphomatoid papulosis, discussed below) thought to be precursors of malignant disease [86].

Despite the difference in histopathology between human CTCL and lymphoma developed in mice carrying the IL-7 transgene, IL-7, and IL-2 have also been documented as being paracrine growth factors for CTCL or Sezary lymphoma cells [87]. Whereas IL-2 only had a low proliferative capacity (a two- to three-fold increase in thymidine uptake) on peripheral blood mononuclear cells isolated from patients with Sezary syndrome, IL-7 constantly induced a significant (3- to 40-fold increase) proliferative response, and was used successfully to generate IL-7-dependent cell lines, suggesting that IL-7 may play an important role in the pathogenesis of cutaneous T cell lymphoma.

Cytokine levels in serum or skin exudates in mycosis fungoides patients have also been studied [88, 89]. The results, however, cannot be used to confirm actual cytokine secretion by the tumor cells. Serum IL-4 enzyme-linked immuno-

- **Angiocentric T-cell lymphoma**

In the early stages, angiocentric T-cell lymphoma is characterized by a polymorphous cellular composition with variable, usually small numbers of large lymphoid cells or immunoblasts and a prominent inflammatory (reactive) background. As the disease progresses, increased atypical and monomorphic large tumor cells become evident, and the inflammatory background becomes inconspicuous. The disease frequently shows prominent vascular invasion.

The cytokines responsible for the prominent cellular reaction in this type of lymphoma, however, are not known. Ohnishi and his associates [84] have shown that IL-4 is frequently detected, but it is unlikely that IL-4 alone is responsible for such a prominent and diversified reaction in angiocentric lymphoma.

**AILD-type T-cell lymphoma**

AILD-type lymphoma is a lymphoproliferative disease characterized by generalized lymphadenopathy, fever, weight loss, and rash [90]. Hist-
logical examination of lymph nodes reveals replacement of the architecture by a diffuse, heterogeneous cellular infiltrate composed of immunoblasts, various lymphoid cells with an irregular nucleus and clear cytoplasm, reactive lymphocytes, and plasma cells. Arborizing vascular proliferation is often present. Approximately 30% of patients with AILD-type T-cell lymphoma have polyclonal hypergammaglobulinemia with a marked plasmacytoid cellular reaction in tissues, bone marrow, and blood.

We have shown that the plasmacytoid B-cell reaction in AILD-type T-cell lymphoma is probably due to excess secretion of IL-6 by lymphoma cells [91]. IL-6 was detected in both small and large tumor cells, but appeared to be particularly abundant in immunoblastoid cells. Expression of IL-6 mRNA, but not IL-4 mRNA, has also been demonstrated in some AILD lymphomas [83]. There is a strong correlation between the extent of IL-6 expression in lymphoma cells and the number of plasma cells in tissue [91]. In culture, the plasmacytoid cells isolated from patients with AILD-type T-cell lymphoma maintained a very low level of Ki-67 expression, but their viability was maintained only when IL-6 was added to the culture medium [91]. IL-6 has been shown to prevent an anti-CD95-induced apoptosis in cultured myeloma plasma cells (in preparation).

**Anaplastic large-cell lymphoma associated with lymphomatoid papulosis**

The term lymphomatoid papulosis (LyP) was first used by Macaulay [92] in 1968 to describe an uncommon dermatosis characterized by self-healing crops of papules and papulonodules which are clinically benign, but which have histological features of malignant lymphoma. The lesions of LyP typically involve the trunk and extremities and may be in various stages of evolution. The lesions may ulcerate, but usually resolve spontaneously (ie, regress), despite the presence of CD30+ clonal T cells. In 10 to 20% of patients, the lesion may progress to full-blown CD30+ anaplastic large cell lymphoma (ALCL) [12, 93].

It has been proposed that regression of LyP occurs when the T-cell clone and host immunity are in balance, whereas malignant lymphoma supervenes when the clonal population overcomes the host immune response by unknown mechanisms [93]. Kadin et al [12] further suggested that the progression of LyP to lymphoma may result from failure of TGF-β to limit the growth of tumor cells. Two cell lines (Mac-2A, -2B) derived from a CD30+ lymphoma which evolved from LyP lacked TGF-β receptors, whereas a cell line, Mac-1, which originated from an early stage of the disease expressed TGF-β receptors [57]. The proliferation and colony formation of Mac-1 was suppressed 45-70% by TGF-β1, whereas similar conditions induced no growth suppression of Mac-2A and -2B cells. This result may indicate that loss of TGF-β receptors correlates with tumor progression in LyP. The growth inhibition by TGF-β seems to explain the slow progression or a wax and waning type of growth pattern of ALCL during the early stage of this disease.

In addition to the TGF-β receptor, most cases of ALCL also express two cytokine receptors CD30 and c-kit [31]. The proliferation of ALCL cells is apparently affected by the pleiotropic activity of CD30L, which is cytotoxic to an ALCL cell line, Karpas 299 [53], but which enhances the proliferation of another cell line, SU-DHL-1. Yet, CD30L was ineffective in inducing either proliferation or cytotoxicity in three other ALCL lines established from Dr M Kadin's laboratory (Harvard University). The precise role of CD30 in ALCL, however, should be re-evaluated in short-term primary cultures. Similarly, the growth regulatory function of c-kit ligand in ALCL has yet to be determined.

