

# Interleukin 13 and interleukin 13 receptor are frequently expressed by Hodgkin and Reed-Sternberg cells of Hodgkin lymphoma

Brian F. Skinnider, Andrew J. Elia, Randy D. Gascoyne, Lorenz H. Trümper, Frederike von Bonin, Ursula Kapp, Bruce Patterson, Bryan E. Snow, and Tak W. Mak

**Hodgkin lymphoma (HL) is characterized by the abnormal expression of multiple cytokines, accounting for its unique clinicopathologic features. We have previously shown that interleukin-13 (IL-13) is secreted by HL cell lines and may serve as an autocrine growth factor. To determine the frequency of IL-13 expression in lymphoma patients, tissue sections from 36 patients with classical HL, 5 patients with nodular lymphocyte predominance HL (NLPHL), and 23 patients with non-Hodgkin lymphoma (NHL) were subjected to in situ hybridization. In 31 of 36 cases (86%) of classical HL**

**patients of all histologic subtypes, between 25% to almost 100% of Hodgkin and Reed Sternberg (HRS) cells were positive for IL-13 expression. In contrast, in no case of NLPHL and in only 4 of 23 NHL cases (1 of 5 T-cell-rich B-cell lymphomas, 2 of 5 anaplastic large cell lymphomas, and 1 of 5 peripheral T-cell lymphomas) did the neoplastic cells express IL-13. The expression of the IL-13 receptor chain  $\alpha 1$  (IL-13R $\alpha 1$ ) was also analyzed by in situ hybridization. In 24 of 27 (89%) cases of classical HL, between 25% to 75% of HRS cells, as well as a high frequency of lymphocytes and histo-**

**cytes, were positive for IL-13R $\alpha 1$  expression. These results were confirmed by the construction of complementary DNA libraries from single HRS cells, followed by polymerase chain reaction analysis, in which IL-13R $\alpha 1$  transcripts were found to be present in all 6 cases of HL. These data indicate that expression of IL-13 and IL-13R $\alpha 1$  is a common feature of HRS cells in HL, consistent with the hypothesis that IL-13 may play a role in autocrine growth in classical HL. (Blood. 2001;97:250-255)**

© 2001 by The American Society of Hematology

## Introduction

Hodgkin lymphoma (HL) is unusual among malignancies in that the neoplastic cells, the Hodgkin and Reed-Sternberg (HRS) cells, make up only a small proportion of the clinically detectable mass. The bulk of the tumor is composed of a reactive infiltrate of lymphocytes, eosinophils, plasma cells, histiocytes, and fibroblasts. Although HL has distinct clinical features and responses to treatment that warrant its distinction from the non-Hodgkin lymphomas (NHL), within HL there are variations in the composition of the inflammatory infiltrate, the morphology of the HRS cells, and the presence of the Epstein-Barr virus (EBV).<sup>1</sup> The clinicopathologic features of HL are consistent with an abnormal pattern of cytokine expression of the HRS cells. These cells can express a variety of cytokines, including interleukin-2 (IL-2), IL-5, IL-6, IL-7, IL-9, IL-10, granulocyte-macrophage colony-stimulating factor, lymphotoxin- $\alpha$ , and transforming growth factor- $\beta$  (TGF- $\beta$ ).<sup>2</sup> Some cytokines show variable expression that correlates with specific clinicopathologic features of HL. For example, IL-5 expression is associated with tissue eosinophilia,<sup>3</sup> IL-10 is associated with EBV-positive HL,<sup>4</sup> and TGF- $\beta$  with sclerosis.<sup>5</sup> However, abnormal expression of no one cytokine has been consistently demonstrated in all forms of HL.

IL-13 is a pleiotropic cytokine secreted by activated T cells that shares immunoregulatory characteristics with IL-4.<sup>6,7</sup> IL-13 exerts its effects primarily on B cells and monocytes, stimulating growth

and immunoglobulin (Ig) class switching in B cells, and up-regulation of the low-affinity IgE receptor and MHC class II on B cells and monocytes. We have recently shown that IL-13 is expressed by HL cell lines and in HRS cells from 4 cases of nodular sclerosis HL (NSHL).<sup>8</sup> In that study, antibody-mediated neutralization of IL-13 inhibited proliferation of an HL cell line, suggesting that IL-13 might function as an autocrine growth factor for HL. As well as IL-13, the HL cell lines examined expressed NF-IL3. Expression of the NF-IL3 gene is up-regulated by IL-4,<sup>9</sup> a cytokine that shares signaling pathways with IL-13.<sup>10</sup> The expression of NF-IL3 by HL cells thus supports the existence of autocrine signaling by IL-13 in HL.

IL-13 signals are transduced via the IL-13 receptor (IL-13R) complex, which varies in its composition among cell types.<sup>11,12</sup> Two distinct IL-13R chains have been identified, IL-13R $\alpha 1$  and IL-13R $\alpha 2$ .<sup>11</sup> IL-13R $\alpha 1$  associates with IL-4R $\alpha$  to form a high-affinity IL13R complex, engagement of which triggers proliferation through activation of JAK1 and subsequent phosphorylation of STAT6.<sup>13</sup> This sharing of the IL-4 $\alpha$  chain accounts for the overlapping biologic effects of IL-13 and IL-4.<sup>10,13</sup> Little is known about the IL-13R $\alpha 2$  chain, which was not expressed in several hematopoietic cell lines<sup>12</sup> and which appears to be a decoy receptor.<sup>14</sup> In the mouse, IL-13 can elicit a mitogenic response through IL-13R $\alpha 1$  but not through IL-13R $\alpha 2$ .<sup>15</sup>

From the Amgen Institute, Ontario Cancer Institute, the Department of Medical Biophysics, and the Department of Immunology, University of Toronto, Ontario, Canada; the Department of Pathology, British Columbia Cancer Agency, Vancouver, British Columbia, Canada; the Department of Internal Medicine I, University of Saarland, Homburg, Germany; the Department of Hematology/Oncology, University Medical Center, Freiburg, Germany; and the Department of Oncologic Pathology, Ontario Cancer Institute, Toronto, Ontario, Canada.

