High Expression of the CC Chemokine *TARC* in Reed-Sternberg Cells

A Possible Explanation for the Characteristic T-Cell Infiltrate in Hodgkin's Lymphoma

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Hodgkin's lymphoma is characterized by the combination of Reed-Sternberg (R-S) cells and a prominent inflammatory cell infiltrate. One of the intriguing questions regarding this disease is what is causing the influx of T lymphocytes into the involved tissues. We applied the serial analysis of gene expression (SAGE) technique on the Hodgkin's lymphoma-derived cell line L428 and on an Epstein-Barr virus (EBV)-transformed lymphoblastoid B-cell line. A frequently expressed tag in L428 corresponded to the T-cell-directed CC chemokine *TARC*. Reverse transcription polymerase chain reaction analyses demonstrated expression of *TARC* in nodular sclerosis (NS) and mixed cellularity (MC) classical Hodgkin's lymphomas but not in NLP Hodgkin's lymphoma, anaplastic large-cell lymphomas, and large-B-cell lymphomas with CD30 positivity. Two of five cases of T-cell-rich B-cell lymphoma (TCRBCL) were *TARC* positive. RNA *in situ* hybridization (ISH) showed a strong signal for *TARC* in the cytoplasm of R-S cells, and immunohistochemical staining confirmed the presence of the *TARC* protein in the R-S cells of NS and MC Hodgkin's lymphomas. The lymphocytic and histiocytic (L&H)-type cells of nodular lymphocyte predominance Hodgkin's lymphoma and the neoplastic cells of non-Hodgkin's lymphomas with the exception of two cases of TCRBCL did not stain for *TARC*. *TARC* is known to bind to the CCR4 receptor, which is expressed on activated Th2 lymphocytes. The immunophenotype of lymphocytes surrounding R-S cells is indeed Th2-like, and by RNA ISH these lymphocytes showed a positive signal for the chemokine receptor *CCR4*. The findings suggest that production of *TARC* by the R-S cells may explain the characteristic T-cell infiltrate in classical Hodgkin's lymphoma. (Am J Pathol 1999, 154:1685-1691)

Hodgkin's lymphoma is characterized by the presence of a minority of neoplastic cells, the so-called Reed-Stern-

berg cells and their mononuclear variants (R-S cells) and a vast majority of reactive cells, including predominantly T lymphocytes, eosinophils, histiocytes, and plasma cells. The R-S cells have recently been demonstrated to have clonal rearrangements of the immunoglobulin genes with numerous somatic mutations of the variable regions, suggesting a germinal center B cell origin.¹ The presence of otherwise crippling mutations such as stop codons suggests rescue of the R-S precursors from apoptosis by a transforming event such as the Epstein-Barr virus (EBV), which indeed is present in a high proportion of cases of classical Hodgkin's lymphoma.

The second characteristic feature of Hodgkin's lymphoma is the presence of CD4-positive T lymphocytes that strongly bind to the R-S cells and have phenotypic features of Th2 helper T cells.^{2,3} One of the intriguing questions is what causes the influx of T lymphocytes into the involved tissues. It has been hypothesized that R-S cells secrete potent cytokines that stimulate their own growth, allow them to evade immune surveillance, and cause the systemic symptoms of Hodgkin's disease.^{4,5}

To gain insight into the genes that are expressed in R-S cells as opposed to normal EBV-transformed B cells, we applied serial analysis of gene expression (SAGE).⁶ This technique allows the construction of a comprehensive expression profile and results in the quantitation of expression levels of the corresponding genes. We analyzed a Hodgkin's lymphoma-derived cell line (L428) and an EBV-transformed lymphoblastoid B-cell line (RAY) for comparison. Differentially expressed genes were further analyzed in tissues involved by Hodgkin's lymphoma and some related lymphoma types.

Materials and Methods

Cell Lines

The L428, L540, L591, and L1236 Hodgkin's lymphoma-derived cell lines^{7,8} were made available to us by Dr.

