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Expression of Interleukin-2Rα and Interleukin-2Rβ in Hodgkin's Disease

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Hodgkin and Reed–Sternberg cells, the putative malignant cells of Hodgkin's disease (HD), carry regularly the CD25 antigen that forms one chain of the interleukin-2 (IL-2) receptor (IL-2Rα). To analyze the putative role of IL-2R expression in Hodgkin's disease, we have investigated the expression of both IL-2Rα and IL-2Rβ chains in HD-derived cell lines and in primary specimens from patients with HD. Expression of IL-2Rα and IL-2Rβ was detected in all HD-derived cell lines. In addition, soluble IL-2Rα molecules were demonstrated in the supernatants of three of these cultured cell lines. In primary tissues, IL-2Rα and IL-2Rβ were seen in some but not all cases. Staining was detected in Hodgkin and Reed–Sternberg and in lymphoid cells. There was a remarkable difference in the pattern of expression, in that IL-2Rα- but not IL-2Rβ-positive cells from HD patients were clustered in frozen sections. We conclude from these data that IL-2R expression might be involved in the biology of HD. (Am J Pathol 1993, 142:1714–1720)

The nature of the Hodgkin (H) and Reed–Sternberg (RS) cells is still controversial. Genetic analyses revealed that in some cases rearrangements of immunoglobulin and T cell receptor (TCR) genes occurred.[1–3] Recently, several investigators demonstrated the presence of Epstein–Barr virus in the tumor cells in 30 to 50% of the cases.[4–5] It has been shown that in the majority of cases of H and RS cells can be stained with the anti-CD25 antibodies, which detect the human interleukin-2 receptor-α (IL-2Rα) chain.[6–7]

IL-2 is a 15-kd polypeptide growth factor that promotes cell-cycle progression of activated lymphocytes. The human high-affinity IL-2R contains two IL-2 binding subunits, the low-affinity (CD25, IL-2Rα) and the intermediate-affinity IL-2R. The IL-2Rα molecule, a 55-kd glycoprotein binds IL-2 with low affinity (k_D 10 nmol/L) and is expressed as a consequence of TCR-mediated T-cell activation. CD25 molecules can also be found on activated B cells and monocytes.[8–9] The intermediate-affinity IL-2R (IL-2Rβ, k_D 1 nmol/L) has a molecular weight of 70 to 75 kd and is responsible for signal transduction by and internalization of the high-affinity IL-2R. The association of the 70- to 75-kd IL-2Rβ chain with the 55-kd IL-2Rα chain forms the functional high-affinity IL-2R complex.[10–12]

We have previously analyzed the expression and structure of the IL-2R in one Hodgkin cell line, L540, in more detail. Our data showed that the cells bear the specific 3.5- and 2.4-kb messenger (m)RNA and the 55-kd protein of the IL-2Rα chain but do not express IL-2. Scatchard plot analysis revealed the presence of 2,000 high-affinity IL-2Rs per cell. Cross-linking experiments directly demonstrated the high-affinity IL-2R to consist of the IL-2Rα and the IL-2Rβ chains. IL-2 was rapidly internalized by these receptors, demonstrating that they can be functional. However, we were unable to stimulate the proliferation of L540 cells *in vitro* with exogenous IL-2.[13] In this study we have analyzed the expression of both chains of the IL-2R in Hodgkin's disease-(HD) derived cell lines and in primary tissues. Our results demonstrate that both IL-2Rα and IL-2Rβ chains are expressed in HD-derived cell lines and in primary H and RS cells.

Materials and Methods

Cells and Cell Culture

The HD-derived cell lines have been described previously.[14] The cell lines used here fulfil a number of criteria to consider them as true tumor cell lines of HD. These criteria include the confirmation of the

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histological diagnosis by two independent pathologists, aneuploidy of the cells, and monoclonality. Advanced clinical disease, the nodular sclerosing subtype, and derivation from effusions were favorable for the establishment of a cell line. Thus, the lines may not be representative for early stages of HD. Immunophenotypic analyses revealed that all cell lines used in this study express the CD30 antigen that is a characteristic marker of primary H and RS cells. In addition, all cell lines have clonal rearrangements of immunoglobulin or TCR genes that reveal the clonal origin of the cell lines. Except in one cell line (L591), Epstein–Barr virus was not detected in the cells. The cells were grown as suspension culture in RPMI 1640 (Gibco, Grand Island, NY) supplemented with 10% fetal calf serum, 100 U/ml penicillin, and 100 μg/ml streptomycin.