Submitted April 14, 2000; accepted September 1, 2000.

**Reprints:** Tak W. Mak, Ontario Cancer Institute/Amgen Institute, 620 University Ave, Suite 706, Toronto, Ontario, Canada M5G 2C1; e-mail: tmak@amgen.com.

The publication costs of this article were defrayed in part by page charge payment. Therefore, and solely to indicate this fact, this article is hereby marked "advertisement" in accordance with 18 U.S.C. section 1734.

© 2001 by The American Society of Hematology

To extend our investigations of the role of IL-13 in the pathogenesis of HL, we analyzed the expression patterns of IL-13 and IL-13R $\alpha$ 1 in a series of lymphomas, including those from patients with HL and NHL. We report here that IL-13 was expressed in 86% of patients with HL examined, a substantially higher frequency than was observed in patients with NHL. Furthermore, IL-13R $\alpha$ 1 was expressed in 89% of patients with HL, reinforcing the potential for IL-13 to act as an autocrine growth factor in HL.

## Materials and methods

### Tissues and cell lines

Formalin-fixed paraffin-embedded biopsy specimens representing the following diseases were investigated: 36 cases of classical HL (23 cases of nodular sclerosis HL [NSHL], including 6 cases of syncytial NSHL,<sup>16</sup> 10 cases of mixed cellularity HL [MCHL], and 3 cases of lymphocyte-depletion HL [LDHL]), 5 cases of nodular lymphocyte predominance HL (NLPHL), 5 cases of T-cell-rich B-cell lymphoma (TCRBCL), 8 cases of diffuse large B-cell lymphoma (DLBCL), including 4 cases with anaplastic morphology (3 of which were CD30<sup>+</sup>), 5 cases of anaplastic large-cell lymphoma (ALCL) with a T/null cell phenotype, and 5 cases of peripheral T-cell lymphoma, unspecified (PTCL). All cases were diagnosed according to the Revised European-American Lymphoma Classification.<sup>17</sup> The cases of ALCL and TCRBCL were selected from previous studies.<sup>18,19</sup>

HL cell lines L540Cy, L428, HD-LM2, and KM-H2 were provided by Dr H. G. Drexler (Braunschweig, Germany). HL cell line L1236 and Burkitt lymphoma (BL) cell line BL-31 were provided by Prof V. Diehl (Cologne, Germany). A previous study<sup>12</sup> demonstrated the presence of IL-13R $\alpha$ 1 messenger RNA (mRNA) in a BL cell line; BL-31 was therefore used as a positive control. Complementary DNA (cDNA) was prepared from cell lines using an mRNA purification kit (Pharmacia, Freiburg, Germany) and subjected to polyA amplification as described.<sup>20</sup>

### Probes

The IL-13 probe was a 388-base pair (bp) fragment that has been previously described.<sup>8</sup> The IL-13R $\alpha$ 1 probe was an 810-bp *EcoRI-ScaI* fragment cloned from the IMAGE consortium clone number 755037.<sup>21</sup> The cDNA was subcloned into pBluescript SK (Stratagene, La Jolla, CA), and sense and antisense probes were synthesized from the linearized vector with T3 or T7 RNA polymerase, labeled with [<sup>32</sup>P]-UTP and processed as previously described.<sup>22</sup>

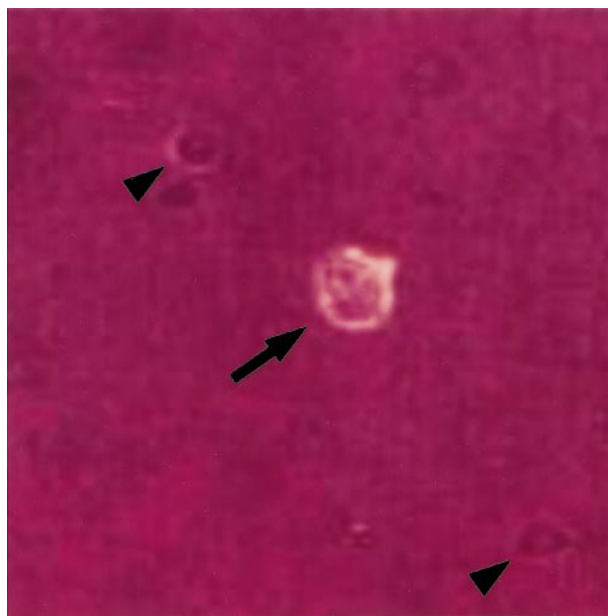
### In situ hybridization

The in situ hybridization procedure was performed as previously described.<sup>22</sup> Sections were deparaffinized in xylene, rehydrated in series of decreasing ethanol concentrations, washed in saline for 5 minutes, and washed 2  $\times$  5 minutes in phosphate-buffered saline (PBS). After fixation in 4% paraformaldehyde for 20 minutes and another 5-minute wash in PBS, sections were treated with 20  $\mu$ g/mL proteinase K for 7.5 minutes at room temperature. After 5 minutes in 4% paraformaldehyde and another 2  $\times$  5-minute washes in PBS, sections were acetylated for 10 minutes in a 0.1 M triethanolamine pH 8.0/0.25% acetic anhydride solution, washed for 5 minutes in PBS, and dehydrated. Approximately 150  $\mu$ L hybridization solution (50% formamide, 0.3 M NaCl, 20 mM Tris-HCl, pH 8.0, 5 mM EDTA, 10 mM NaPO<sub>4</sub>, pH 8.0, 10% dextran sulfate, 1  $\times$  Denhardt's, 0.5 mg/mL yeast transfer RNA [tRNA], 10 mM DTT, and approximately 75 000 cpm/ $\mu$ L of labeled sense or antisense probe) was applied to each slide. Slides were overlain with coverslips and incubated overnight in a humid chamber at 55°C. The coverslips were removed by dipping slides into 5  $\times$  SSC containing 0.1%  $\beta$ -mercaptoethanol. The slides were then immersed for 30 minutes in washing buffer (50% formamide, 2  $\times$  SSC, 0.1%  $\beta$ -mercaptoethanol) at 62°C, rinsed 2  $\times$  15 minutes in NTE (0.5 M NaCl, 10 mM Tris-HCl, pH 7.5, 5 mM EDTA) at 37°C, treated for 30