Many ALCL cells express IL-6 and IL-9; the latter is well known for its role in the proliferation of T cells [74, 80, 94]. It is possible that the expression of IL-6, IL-9, CD30, c-kit receptor, and TGF-β receptor represents interacting and sometimes opposing regulatory forces controlling the growth of some, if not all, ALCL cells.

**HTLV-I-positive adult T-cell leukemia/lymphoma (ATL)**

ATL is characterized by a clonal expansion of CD4+ lymphocytes associated with a monoclonal integration of HTLV-I provirus in the tumor cells. This lymphoproliferative malignancy occurs mainly in HTLV-I high endemic areas (Japan, Caribbean, South America, and Africa).

IL-2 and CD30L may play important roles in the growth regulation of HTLV-I-positive ATL cells, which often express the IL-2 receptor (CD25) and CD30. Expression of CD30 is detected in approximately 20% of incipient ATL. Recently, we have documented that HTLV-I-positive T-cell lines in culture often express CD30L mRNA (manuscript in preparation). This
suggests possible autocrine CD30/CD30L loop operated in ATL cells. Similarly, a proliferation by an IL-2/IL-2R autocrine mechanism may play a role in ATL progression, because the simultaneous expression of IL-2 and IL-2R is seen in 16% of patients with ATL [95].

Conclusion

The clinical and histological alterations in patients with lymphomas can be attributed, at least in part, to abnormal cytokine secretion by lymphoma cells or by the accompanying reactive cells. The cellular reaction may also be potentiated by the presence of other soluble or insoluble cell-associated antigens, adhesion molecules, homing substances, or cellular metabolites.

HD is characterized by an abundant, but varied cytokine secretion, not only by tumor cells, but also by reactive cells. The heterogeneity of cytokine secretion by H-RS cells may explain the heterogeneity of different types of HD. In contrast to HD, the majority of non-HD lymphomas do not produce cytokines to an extent that causes a significant histological reaction. Exceptions, however, may be observed in a few types of lymphoma, such as AILD-type T-cell lymphoma and TriB lymphoma.

Although many cytokines have been identified, many others still await discovery. It is difficult to explain the clinical or histopathological alterations in patients with lymphoma entirely on the basis of the expression of a few selected cytokines currently identified. It may also be difficult to understand the effects of several cytokines at the cellular level. Despite these difficulties, the determination of cytokine expression in lymphoma offers significant insights into the pathobiology of lymphomas.

References

25. Ruco LP, Pomponi D, Pigott R et al. Cytokine production (IL-1, IL-1ß, and TNF-α) and endothelial cell activation

26 Hsu PL, Hsu SM. Production of tumor necrosis factor-α and lymphotixin (TNF-β) by cells of Hodgkin's neoplastic cell lines HDLM-1 and KM-H2. Am J Pathol 1989;135:735


33 Murray HW. Interferon-γ, the activated macrophage and host defence against microbial challenge. Ann Intern Med 1988;108:595


36 Barker JNWN, Jones ML, Mitra RS et al. Modulation of keratinocyte-derived interleukin-8 which is chemotactic for neutrophils and T lymphocytes. Am J Pathol 1991;139:869


43 Ruegamer JJ, Ho SN, Augustine JA et al. Regulatory effects of transforming growth factor-β on IL-2 and IL-4-dependent T cell-cycle progression. J Immunol 1990;144:1767


48 Bristol LA, Ruscetti FW, Brody DT et al. IL-1α induces expression of active transforming growth factor-β in non proliferating T cells via a post-transcriptional mechanism. J Immunol 1990;145:4108


53 Smith CA, Gruss HJ, Davis T et al. CD30 antigen, a marker for Hodgkin's lymphoma, is a receptor whose ligand and defines an emerging family of cytokines with homology to TNF. Cell 1993;73:1349


56 Frederik MS, Steinman RM. The distinct surface of human blood dendritic cells. Proc Natl Acad Sci USA 1990;87:7698

57 Kadin ME, Cavaile-Coil M. Transforming growth factor beta mediates regression of skin lesions in lymphomatoid papulosis and Ki-1+ cutaneous lymphomas. Lab Invest (abstract) 1991;65:75A


70 Black K, Garrett IR, Mundy GR. Chinese hamster ovarian cells tansfected with the murine interleukin-6 gene cause hypercalcemia as well as cachexia, leukocytosis, and thrombocytosis in tumor-bearing nude mice. Endocrinol 1991;128:2657


75 Weiss C, Stehle B, Ho AD et al. Serum levels of tumor necrosis factor-\( \alpha \) in hairy cell leukemia. Blood 1990;75:321


88 Lawlor F, Smith NP, Camp KD et al. Skin exudate levels of interleukin 6, interleukin 1 and other cytokines in mycosis fungoides. Br J Dermatol 1990;123:297


91 Waldron JA Jr, Fink L, King C et al. Significance of interleukin-6 in angioimmunoblastic lymphadenopathy (AILD)-type T-cell lymphoma. Lab Invest (Abstr) 1992;66:89A


93 Agnarsson BA, Kadim ME. Host response in lymphomatoid papulosis. Hum Pathol 1989;20:747