Immunofluorescent Staining

Fluorescein isothiocyanate–conjugated monoclonal anti-IL-2Rα antibodies were purchased from Becton-Dickinson (Mountain View, CA). Anti-IL-2Rβ antibodies were a gift from Dr. Taniguchi.[15] Cells (10^6) of the L540 cell line were washed in phosphate-buffered saline–bovine serum albumin (1% bovine serum albumin, 0.02% sodium azide). The cells were incubated with anti-IL-2R antibodies for 30 minutes on ice, washed, and subsequently incubated with fluorescein isothiocyanate–labeled goat anti-mouse Ig. Staining was analyzed by flow cytometry (FACscan, Becton-Dickinson).

Immunocytological and Immunohistological Analyses

Immunocytological staining of smears from HD-derived cell lines and immunohistological staining of frozen sections were performed using the alkaline phosphatase-anti-alkaline phosphatase (APAAP) method as described previously.[16] For control staining anti-peroxidase antibodies were used. Anti-IL-2Rα antibodies were purchased from Dianova (7); anti-IL-2Rβ antibody Tu27 was a gift from Dr. Taniguchi.[15]

Enzyme-Linked Immunosorbent Assay (ELISA)

Cytokine levels were determined by means of commercially available sandwich ELISAs according to the manufacturer's description. The IL-2 ELISA was purchased from Genzyme Corporation (Boston, MA) and the ELISA to detect soluble IL-2 receptors from T Cell Sciences Inc. (Cambridge, MA). The sensitivity of the ELISA for IL-2 was 20 pg/ml.

Results

Analysis of HD-Derived Cell Lines

It has been shown previously that different HD-derived cell lines can be stained with anti-IL-2Rα monoclonal antibodies.[3,17] In this experiment, staining of the L540 cell line with anti-IL-2Rα and anti-IL-2Rβ was evaluated by flow cytometry. As shown in Figure 1, both chains of the IL-2R were expressed on L540 cells. In addition, the HD-derived cell lines L428, HDLM2, KMH2, and L591 were analyzed for the expression of IL-2Rα and IL-2Rβ using the APAAP method. The results in Table 1 demonstrate that all five HD-derived cell lines analyzed expressed the IL-2Rβ and the IL-2Rα antigens. In addition, expression of the CD30 antigen was detected in all cell lines. Soluble IL-2 receptors could be demonstrated in the supernatants of three HD-derived cell lines (L540, L591, and HDLM2, Table 1). However, production of IL-2 was not detected in any of the HD-derived cell lines.

Staining of Primary Tissues

Frozen sections of 17 patients with HD were analyzed for expression of IL-2R chains using the APAAP method. Control staining was performed with anti-peroxidase antibodies that were negative in all cases and for CD30, which showed expression in H and RS cells in all cases. In the primary specimen, IL-2Rα expression was detected in 11 of 17 cases in H and RS cells and in all cases in lymphocytic cells (Table 2). Frequently, clusters of IL-2Rα-positive cells were detected, which consisted of H/RS and lymphocytic cells. Representative examples are given in Figure 2, A and B.

Expression of IL-2Rβ was found in seven of 13 cases in H and RS cells and in 13 of 13 cases in lymphocytic cells. Significantly lower numbers of lymphoid cells stained positive for IL-2Rβ however. In contrast to IL-2Rα, clusters of stained cells could rarely be observed with anti-IL-2Rβ antibodies. An example of the staining pattern is given in Figure 3, A and B. In five cases, both IL-2Rα and IL-2Rβ were expressed in H and RS cells, in five cases IL-2Rα chains only, in one case IL-2Rβ only, and in five cases none of the two chains were detected. There was no obvious correlation with the histological subtype of the disease, although, as shown in Table 2,

