the Epo receptor, thereby promoting leukemogenesis. In addition to growth-regulating genes, the cloning of translocations and retroviral integration sites from human and animal leukemias has identified genes with homology to known transcription factor.

On the other hand, aplastic anemia is a disorder of multipotential hemopoietic cells, although there is a possibility that inhibitory cells and factors for hematoipoiesis are involved. It is more likely that the responsiveness of hemopoietic stem cells to stromal cells is defective rather than soluble hemopoietic factors.

4. Hematopoietic stem cell transplantation

In order to extend the application of bone marrow transplantation, in vitro expansion of hematopoietic stem cells and tumor cell purging by the introduction of stromal cells and stromal cell-derived factor(s) are necessary. From the results of our preliminary experiments, it appears that the combination of SCF and IL-6 can expand murine CFU-C and CFU-S. It is expected that tumor cells can be removed from autograft in a long-term culture of hematopoietic cells. On the other hand, we found that some monoclonal antibodies against carbohydrate antigens are expressed on leukemic cells but not on normal hematopoietic progenitors (CD34+ cells). In the near future, more effective purging of tumor cells will be possible using such monoclonal antibodies which spare normal hematopoietic stem cells.

2. Interleukin-2 and Its Receptor in Adult T Cell Leukemia

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Interleukin-2 (IL-2) found as a T cell growth-promoting factor is one of the cytokines cDNAs of which were cloned at an early phase of the successful application of molecular biological techniques to clarify the structure of cytokines. The development of monoclonal antibodies which recognize the IL-2 receptor (IL-2R) greatly facilitated the study of IL-2R. IL-2R is abnormally expressed on leukemic cells of adult T cell leukemia (ATL) which is induced by HTLV-I infection, showing various clinical manifestations and with poor prognosis. Here, 1) the data obtained from the study of IL-2 receptors expressed on ATL cells, 2) the possible role of abnormally expressed IL-2R in the leukemogenesis, 3) the serum soluble IL-2R level as a useful indicator during the clinical course, and 4) the basic study aimed at the clinical application of IL-2R-oriented selective cytoreduction are discussed.

I. IL-2 and its receptor

Human IL-2, a glycoprotein with a molecular weight (MW) of approximately 15,000 and composed of 133 amino acids, is produced by activated T cells and exerts various bioactivities depending on the target cells (T cells, B cells, NK cells, LAK cells and monocytes). It also enhances the production of IFN-γ and TNFα. The IL-2R, specific binding sites on the cell surface, consist of at least two distinct subunit proteins. The α chain (Tac) is an acidic glycoprotein with MW of 55,000 consisting of 251 amino acids and the β chain is a glycoprotein with MW of 75,000 consisting of 525 amino acids. Both the association and dissociation of IL-2 with the α chain are very rapid and the α chain functions as a low affinity receptor if present solely. In contrast, solely-expressed β chains function as intermediate affinity receptors resulting from the slow association and dissociation of IL-2. IL-2 has been considered to consist of 6 α-helical cylinders and binds
Interleukin-2 and Its Receptor in ATL

respectively. Studies have indicated that the \( \beta \) chain but not the \( \alpha \) chain mediate the signal transduction initiated by IL-2 binding to the receptor citations. In addition to the \( \alpha \) and \( \beta \) chain, recent studies have suggested that another subunit or receptor-associated molecule is required for the complete binding of IL-2 to the receptor and the signal transduction.

### II. IL-2 production and IL-2 receptor expression in ATL

Leukemic cells from the majority of ATL patients are the monoclonally proliferated CD4(+) T cells infected with human T cell leukemia virus, type I (HTLV-I) which express IL-2R on their cell surface. Our studies on IL-2R expression in ATL (3–6) can be summarized as follows. 1) Peripheral blood leukemia cells from ATL patients expressed both IL-2 receptor \( \alpha \) chain and \( \beta \) chain as examined by an immunofluorescence technique using anti-Tac antibody and 2R-B antibody followed by flow cytometric analysis. This is in contrast to the very weak expression of the \( \alpha \) chain in a certain population of CD4(+) T cells and no expression of the \( \beta \) chain in CD4(+) T cells from healthy individuals. 2) Radiolabeled-IL-2 binding assay of ATL cells demonstrated 150–1,500/cell high affinity and 1,600–22,700/cell low affinity binding sites. Thus, ATL cells express IL-2R constitutively without any

#### Table 1. IL-2 Receptor Expression in ATL

<table>
<thead>
<tr>
<th>ATL case</th>
<th>IL-2 Binding</th>
<th>Positive cells (%)</th>
<th>Proliferative response to IL-2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>High (/cell)</td>
<td>Low (/cell)</td>
<td>Tac (( \alpha ))</td>
</tr>
<tr>
<td></td>
<td>Kd:10–25pM</td>
<td>Kd:17–77nM</td>
<td>( \alpha )</td>
</tr>
<tr>
<td>1,400</td>
<td>22,700</td>
<td>95</td>
<td>96</td>
</tr>
<tr>
<td>250</td>
<td>10,000</td>
<td>81</td>
<td>95</td>
</tr>
<tr>
<td>1,200</td>
<td>14,000</td>
<td>98</td>
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</tr>
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</tr>
<tr>
<td>180</td>
<td>1,600</td>
<td>76</td>
<td>11</td>
</tr>
</tbody>
</table>

particular exogenous stimuli. 3) Southern blot hybridization analysis of the α and β chain failed to demonstrate the gross rearrangement or the obvious amplification of their genes. 4) Abnormalities of the amino acid sequence of IL-2R α chain were not demonstrable although aberrant glycosylation was detected in some HTLV-I-infected cell lines and ATL cases. 5) A close association between IL-2R α chain mRNA expression and HTLV-I viral RNA expression was found in short-term-cultured leukemic cells from ATL patients. These findings strongly suggest a close association of IL-2R expression and HTLV-I infection. Indeed, several studies have clarified the mechanism underlying the constitutive expression of IL-2R in HTLV-I-infected T cells. They have demonstrated that the gene transcription of IL-2R α chain is activated through the DNA-binding protein, NF-κB by p40^tax encoded by pX and part of the env gene of HTLV-I. It remains to be clarified whether IL-2R β chain expression is induced by HTLV-I.