minutes at 37°C with 20  $\mu$ g/mL RNaseA, and washed for 15 minutes in NTE. The slides were then incubated for 25 minutes in washing buffer at 62°C, 15 minutes in 2  $\times$  SSC at 37°C, 15 minutes in 0.1  $\times$  SSC at 37°C, and dehydrated. The slides were coated with Kodak NTB 2 emulsion (Eastman Kodak, Rochester, NY) and stored at 4°C. After exposure for 10 to 14 days, the slides were developed in Kodak D19 (Eastman Kodak) and counterstained with toluidine blue. The specificity of the hybridization signal was verified by the corresponding lack of signal when sense RNA probes were used. Cells were scored as positive if the number of silver grains was at least 4-fold over background. Control  $\beta$ -actin mRNA was detected using a commercially available kit (Dako, Carpinteria, CA), following the manufacturer's instructions.

### Single-cell reverse transcriptase-polymerase chain reaction

Single-cell suspensions were prepared from pathologic lymph nodes freshly isolated from 6 patients with HL who were not included in the in situ hybridization analysis. Individual HRS cells were identified by their CD30 expression and distinctive morphologic features (Figure 1) as previously described.<sup>23</sup> In this study,<sup>23</sup> the correct identity of the HRS cells in an EBV<sup>+</sup> HL case was supported by demonstrating an EBV gene expression pattern that is seen in HL, BL, and nasopharyngeal carcinoma.<sup>24</sup> The cDNA of individual HRS cells was synthesized using the global polyA-polymerase chain reaction (PCR) method as previously described.<sup>20</sup> Only cases with a positive  $\beta$ -actin signal were used for subsequent IL-13R $\alpha$ 1 PCR. IL-13R $\alpha$ 1 cDNA was amplified using the following primers: 5' CAT GAA GAG GAT GCT GTG AAA TTC CCA ACA AAC 3' and 5' GTT AAA CAG AAA CAA TCC CTG GTT GAA GAC TAC C 3'. PCR analysis was performed in a 50  $\mu$ L volume containing 200 nM of each primer, 1.5 mM MgCl<sub>2</sub>, 1.5 units AmpliTaq Gold (Perkin Elmer, Boston, MA), with 4% DMSO. PCR conditions were as follows: denaturing at 94°C for 1 minute, annealing at 58°C for 1 minute, and extension at 72°C for 2 minutes, for 40 cycles. Products were analyzed by agarose gel electrophoresis. PCR products were confirmed by hybridization with a [<sup>32</sup>P]-end-labeled internal oligonucleotide (5' ATG GGA AAT CCA CTG ATA CAG ACA CCT CCA AGA GC 3'), as previously described.<sup>20</sup> Appropriate positive (cDNA from BL-31 or KM-H2 cells) and negative (cells with no reverse transcriptase; H<sub>2</sub>O) controls were included.



**Figure 1. Identification of single HRS cells in cell suspension.** A single cell suspension was prepared from a lymph node involved by HL, stained with an anti-CD30 antibody coupled to phycoerythrin, and viewed under an inverted fluorescence microscope as previously described.<sup>23</sup> A single HRS cell is identified by its large size, bilobed nucleus with prominent nucleoli, and CD30 expression (arrow). Note smaller CD30-negative cells in the background (arrowheads).

**Table 1. Expression of IL-13 and IL-13R $\alpha$ 1 in Hodgkin lymphomas and non-Hodgkin lymphomas**

Diagnosis	IL-13 <sup>+</sup> cases*/total cases (%)	IL-13R $\alpha$ 1 <sup>+</sup> cases*/total cases (%)
Classical Hodgkin lymphoma	31/36 (86%)	24/27 (89%)
Nodular sclerosis	21/23 (91%)	16/17 (94%)
Mixed cellularity	8/10 (80%)	5/7 (71%)
Lymphocyte depletion	2/3 (67%)	3/3 (100%)
Nodular lymphocyte predominance Hodgkin lymphoma	0/5	ND
Diffuse large B-cell lymphoma	0/8	ND
T-cell-rich B-cell lymphoma	1/5 (20%)	ND
Anaplastic large-cell lymphoma	2/5 (40%)	ND
Peripheral T-cell lymphoma, unspecified	1/5 (20%)	ND

\*Cases with IL-13 or IL-13R $\alpha$ 1 positive malignant cell population (HRS cells in classical HL, L&H cells in NLPHL, and large B-cells in TCRBCL); ND = not done.

## Results

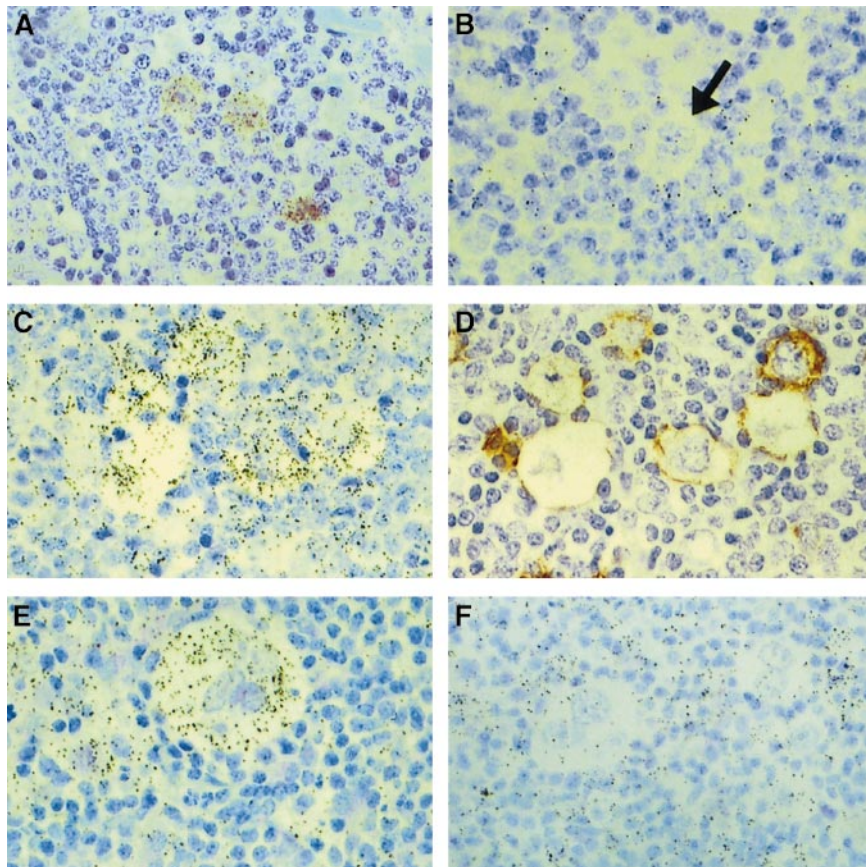
### IL-13 is expressed by HRS cells in a large majority of patients with HL