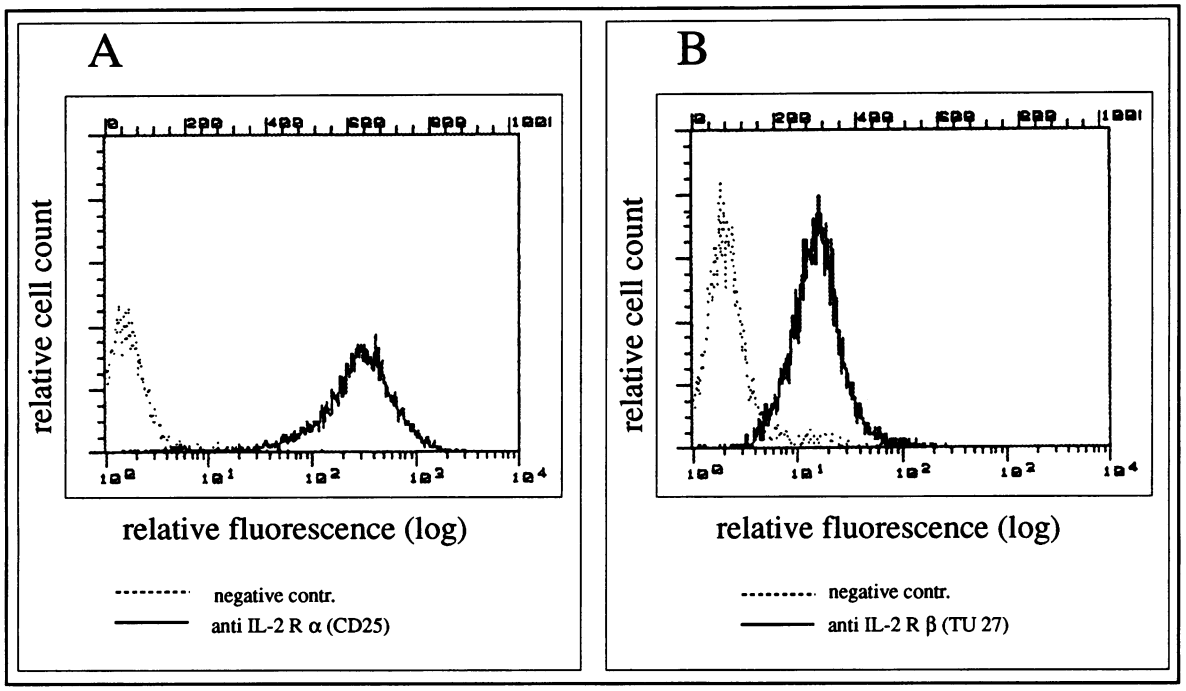


Figure 1. *Immunofluorescent staining of L540 cells with anti-IL-2Rα (A) and anti-IL-2Rβ antibodies (B). 10^5 cells of the Hodgkin's derived cell line L540 were incubated with IL-2Rα and IL-2Rβ specific antibodies for 30 minutes, washed, and reincubated with fluorescein isothiocyanate–labeled goat anti-mouse Ig. Fluorescent staining was evaluated by flow cytometry (FACScan, Becton-Dickinson).*

Table 1. *Expression of IL-2Rβ and IL-2Ra in HD-Derived Cell Lines*

HD-derived cell line	IL-2Rβ*	IL-2Rα*	CD30*	Soluble IL-2Rα† U/ml
L540	+	+	+	400
L428	+	(+)	+	<10
HDLM2	+	+	+	<10
KMH2	+	(+)	(+)	450
L591	+	+	+	40

+: strong membrane staining (>80% of cells positive).
(+): weak membrane staining (<50% of cells positive).
* Expression of IL-2Rβ, IL-2Rα, and CD30 molecules were analyzed by staining with monoclonal antibodies using the APAAP method.
† Expression of soluble IL-2Rα molecules in the supernatants was analyzed by ELISA.

lymphocyte-depleted regions seemed to contain IL-2Rβ-positive cells more frequently. Large case numbers, however, are needed for detailed analysis.

Discussion

Constitutive expression of normal or abnormal growth factor receptors may lead to unlimited proliferation in certain tumor cells. Autocrine stimulation of malignant T cells have been demonstrated in a case of HTLVI-induced T cell leukemia, which produce and respond to IL-2.[15] In addition, a variety of B cell, T cell, and histiocytic malignancies as well as HD exhibit constitutive expression of the IL-2Rα chain.[6,17,19,20]

Here we demonstrate that cell lines derived from HD constitutively express both chains of IL-2R (Table 1). In addition, IL-2Rα and IL-2Rβ molecules were detected in H and RS cells and in reactive lymphoid cells in primary specimen from patients with HD (Table 2). In a previous paper,[13] we have shown that in the cell line L540 both IL-2Rα and IL-2Rβ chains were expressed. The two chains formed the high-affinity IL-2R complex and could bind and internalize exogenous IL-2. In the present paper, we confirmed that both IL-2Rα and β chains were produced by L540 cells and further demonstrated that this is true for the additional HD-derived cell lines analyzed. In primary H and RS cells, expression of IL-2Rα and β chains was more heterogeneous: in some cases H and RS cells expressed IL-2Rβ or IL-2Rα molecules only; in other cases both receptor molecules were present or both were missing. The discrepancy in the expression pattern between HD-derived cell lines and primary lesions could be reflected by the biological characteristics of the cell lines, which may not be representative for all stages of HD or by a difference in the numbers of receptor molecules on the cells.