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Table 1. Primer Sequences Used for the Amplification of *TARC*, *CCR4*, *CCR8*, and *GAPDH*

Gene	Forward primer	Reverse primer	PCR product (bp)
<i>TARC</i>	ACCTGCACACAGAGACTCC	ATCTGGGCCCTTTGTGCCC	510
<i>CCR4</i>	TTGGACTATGCCATCCAGGC	AATTCCTCTGGAGAAACCC	590
<i>CCR8</i>	AGTTCAGCATGAAGGATGCC	CTGTAGTCCTCCATAAGCC	430
<i>GAPDH</i>	CCATCACTGCCACTCAGAAGACT	TTACTCCTTGAGGCCATGTAGG	469

Volker Diehl and co-workers (Cologne, Germany). The RAY and POP lymphoblastoid cell lines were initiated by transforming peripheral blood B cells with EBV. Large-B-cell non-Hodgkin lymphoma cell lines VER and Rose were established in our laboratory (unpublished data). VER was derived from a large-cell B-cell lymphoma with t(8;14) secondary to Hodgkin's lymphoma. Rose was derived from a large-cell B-cell lymphoma with t(14;18) derived from a transformed follicular lymphoma. Anaplastic large-cell lymphoma cell line KARPAS 299 was obtained from American Type Culture Collection, Rockville, MD.⁹

Tissues

Frozen tissue specimens and cell suspensions of lymph nodes involved by Hodgkin's and non-Hodgkin's lymphomas came from the tissue bank of the Department of Pathology. We randomly selected eight cases of nodular sclerosis (NS), four cases of mixed cellularity (MC), and six cases of nodular lymphocyte predominance (NLP) subtype. In addition, four cases of anaplastic large-cell lymphoma (ALCL) (T/0), six cases of large-B-cell lymphoma with CD30 positivity, and five T-cell-rich B-cell lymphomas (TCRBCL) were studied. As control tissues we included a lymph node with dermatopathic lymphadenopathy, a lymph node with follicular and diffuse hyperplasia, and a case with progressively transformed germinal centers.

Purification of R-S Cells

A cell suspension of one of the lymph nodes involved by NS Hodgkin's lymphoma was incubated with anti-CD30 (Mab BerH2, gift of Dr. Harald Stein, Berlin, Germany), and the CD30-positive R-S cells were isolated by binding to sheep anti-mouse Ig-labeled magnetic beads (Dyna, Oslo, Norway). The cells labeled with magnetic beads were isolated with the equipment provided by the manufacturer. The nonbound cells were saved as the depleted population. The bound cells were washed three times to remove the contaminating CD30-negative cells and kept as the enriched cells. The efficiency of the purification was checked in cytopins from the starting lymphoma cell population, the enriched CD30-positive cells, and the depleted cell population. RNA was isolated from the enriched CD30-positive R-S cell population and from the CD30-depleted cell population.

SAGE Procedure

A detailed protocol for the SAGE procedure and the SAGE300 computer program (version 3.03) used for the analysis of the tags were kindly provided by Dr. Kinzler (Johns Hopkins Oncology Center, Baltimore, MD).⁶ mRNA was isolated from the L428 and RAY cell lines (starting from 1×10^7 to 2×10^7 cells), converted into double-stranded (ds)-cDNA (TimeSaver cDNA synthesis kit, Amersham Pharmacia Biotech, Rainham, UK), and digested with *Nla*III. The 3'-primed ds-cDNA fragments were isolated and ligated to a linker. A second restriction enzyme digest with *Bsm*FI was performed, resulting in 50-bp fragments containing the linker sequences and 10 to 12 gene-specific bases that originate downstream from the *Nla*III restriction site (the so-called tags). After filling in of the sticky ends, the blunt-ended DNA fragments were pooled and ligated (resulting in the so-called ditags). Large-scale amplification of the ditags then gave polymerase chain reaction (PCR) products of ~100 bp consisting of two 10- to 12-nucleotide fragments from two different genes flanked by the linker sequences. These PCR products were digested with *Nla*III to purify the gene-specific sequences from the linkers. The 24- to 28-bp fragments containing two gene-specific sequences were ligated overnight to obtain concatemers. The concatemers were cloned in the pUC18 vector (digested with *Sph*I) and transformed to *Escherichia coli* DH5- α cells (Gibco BRL, Paisley, UK). The clones were sequenced on an automated sequencer (ALF, Amersham Pharmacia Biotech). The sequence files were analyzed with the SAGE300 computer program.⁶ The tag sequences were used to screen for homologies with genes present in the GenBank. In case a gene corresponded to a tag, the 15th base that usually is present in the ditag sequence was used to confirm the homology.