IL-13 expression was analyzed by in situ hybridization in tissue samples from 36 patients with classical HL, 5 patients with NLPHL, and 23 patients with NHL. IL-13 transcripts were detected in HRS cells in 31 of 36 (86%) patients with classical HL (Table 1, Figure 2A). In contrast, the lymphocytic and histiocytic (L&H) cells in all 5 patients with NLPHL were negative for IL-13 (Table 1; Figure 2B). Similarly, only 1 of 5 patients with TCRBCL demon-

strated IL-13 expression in less than 25% of the malignant cell population, and only 2 of 5 patients with ALCL were IL-13 positive (25% to 50% of cells). Only 1 of 5 patients with PTCL had scattered malignant cells (less than 10%) that expressed IL-13, and all 8 patients with DLBCL were negative for IL-13.

Among the classical HL cases, no significant differences in IL-13 expression were detected among subtypes of classical HL: 21 of 23 (91%) of nodular sclerosis cases, 8 of 10 (80%) of mixed cellularity cases, and 2 of 3 (67%) of lymphocyte depletion cases contained positive HRS cells ( $P$  = not significant) (Table 2). Nearly all cells expressing IL-13 demonstrated the morphology of HRS cells, with only rare small lymphocytes in 19 of 36 (53%) cases also staining positively. Staining of serial sections with either anti-CD30 or anti-CD15 to identify HRS cells showed that the percentage of IL-13-expressing HRS cells varied from case to case, ranging from 25% to almost 100%. A study of the slides in the same hybridization batch and exposed for the same length of time revealed that there was no significant difference among the different subtypes of classical HL in the percentage of HRS cells staining positively for IL-13. Similarly, although the intensity of the IL-13 signal varied from case to case, there was no significant difference in the distribution of signal intensity among subtypes of classical HL.

All 5 IL-13 negative cases showed a positive signal for IL-13R $\alpha$ 1 (see below), excluding the possibility of false-negative results due to degradation of RNA before fixation or during tissue processing. Selected NHL cases that were negative for IL-13 expression were analyzed for  $\beta$ -actin mRNA by in situ hybridization. All of these were positive for  $\beta$ -actin expression (data not shown), again suggesting that the RNA was intact.



**Figure 2. IL-13 and IL-13R $\alpha$ 1 expression in Hodgkin lymphoma.** In situ hybridization for IL-13 (A, B) and IL-13R $\alpha$ 1 (C, E, F); black-colored silver grains denote mRNA expression. HRS cells from a case of mixed cellularity HL (A) express IL-13, whereas an L&H cell (arrow) from a case of NLPHL (B) is negative for IL-13. HRS cells from 2 cases of nodular sclerosis HL (C, E) express IL-13R $\alpha$ 1, whereas HRS cells from a case of mixed cellularity HL (F) are negative for IL-13R $\alpha$ 1. Serial sections from one of the cases of nodular sclerosis (C, D) were hybridized with an IL-13R $\alpha$ 1 antisense probe (C) or stained for CD30 (D), illustrating IL-13R $\alpha$ 1 expression in CD30<sup>+</sup> HRS cells. Note IL-13R $\alpha$ 1 expression in surrounding lymphocytes and histiocytes (C, F).



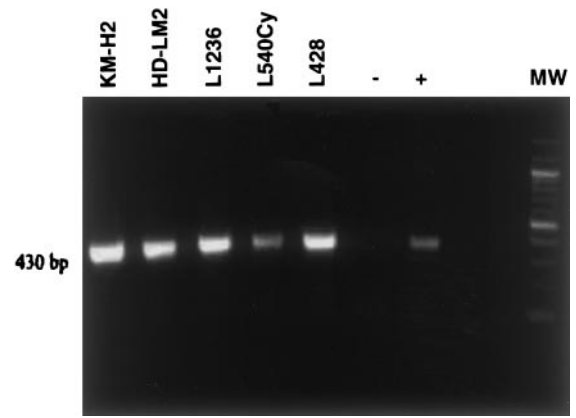
**Table 2. IL-13 and IL-13R $\alpha$ 1 expression in patients with Hodgkin lymphoma**