Table 2. *Expression of IL-2Rα and IL-2Rβ in Hodgkin's Disease*

Case	Sex	Age	Subtype	IL-2Rα		IL-2Rβ	
				Lympho-cytes	H/SR	Lympho-cytes	H/SR
1	m	51	NS	+++	–	+	–
2	m	46	MC	+++	(+)	+++	+
3	m	44	LP	+	+	++	(+)
4	f	17	NS	++	–	++	(+)
5	m	22	nd	+++	+++	++	–
6	f	79	NS	+++	+	nd	nd
7	m	37	NS	+++	–	+++	–
8	f	79	NS	++	–	++	–
9	m	38	NS	+	+	+++	–
10	m	20	NS	(+)	–	nd	nd
11	m	15	NS	++	+	+++	+
12	m	30	MC	+	–	+	–
13	m	63	MC	+++	+	+++	+
14	m	52	NS	+++	(+)	+	+
15	f	48	NS	++	+	++	+
16	f	31	NS	++	+	nd	nd
17	m	31	NS	+++	+	nd	nd

NS: nodular sclerosis; MC: mixed cellularity; LP: lymphocytic predominance; LD: lymphocytic depletion; nd: not done; +++: very strongly positive; ++: strongly positive; +: positive; (+): weakly positive; –: negative.

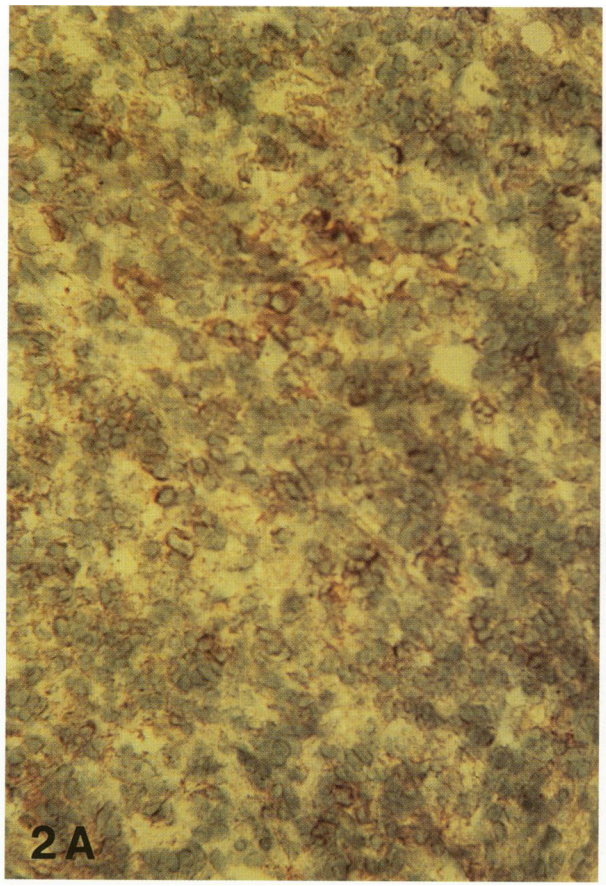
Our results are in contrast with a previous report from Hsu and coworkers, who did not detect the IL-2Rβ molecules in cultured or primary H and RS cells.[21] The cell lines used by Hsu et al (HDLM-1 and KMH2) were different from those analyzed here, however. Hsu et al detected IL-2Rα on H/RS cells in 6 out of 10 cases but no immunoreactivity with anti-IL-2Rβ antibodies. It is possible that this difference is due to differences in the sensitivity of the assay or to the heterogeneity of IL-2Rβ chain expression in cases with HD. In fact, we have detected strong expression of IL-2Rβ in primary H and RS cells in five of 13 cases and weak expression in two of 13 cases.