It is quite possible that the abnormally expressed IL-2R is related to the neoplastic transformation or the neoplastic cell growth in ATL because IL-2 is the factor mainly responsible for the proliferation of T cells. First of all, leukemic cells may continuously proliferate by IL-2 autocrine mechanism. However, it is unlikely that an IL-2 autocrine mechanism is truly operating because IL-2 activity was not detectable in the culture supernatant of ATL cells. In addition, our Northern blot hybridization studies did not show IL-2 mRNA expression in peripheral blood leukemic cells from 20 ATL patients although mRNA expression of IL-1α, IL-1β and IL-3 was detectable in 3, 7 and 1 cases, respectively (7). However, it is still possible that the leukemic cells actively proliferate somewhere in the body by utilizing IL-2 molecules produced and secreted by the leukemic cells. Another possible association of IL-2R with the leukemogenesis of ATL is that the constitutively-expressed IL-2R induced by HTLV-I infection plays a key role at a certain stage of neoplastic transformation although it is no longer involved in the neoplastic cell growth in ATL patients. Critical changes which eventually lead to the development of ATL may occur during continuous or repetitive cell proliferation resulting from the continuous (constitutive) expression of IL-2R in HTLV-I-infected T cells.

III. Soluble IL-2 receptor α chain as one of the useful diagnostic indicators

The soluble form of IL-2R α chain (s-IL-2R α), devoid of the transmembrane and intracytoplasmic...
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Effect of Ricin A-2R-B on the growth of ED515-I Cells

Fig. 3. Suppressive effect of ricin A chain-conjugated 2R-B antibody on the proliferation of HTLV-I-infected ED515-I cells.

portion, is detectable by the sandwich ELISA method using two monoclonal antibodies which recognize different epitopes of the α chain. The serum level of s-IL-2R α may be a useful indicator of 1) the number of activated T cells in vivo in infections, rejection, or graft versus host disease following organ transplantation, and 2) the quantity of neoplastic cells in lymphoproliferative diseases. The level of s-IL-2R α is remarkably high in ATL patients compared to that in healthy individuals (8). It is not uncommon for the serum s-IL-2R α level to be 100-fold higher than normal in the acute type or lymphoma type of ATL. The serum s-IL-2R α level is not only a useful indicator in lymphoma type ATL obtaining the neoplastic cells for analysis is difficult but also it correlates well with the clinical manifestations, serum LDH level and the number of peripheral blood leukemic cells.

IV. IL-2 receptor-oriented selective cytoreduction therapy in ATL

It is theoretically possible for the modified or cytotoxin-conjugated form of IL-2 or anti-IL-2R antibodies to eliminate cells expressing IL-2R. We have developed ricin A chain-conjugated anti-Tac antibody and 2R-B antibody and examined their selective cytotoxicity in vitro against HTLV-I-infected Hut102 cells and ED-515-I cells. Both ricin A-conjugated antibodies reduced the number of viable cells to 1/15 of the control 72 h after the initiation of the culture and markedly suppressed protein synthesis of these cells as assayed by 3H-leucine incorporation into cells. In contrast, neither reagent showed significant cytotoxicity against cell lines not expressing IL-2R. Waldmann et al reported that the administration of anti-Tac antibody results in the clinical remission in 3 of 9 ATL patients (9). Thus, the IL-2R-oriented approach to the development of a new and effective treatment of ATL is probably one of the promising trials inasmuch as current therapy employed in the treatment of ATL is disappointing ineffective.

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REFERENCES


3. Cytokines and Liver Disease

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In the past few years, many cytokines have been identified, and advances in molecular biology have facilitated study at the gene level. Researchers can now study the effects of cytokines by molecular cloning in vitro and in vivo as well as the relationship between cytokines and diseases. Although an immunological mechanism is thought to be related to the occurrence and progression of not only autoimmune hepatitis but also viral hepatitis and drug- or alcohol-induced liver disease, the relationship between cytokines and liver disease is still not well understood.

At this symposium, I discussed several cytokines which have been implicated in chronic hepatitis, liver cirrhosis and hepatocellular carcinoma (HCC), with particular reference to the following six areas of research: 1) serum levels, 2) production by peripheral mononuclear cells, 3) intrahepatic localization, 4) cytokines and liver injury, 5) production by hepatoma cells, and 6) cytokine therapy.

1) Serum levels

The issue of whether circulating cytokines are detectable in viral hepatitis remains controversial. Early reports had suggested that patients with viral hepatitis do not produce measurable amounts of circulating interferon (IFN) (1). In some studies, with newer assay methods, however, circulating IFN-α and/or IFN-γ have been found in certain patients with acute hepatitis and chronic type B hepatitis (2, 3).

We detected a low serum tumor necrosis factor (TNF) level in a few patients, as have other researchers (4, 5) but were unable to detect serum interleukin-1α (IL-1α) and IL-6 by ELISA.

Thus, there are few cases to explain the relationship between circulating cytokine and pathogenesis in liver disease, other than the cholestatic factor described by Mizoguchi et al (6) in patients with intrahepatic cholestasis due to various etiologies such as drug-induced hepatitis, alcoholic liver injury or viral hepatitis. Since cytokines per se should act on