Case no.	Diagnosis	Age/sex	Stage	IL-13 + HRS cells	IL-13R $\alpha$ 1 + HRS cells
1	NSHL	16/M	IV B	+	+
2	NSHL	24/F	II A	+	+
3	NSHL	15/F	II A	+	+
4	NSHL	43/M	IV B	+	+
5	NSHL	33/M	III B	+	+
6	NSHL	30/M	II A	+	+
7	NSHL	32/F	II B	+	+
8	NSHL	25/F	III B	+	ND
9	NSHL	27/F	II A	+	+
10	NSHL	22/M	III B	+	U
11	NSHL	34/M	III A	+	+
12	NSHL	19/M	III A	+	+
13	NSHL	81/F	II A	+	ND
14	NSHL	36/M	III B	+	+
15	NSHL	31/F	IV A	+	ND
16	NSHL	NA	NA	+	ND
17	NSHL	NA	NA	+	+
18	NSHL, syn	64/M	III B	-	-
19	NSHL, syn	20/F	II A	+	+
20	NSHL, syn	35/M	I B	-	+
21	NSHL, syn	45/M	III A	+	+
22	NSHL, syn	41/F	II A	+	U
23	NSHL, syn	25/M	III B	+	+
24	MCHL	NA	NA	+	+
25	MCHL	NA	NA	-	-
26	MCHL	28/M	II A	+	+
27	MCHL	28/M	II B	+	ND
28	MCHL	42/M	I A	+	+
29	MCHL	NA/M	II A	+	ND
30	MCHL	42/F	III A	+	+
31	MCHL	NA/F	II A	+	+
32	MCHL	45/M	I A	+	ND
33	MCHL	88/M	I A	-	-
34	LDHL	54/F	IV B	+	+
35	LDHL	65/M	NA	-	+
36	LDHL	72/F	IV B	+	+

NSHL = nodular sclerosis Hodgkin lymphoma; syn = syncytial variant; MCHL = mixed cellularity Hodgkin lymphoma; LDHL = lymphocyte-depletion Hodgkin lymphoma; U = uninterpretable; ND = not done; NA = not available.

**IL-13R $\alpha$ 1 is expressed by HRS cells expressing IL-13**

To determine whether IL-13-expressing HRS cells also expressed IL-13R $\alpha$ 1, and thus could become subject to autocrine stimulation, IL-13R $\alpha$ 1 expression was examined by in situ hybridization in 29 cases of classical HL and was interpretable in 27 cases (see below). Twenty-four cases (89%) contained HRS cells positive for IL-13R $\alpha$ 1 expression (Figure 2C-F). No significant differences in IL-13R $\alpha$ 1 expression were detected among subtypes of classical HL: 16 of 17 (94%) of nodular sclerosis cases, 5 of 7 (71%) of mixed cellularity cases, and 3 of 3 (100%) of lymphocyte-depletion cases contained positive HRS cells ( $P = NS$ ). The percentage of IL-13R $\alpha$ 1 positive HRS cells varied from 25% to 75% in different cases. In all cases, IL-13R $\alpha$ 1 expression was not limited to HRS cells, but was also present in a large proportion of cells in reactive infiltrates that could be morphologically defined as lymphocytes and histiocytes (Figure 2C,F). IL-13R $\alpha$ 1 transcripts could not be detected in the fibroblasts within the sclerotic bands in cases of NSHL. As a positive control, a benign reactive tonsil was hybridized with the IL-13R $\alpha$ 1 probe. Positive signals were obtained for cells morphologically resembling histiocytes (including tingible-body macrophages in reactive germinal centers) and numerous lymphocytes outside the germinal center (data not



**Figure 3. IL-13R $\alpha$ 1 expression by HL cell lines.** The cDNA was prepared from HL cell lines (KM-H2, HD-LM2, L1236, L540Cy, and L428) and amplified by PCR using IL-13R $\alpha$ 1-specific oligonucleotides, followed by agarose gel electrophoresis. The presence of the 430-bp PCR product indicates IL-13R $\alpha$ 1 gene expression in all 5 HL cell lines examined. Negative control (-): H<sub>2</sub>O; positive control (+): cDNA from BL-31 cells. MW = 100-bp ladder.

shown). Two cases of HL that contained reactive germinal centers with tingible-body macrophages did not show the IL-13R $\alpha$ 1 signal in any cell population, and were therefore considered noninterpretable.

Of the 27 cases of classical HL analyzed for both IL-13 and IL-13R $\alpha$ 1, 22 cases were IL-13<sup>+</sup> / IL-13R $\alpha$ 1<sup>+</sup>; 3 cases were IL-13<sup>-</sup> / IL-13R $\alpha$ 1<sup>-</sup>; and 2 cases were IL-13<sup>-</sup> / IL-13R $\alpha$ 1<sup>+</sup> (Table 2). The relationship between IL-13 and IL-13R $\alpha$ 1 expression was statistically significant ( $P = .003$ ).

**Expression of IL-13R $\alpha$ 1 as determined by PCR of individual HRS cell cDNA libraries**

To confirm the presence of IL-13R $\alpha$ 1 mRNA in individual HRS cells, cDNA libraries of mRNA from HRS cells were constructed and analyzed by PCR with IL-13R $\alpha$ 1-specific primers. First, the presence of IL-13R $\alpha$ 1 sequences in 5 HRS cell lines (KM-H2, HD-LM2, L1236, L540Cy, and L428) was examined. IL-13R $\alpha$ 1 transcripts were detected in all 5 cell lines (Figure 3). These studies were extended to construct cDNA libraries from single HRS cells from 6 patients with HL, including 4 patients with NSHL and 2 patients with MCHL. In all 6 patients, IL-13R $\alpha$ 1 sequences were detected in 30% to 85% of HRS cells (Table 3; Figure 4) and were confirmed by hybridization with an internal oligonucleotide (data not shown).

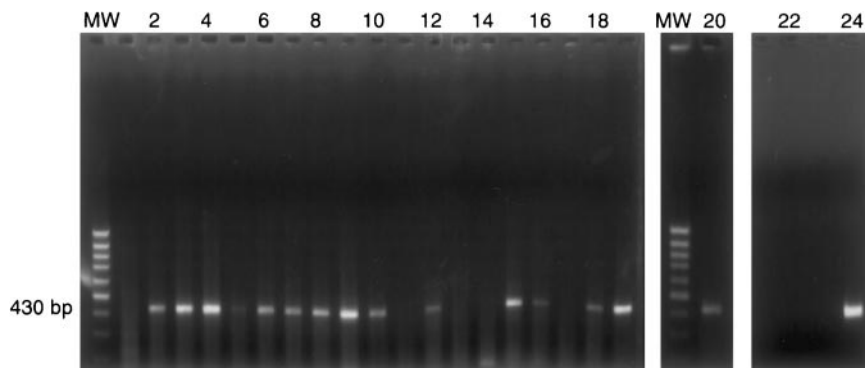
**Discussion**

We have shown that IL-13 is expressed in a variable percentage of HRS cells in 86% of the classical HL cases examined. Transcripts