The pattern of IL-2Rα and IL-2Rβ expression in primary lesions with HD shows a remarkable difference: whereas clusters of IL-2Rα chain-positive cells were frequently detected, IL-2Rβ expression was found preferentially in single scattered cells. Clusters of IL-2Rα cells consisted of H and RS cells and of reactive lymphocytes. This pattern may be due to intimate interactions between the tumor cells and reactive bystander cells. Thus, activated T cells could secrete IL-2, which in turn stimulates the growth of IL-2R-positive H and RS cells in a paracrine way. Similarly, clusters of IL-2Rα cells were detected in two cases with non-Hodgkin's lymphomas, whereas IL-2Rβ-positive cells were also scattered in these three cases.

The cell lines analyzed in this paper, however, do not resemble mature T cells in that they do not bear surface CD3 antigens or TCR molecules. TCRβ and TCRα genes are rearranged in L540 cells,[3] whereas TCR Cδ genes are deleted on both alleles (data not shown). Northern blot experiments revealed the presence of TCRα mRNA but not TCRβ or TCRγ mRNA.[3] This genotype has not been detected in normal immature T cells so far. In L428 cells, TCRβ but also IgH and IgL chains are rearranged and mRNAs specific for IgH chains were found. Co cells resemble immature T cells and express TCRβ but not TCRα mRNA and cytoplasmic CD3 antigens.[3] KMH2, Dev, and L591 cells have rearranged immunoglobulin heavy and light chains. HDLM2 cells have rearranged TCRβ and TCRγ genes but do not express the CD3 antigen.

We have also detected soluble IL-2Rα molecules in the supernatant of three out of five HD-derived cell lines (L540, L591, and HDLM2, Table 2). Gause and coworkers analyzed the expression of soluble IL-2R in serum of patients with HD.[22] In patients with B symptoms, the levels of soluble IL-2R were significantly higher than in patients without B symptoms. Patients in stage IVB had highest concentrations of the protein. Interestingly, all patients with low IL-2R levels achieved complete remission with no relapse after a median of 20 months.