RT-PCR on Hodgkin Lymphoma Tissues for TARC, CCR4, and CCR8

Total RNA was isolated with Trizol (Gibco BRL, Gaithersburg, MD) from cell suspension and from cryostat tissue sections. The cDNA syntheses was primed with oligo(dT) using the protocol provided by the manufacturer (MBI Fermentas, St. Leon-Rot, Germany). Primer sequences used for the amplification are listed in Table 1. PCR for all primer sets was performed with 1 U of *Taq* polymerase (Pharmacia Biotech) and the reaction buffer provided by the manufacturer. The PCR program consisted of 33 cycles with a denaturation step of 30 seconds at 94°C, an

annealing step of 45 seconds at 57°C, and an extension step of 45 seconds at 72°C. The first denaturation step lasted for 5 minutes, and the final extension step lasted for 7 minutes.

In Situ Hybridization for TARC and CCR4

The RT-PCR products were subcloned in the pCRII-TOPO vector (Invitrogen, Carlsbad, NM). Digoxigenin (DIG)-labeled RNA probes were made with the DIG RNA labeling kit (Sp6/T7) (Boehringer Mannheim, Mannheim, Germany). *In situ* hybridization (ISH) was performed on routinely fixed paraffin-embedded tissue sections using standard laboratory protocols.

Immunohistochemistry

Immunohistochemical staining was performed with an affinity-purified goat anti-human TARC antibody (R&D Systems, Minneapolis, MN) on paraffin tissue sections after heat-induced antigen retrieval. Peroxidase-labeled rabbit anti-goat antibody (DAKO, Copenhagen, Denmark) followed by peroxidase enzyme staining with diaminobenzidine and H₂O₂ were used to visualize the TARC-positive cells. Paraffin sections of 12 cases of NS, 4 cases of MC, and 6 cases of NLP Hodgkin's lymphoma, 7 lymph nodes with follicular and/or diffuse hyperplasia, 1 lymph node with progressively transformed germinal centers, 20 common B-cell non-Hodgkin lymphomas, 6 anaplastic large-cell B-cell lymphomas, 4 T/O anaplastic large-cell lymphomas, and 5 T-cell-rich B-cell lymphomas were stained.

Results

The SAGE technique was applied on the Hodgkin's lymphoma-derived cell line L428 and for comparison on the EBV-transformed lymphoblastoid cell line RAY. For reasons of economy we sequenced only a limited number of tags. For L428 we sequenced 1055 tags (representing 701 independent genes), and for RAY we sequenced 634 tags (representing 474 independent genes). The electronic comparison resulted in the identification of 98 tags that occurred in both libraries and 979 tags that occurred only in the L428 or in the RAY library. Of the L428-specific tags, 576 occurred only once, whereas the remaining 125 tags occurred up to 26 times (Table 2). For RAY, 417 of the tags occurred only once, whereas the remaining 57 tags occurred up to 25 times. Homology screening of the L428 tags to the known human sequences (release 107.0 of the GenBank) resulted in the identification of the corresponding genes for 247 of the tags. Among these genes we detected housekeeping genes, such as *GAPDH* and *β-actin*, and a high number of ribosomal genes, of which 34 of 49 were present in both libraries. In addition, we also identified a tag corresponding to *CD30*, a known R-S cell marker, and *Fascin* for which a high expression has been reported in Hodgkin's lymphoma.¹⁰ Screening of the remaining tags for homology to human