**Table 3. IL-13R $\alpha$ 1 polymerase chain reaction from single Hodgkin and Reed-Sternberg cell complementary DNA libraries**

Case no.	Diagnosis	Age/sex	No. HRS cells examined	No. IL-13R $\alpha$ 1 + HRS cells/no. HRS cells examined (%)
37	NSHL	25/F	20	15/20 (75%)
38	NSHL	28/F	20	6/20 (30%)
39	NSHL	33/M	8	3/8 (37.5%)
40	NSHL	26/M	20	7/20 (37%)
41	MCHL	29/M	20	17/20 (85%)
42	MCHL	30/M	19	12/19 (63%)

NSHL = nodular sclerosis Hodgkin lymphoma; MCHL = mixed cellularity Hodgkin lymphoma.



**Figure 4. IL-13R $\alpha$ 1 expression by individual HRS cells.** Single HRS cells from case 37 were micromanipulated and subjected to polyA PCR as described in "Materials and methods." PolyA cDNA products from 20 individual HRS cells (lanes 1-20) were amplified by PCR with IL-13R $\alpha$ 1-specific oligonucleotides. The 430-bp PCR product indicates IL-13R $\alpha$ 1 gene expression in 15 of 20 single HRS cells. The presence of IL-13R $\alpha$ 1 cDNA was confirmed by hybridization with an internal oligonucleotide. MW = 100-bp ladder; lanes 1-20: single HRS cells; lane 21: negative control (single HRS cell, no reverse transcriptase); lane 22: blank; lane 23: negative control (H<sub>2</sub>O); lane 24: positive control (cDNA from KM-H2 cells).

for IL-13 are present in HRS cells of NSHL, MCHL, and LDHL, with no significant differences in the intensity of signal or percentage of positive cells between HL subtypes. The HRS cells appear to be the dominant source of IL-13, with rare lymphocytes showing a positive signal in approximately half of the cases. There were no morphologic differences between IL-13 positive and IL-13 negative cases, either in the presence of fibrosis or composition of reactive infiltrate. It is possible that IL-13 is still present in these "negative" cases but at levels undetectable by *in situ* hybridization. Alternatively, some cases of HL may be truly negative for IL-13, in which case other cytokines presumably play a role in their pathogenesis. Our data suggest that IL-13 expression is a basic property of HRS cells in the majority of cases of HL, regardless of histologic subtypes.

IL-13 mRNA was not detected in the L&H cells of NLPHL, which shows clinical, morphologic, and immunophenotypic features distinct from those of classical HL.<sup>25,26</sup> HRS cells in classical HL are typically CD15<sup>+</sup>CD30<sup>+</sup> and are negative for B-cell markers in the majority of cases.<sup>1</sup> Classical HL is believed in many cases to be a B-lineage lymphoproliferative disorder in which the HRS cells demonstrate clonal rearrangement and somatic mutations of the Ig heavy chain genes but do not express Ig.<sup>27,28</sup> The L&H cells of NLPHL, on the other hand, demonstrate a B-cell phenotype, produce functional Ig, and are typically CD15<sup>-</sup>CD30<sup>-</sup>. The absence of IL-13 in NLPHL further emphasizes the difference between these 2 distinct disease entities.

IL-13 is produced predominantly by activated T cells and acts on several cell types.<sup>6,7</sup> In B cells, IL-13 promotes survival and proliferation, as well as Ig class switching to IgG4 and IgE.<sup>6,7,29</sup> IL-13 plays an important role in Th2 cell differentiation in the mouse,<sup>30</sup> but its exact role in T-cell development in humans is unclear. Although some investigators have reported that human T cells lack surface IL-13 receptors,<sup>31</sup> others have shown that T cells can respond to IL-13 *in vitro*.<sup>32</sup> Human fibroblasts express the IL-13 receptor,<sup>10,14</sup> and IL-13 has been shown to play a role in development of fibrosis in asthma<sup>33</sup> and parasite-associated hepatic damage.<sup>34</sup> In view of these effects, many features of HL, including the reactive infiltrate of T and B cells, histiocytes, as well as the fibrosis in NSHL, may be attributable to IL-13 expression. Indeed, it has been shown that in transgenic mice that overexpress IL-13 in the lung, elevated levels of IL-13 lead to the presence of a mononuclear infiltrate, eosinophilia, and fibrosis.<sup>33</sup>

Although the effects of IL-13 on target cells have been reasonably well studied, the receptor complex through which it acts is much less well understood. In the best characterized receptor complex, IL-13R $\alpha$ 1 associates with IL-4 $\alpha$ , but the IL-13 receptor structure varies among different cell types.<sup>10-13</sup> The precise function of the IL-13R $\alpha$ 2 chain has yet to be defined.<sup>14,15</sup> In this study, *in situ* hybridization of tissue sections showed that HRS cells in

89% of classical HL cases expressed IL-13R $\alpha$ 1. IL-13R $\alpha$ 1 expression was also demonstrated using PCR of cDNA libraries made from single HRS cells picked from cell suspensions of lymph nodes with HL involvement. The presence of IL-13R $\alpha$ 1 on HRS cells that also express IL-13 suggests the potential existence of autocrine regulatory loops influencing HRS cell proliferation. These results support the findings of our previous study in which it was shown that HL cell lines both produce IL-13 and express the IL-13 receptor, and that neutralization of IL-13 can lead to inhibition of proliferation.<sup>8</sup> However, whereas cells in the majority of HL cases in this study expressed both IL-13 and IL-13R $\alpha$ 1, HRS cells in a few cases expressed neither the cytokine nor the receptor, implying that IL-13 does not act as an autocrine growth factor in all cases. The clinical significance of the absence of IL-13 and IL-13R $\alpha$ 1 in these cases remains to be determined.