Screening of a panel of cytokines on the mRNA and protein level in HD-derived cell lines revealed expression of a variety of growth factors such as IL-1α, IL-4, IL-5, IL-6, IL-8, tumor necrosis factor-α, and tumor necrosis factor-β but not IL-1β, IL-2, IL-3, and G-CSF[16] (SK, MJ, and HT, manuscript in preparation). Production of IL-2 was not detected in HD-derived cell lines. Because the receptors are regularly expressed on H and RS cells, it is possible that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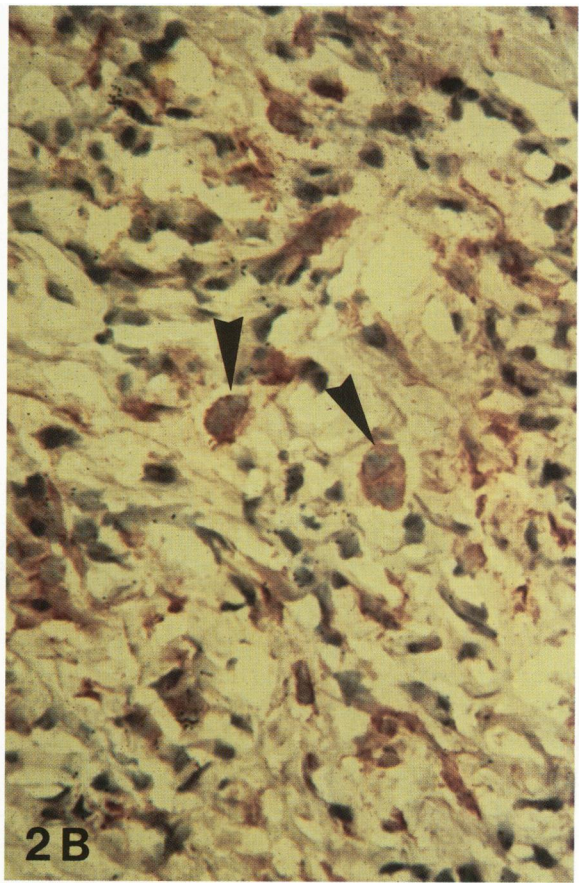
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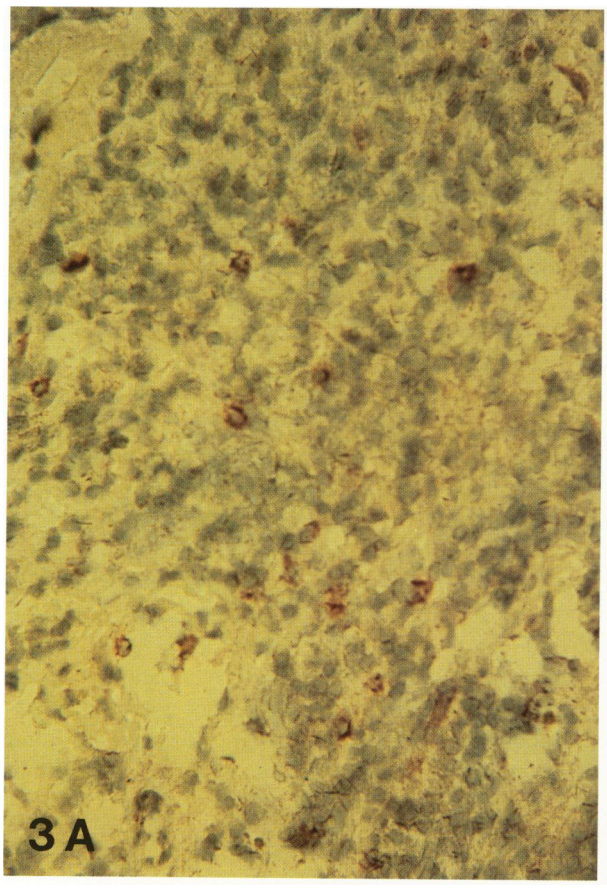
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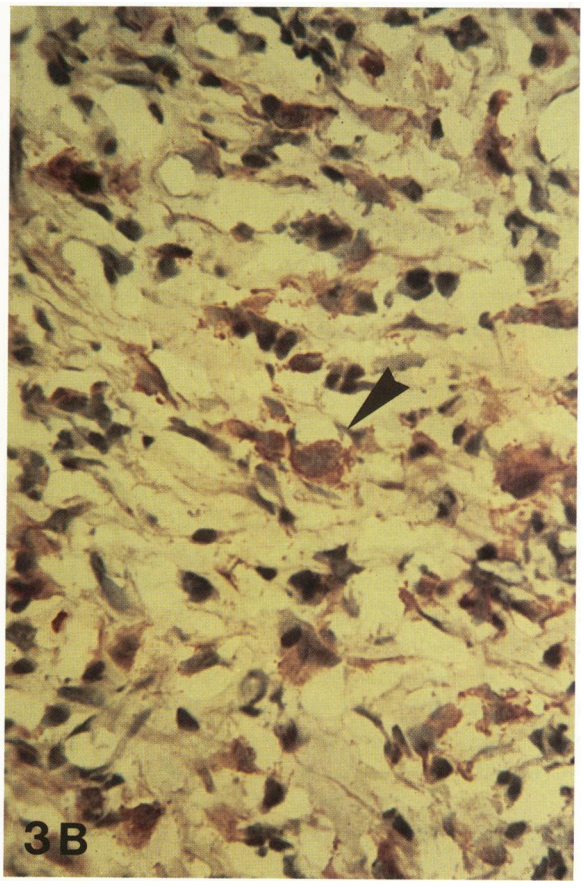
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they are important for the growth *in vivo*. Although autocrine stimulation through IL-2 does not seem to occur in the cells, H and RS cells may be stimulated by IL-2, which is produced by activated T cells. In fact, H and RS cells are frequently surrounded by CD4-positive T cells, which carry activation markers.[23] Also, H and RS cells might activate surrounding T cells by IL-6, which has been detected in HD-derived cell lines and in primary H and RS cells.[16] Thus the histological pattern seen in HD could be reflected by a complex cytokine network that may regulate the interactions between the tumor cells and reactive bystander cells. One should keep in mind however, that IL-2R molecules were not expressed in all cases with HD. There is also a remarkable heterogeneity within HD with respect to differentiation antigens and antigen-receptor rearrangements in H and RS cells, the expression of cytokines, the presence of EBV, and activation of proto-oncogenes. Thus, the heterogenous pattern suggests that HD represents a syndrome rather than a disease entity.

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Figure 2. *(top) Immunohistochemical localization of IL-2Rα-carrying cells in patient no. 11 with HD:* **A:** *diffusely scattered positive lymphoid cells;* **B:** *focal regions of positive RS cells (arrows) and spindle cells (both: APAAP, ×375).*
Figure 3. *(bottom) Immunohistochemical localization of IL-2Rβ-carrying cells in patient no. 11 with HD:* **A:** *only few scattered positive lymphoid cells are noted;* **B:** *one positive RS cell (arrow) and many spindle cells in the focal lymphocyte depleted areas (both: APAAP; ×375).*

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