In addition to HRS cells, many histiocytes and lymphocytes expressed IL-13R $\alpha$ 1. The presence of the receptor on those cells surrounding the IL-13 positive HRS cells would allow a paracrine mechanism to facilitate the accumulation of the mixed inflammatory infiltrate characteristic of HL. However, although IL-13R $\alpha$ 1 is expressed in human fibroblast cell lines,<sup>10,14</sup> IL-13R $\alpha$ 1 expression could not be detected in fibroblasts in the sclerotic bands of NSHL cases examined in this study. The particular role that IL-13 plays in the development of fibrosis in NSHL remains unclear because there was no difference in IL-13 expression between cases of NSHL and MCHL, which by definition lack sclerotic collagenous bands.

IL-13 was also detected in 3 NHL cases that share some morphologic or immunophenotypic features with HL, including 1 of 5 cases of TCRBCL and 2 of 5 cases of ALCL. TCRBCL is an aggressive NHL that morphologically resembles HL because of its characteristic prominent reactive component of benign T cells and histiocytes,<sup>19,35</sup> suggesting that it is also a malignancy with abnormal cytokine expression. Indeed, the neoplastic cells of both HL and TCRBCL have been shown to produce the CC chemokine TARC (thymus and activation-regulated chemokine), likely accounting for the prominent T-cell infiltration of both tumors.<sup>36</sup> Although IL-13 transcripts were detected in the malignant cells of only one case of TCRBCL in this study, expression of IL-4, whose biologic effects overlap those of IL-13, was found by others in malignant cells of additional TCRBCL cases.<sup>37</sup> HL shows an opposite expression pattern of these 2 related cytokines, namely, positive expression of IL-13 but negative expression of IL-4.<sup>4</sup> These results suggest that IL-4 and IL-13 may contribute to a malignant phenotype to different degrees in different types of lymphomas.

Because T cells are the predominant source of IL-13 secretion, we analyzed 9 cases of T-cell lymphomas for the presence of IL-13, including cases of ALCL and PTCL. ALCL shares some features with HL, including pleomorphic morphology of the neoplastic cells

and CD30 expression.<sup>18</sup> In situ hybridization studies showed that 2 cases of ALCL and one case of PTCL contained IL-13 positive cells. Unlike HL, these cases contained a homogeneous population of lymphoma cells without a prominent reactive infiltrate. These data do not permit us to conclude that IL-13 plays a defining role in the pathogenesis of subsets of T-cell lymphomas. In some tumors, IL-13 expression may merely reflect the retention of gene expression patterns of the cell of origin.

In conclusion, we have shown that IL-13 and IL-13R $\alpha$ 1 are frequently expressed in HRS cells of HL. IL-13 is important for normal B-cell physiology, inducing survival and proliferation of these cells.<sup>29</sup> Because most cases of classical HL are derived from B cells,<sup>27,28</sup> IL-13 may also function as a growth factor for these tumors. The possible role of IL-13 in autocrine growth of HRS cells and paracrine stimulation of the reactive infiltrate suggests that IL-13 signaling is a potential pharmacological target in the treatment of patients with HL. Inhibition of IL-13 signaling for therapeutic purposes

may not be seriously detrimental to the immune system of patients with HL. Although the biologic activities of IL-13 and IL-4 overlap, IL-4 is not a cytokine associated with HL.<sup>4</sup> Mice deficient for IL-13 (but wild type for IL-4) are still able to develop a Th2 cytokine and Ig response to parasitic infection, although clearance of the pathogen is less efficient.<sup>30</sup> Thus, medical intervention to block IL-13–mediated autocrine stimulation of HRS cells should not compromise an IL-4–mediated Th2 response, allowing the patient some measure of immune protection during antitumor treatment.

## Acknowledgments

We thank J. Ho for excellent technical support; H. G. Drexler, B. Dörken, and V. Diehl for HL-derived and BL-derived cell lines; M. Bray for valuable critical comments; N. Le for statistical analysis; and M. Saunders for scientific editing.

## References

- Harris NL. Hodgkin's disease: classification and differential diagnosis. *Mod Pathol*. 1999;12:159-176.
- Kadin ME, Liebowitz DN. Cytokines and cytokine receptors in Hodgkin's disease. In: Mauch PM, Armitage JO, Diehl V, Hoppe RT, Weiss LM, eds. *Hodgkin's Disease*. Philadelphia, PA: Lippincott Williams & Wilkins; 1999:139-158.
- Samoszuk M, Nansen L. Detection of interleukin-5 messenger RNA in Reed-Sternberg cells of Hodgkin's disease with eosinophilia. *Blood*. 1990;75:13-16.
- Herbst H, Foss H-D, Samol J, et al. Frequent expression of interleukin-10 by Epstein-Barr virus—harboring tumor cells of Hodgkin's disease. *Blood*. 1996;87:2918-2929.
- Kadin ME, Agnarsson BA, Ellingsworth LR, Newcom SR. Immunohistochemical evidence of a role for transforming growth factor beta in the pathogenesis of nodular sclerosing Hodgkin's disease. *Am J Pathol*. 1990;136:1209-1214.
- Zurawski G, de Vries JE. Interleukin 13, an interleukin 4-like cytokine that acts on monocytes and B cells, but not on T cells. *Immunol Today*. 1994;15:19-26.
- Minty A, Chalton P, Derocq JM, et al. Interleukin-13 is a new human lymphokine regulating inflammatory and immune responses. *Nature*. 1993;362:248-250.
- Kapp U, Yeh WC, Patterson B, et al. Interleukin 13 is secreted by and stimulates the growth of Hodgkin and Reed-Sternberg cells. *J Exp Med*. 1999;189:1939-1946.
- Chu CC, Paul WE. Expressed genes in interleukin-4 treated B cells identified by cDNA representational difference analysis. *Mol Immunol*. 1998;35:487-502.
- Murata T, Husain SR, Mohri H, Puri RK. Two different IL-13 receptor chains are expressed in normal human skin fibroblasts, and IL-4 and IL-13 mediate signal transduction through a common pathway. *Int Immunol*. 1998;10:1103-1110.
- Jensen PL. The interleukin 13 receptor complex. *Stem Cells*. 2000;18:61-62.
- Murata T, Obiri NI, Debinski W, Puri RK. Structure of IL-13 receptor: analysis of subunit composition in cancer and immune cells. *Biochem Biophys Res Commun*. 1997;238:90-94.
- Lin JX, Migone TS, Tsang M, et al. The role of shared receptor motifs and common Stat proteins in the generation of cytokine pleiotropy and redundancy by IL-2, IL-4, IL-7, IL-13, and IL-15. *Immunity*. 1995;2:331-339.
- Feng N, Lugli SM, Schnyder B, et al. The interleukin-4/interleukin-13 receptor of human synovial fibroblasts: overexpression of the nonsignaling interleukin-13 receptor alpha2. *Lab Invest*. 1998;78:591-602.
- Donaldson DD, Whitters MJ, Fitz LJ, et al. The murine IL-13 receptor alpha 2: molecular cloning, characterization, and comparison with murine IL-13 receptor alpha 1. *J Immunol*. 1998;161:2317-2324.
- Strickler JG, Michie SA, Wamke RA, Dorfman RF. The "syncytial variant" of nodular sclerosing Hodgkin's disease. *Am J Surg Pathol*. 1986;10:470-477.
- Harris NL, Jaffe ES, Stein H, et al. A revised European-American classification of lymphoid neoplasms: a proposal from the International Lymphoma Study Group. *Blood*. 1994;84:1361-1392.
- Gascoyne RD, Aoun P, Wu D, et al. Prognostic significance of anaplastic lymphoma kinase (ALK) protein expression in adults with anaplastic large cell lymphoma. *Blood*. 1999;93:3913-3921.
- Skinnider BF, Connors JM, Gascoyne RD. Bone marrow involvement in T-cell-rich B-cell lymphoma. *Am J Clin Pathol*. 1997;108:570-578.
- Trumper LH, Brady G, Bagg A, et al. Single-cell analysis of Hodgkin and Reed-Sternberg cells: molecular heterogeneity of gene expression and p53 mutations. *Blood*. 1993;81:3097-3115.
- Lennon G, Auffray C, Polymeropoulos M, Soares MB. The IMAGE Consortium: an integrated molecular analysis of genomes and their expression. *Genomics*. 1996;33:151-152.
- Hui C-C, Joyner AL. A mouse model of Greig cephalopolysyndactyly syndrome: the *extra-toes*<sup>1</sup> mutation contains an intragenic deletion of the *Gli3* gene. *Nat Genet*. 1993;3:241-246.
- Trumper L, Daus H, Merz H, et al. NPM/ALK fusion mRNA expression in Hodgkin and Reed-Sternberg cells is rare but does occur: results from single-cell cDNA analysis. *Ann Oncol*. 1997;8:S83-S87.
- Anagnostopoulos I, Hummel M. Epstein-Barr virus in tumours. *Histopathology*. 1996;29:297-315.
- Mason DY, Banks PM, Chan J, et al. Nodular lymphocyte predominant Hodgkin's disease: a distinct clinicopathological entity. *Am J Surg Pathol*. 1994;18:526-530.
- von Wasielewski R, Werner M, Fischer R, et al. Lymphocyte-predominant Hodgkin's disease: an immunohistochemical analysis of 208 reviewed Hodgkin's disease cases from the German Hodgkin Study Group. *Am J Pathol*. 1997;150:793-803.
- Kuppers R, Rajewsky K. The origin of Hodgkin and Reed/Sternberg cells in Hodgkin's disease. *Annu Rev Immunol*. 1998;16:471-493.
- Marafioti T, Hummel M, Foss H-D, et al. Hodgkin and Reed-Sternberg cells represent an expansion of a single clone originating from a germinal center B-cell with functional immunoglobulin gene rearrangements but defective immunoglobulin transcription. *Blood*. 2000;95:1443-1450.
- Ford D, Sheehan C, Girasole C, et al. The human B cell response to IL-13 is dependent on cellular phenotype as well as mode of activation. *J Immunol*. 1999;163:3185-3193.
- McKenzie GJ, Bancroft A, Grenics RK, McKenzie ANJ. A distinct role for interleukin-13 in Th2-cell-mediated immune responses. *Curr Biol*. 1998;8:339-342.
- Graber P, Gretener D, Herren S, et al. The distribution of IL-13 receptor alpha1 expression on B cells, T cells and monocytes and its regulation by IL-13 and IL-4. *Eur J Immunol*. 1998;28:4286-4298.
- Yu CR, Kirken RA, Malabarba MG, Young HA, Ortaldo JR. Differential regulation of the Janus kinase-STAT pathway and biologic function of IL-13 in primary human NK and T cells: a comparative study with IL-4. *J Immunol*. 1998;161:218-227.
- Zhu Z, Homer RJ, Wang Z, et al. Pulmonary expression of interleukin-13 causes inflammation, mucus hypersecretion, subepithelial fibrosis, physiologic abnormalities, and eotaxin production. *J Clin Invest*. 1999;103:779-788.
- Chiaramonte MG, Donaldson DD, Cheever AW, Wynn TA. An IL-13 inhibitor blocks the development of hepatic fibrosis during a T-helper type 2-dominated inflammatory response. *J Clin Invest*. 1999;104:777-785.
- Greer JP, Macon WR, Lamar RE, et al. T-cell-rich B-cell lymphomas: diagnosis and response to therapy of 44 patients. *J Clin Oncol*. 1995;13:1742-1750.
- van den Berg A, Visser L, Poppema S. High expression of the CC chemokine *TARC* in Reed-Sternberg cells. *Am J Pathol*. 1999;154:1685-1691.
- Macon WR, Cousar JB, Waldron JA Jr, Hsu SM. Interleukin-4 may contribute to the abundant T-cell reaction and paucity of neoplastic B cells in T-cell-rich B-cell lymphoma. *Am J Pathol*. 1992;141:1031-1036.