Table 2. Overview of the 1055 SAGE Tags Identified in the L428 Hodgkin Cell Line

Total number of tags	Frequency	Homology to known sequences		Total number of tags with homology (%)
		Genes	EST	
1	26×	0	0	
1	22×	0	0	
1	21×	1		
1	15×	0	0	
1	11×	1		
1	10×	1		
1	9×	1		
4	7×	4		100
8	6×	6	1	87.5
9	5×	5	3	89
12	4×	10	1	92
26	3×	19	5	92
59	2×	41	12	90
576	1×	158	221	66

EST sequences resulted in the identification of a corresponding sequence for 243 (Table 2). An overview of the most frequently occurring L428-specific tags is given in Table 3. One of the tags corresponded to the T-cell-directed CC chemokine *TARC* (thymus and activation regulated chemokine)¹¹ (GenBank accession number D43767) and was detected nine times in L428 (0.9%) and not in RAY. Primers specific for *TARC* were selected from the nucleotide sequence and used for the amplification of the *TARC* mRNA in L428, RAY, and some other Hodgkin's and non-Hodgkin's lymphoma cell lines (Figure 1). *TARC* was expressed only in the Hodgkin's lymphoma-derived cell lines L428, L491, L540, and L1236 and not in the lymphoblastoid B-cell lines RAY and POP and non-Hodgkin's lymphoma-derived cell lines (Table 4). To determine whether the expression of *TARC* was consistently present in and specific for Hodgkin's lymphoma we analyzed a number of other Hodgkin's and non-Hodgkin's lymphoma tissue and cell suspension samples (Figure 2). *TARC* expression was found in the eight tissues with NS and in the four with MC Hodgkin's lymphoma (Table 5). Hyperplastic tonsils and lymph nodes were negative with the exception of a case of dermatopathic lymphadenopathy that had a high content of Langerhans' and interdigitating reticulum cells.

The level of *TARC* expression varied between the different Hodgkin's lymphoma samples. As this might be caused by variation in the percentage of R-S cells in the different tumor samples and to assess whether the *TARC* signal was derived from the R-S cells or other cell types, R-S cells from a case of NS Hodgkin's lymphoma relatively rich in R-S cells were enriched with anti-CD30 magnetic beads. The original suspension had 2% CD30-positive cells, the enriched fraction had 50% CD30-positive cells, and the depleted fraction contained only sporadic CD30-positive cells (<1 per 10⁶). This resulted in an enhanced signal in the enriched cells and a decreased signal in the depleted population, despite the fact that less cDNA was present in the enriched fraction as shown by the *GAPDH* signals (Figure 3). The findings

Table 3. Overview of the Most Frequently Occurring L428-Specific Tags

Frequency of L428 tags	Sequence	Homology
9×	GGCACAAAGG	T-cell-directed chemokine TARC
6×	AGCACCTCCA	Elongation factor 2
5×	CTGCTATACG	Ribosomal protein L5
5×	TCAGAAGTTT	
5×	TCCCCCGTAC	
4×	AGCCCTACAA	NADH dehydrogenase III
4×	CCTCGGAAAA	Ribosomal protein L38
4×	CTCAACATCT	Acidic ribosomal phosphoprotein PO
4×	GATGCTGCCA	Ribosomal protein L22
4×	TGGTGTTGAG	Ribosomal protein S18
3×	ACACAGCAAG	
3×	CACTACTCAC	
3×	CTCCTCACCT	BAK
3×	GACGTGTGGG	Histone
3×	GGCTGGGGCC	Medullasin
3×	TGAAATAAAA	Nucleolar phosphoprotein B23
3×	9 tags	Ribosomal genes
2×	ACTGGGTCTA	NM23-H2
2×	GGCAGAGGAC	NM23-H1
2×	ATAGTAGCTT	FASCIN
2×	GAAGCAGGAC	Cofilin
2×	CGCAAGCTGG	Lamin C
2×	GGGGAAATCG	Thymosin β -10
2×	GCTGGGGTGG	FADD
2×	CCTATAATCC	Retinoblastoma-related protein p107
2×	3 tags	Ribosomal genes
2×	18 tags	

support that the *TARC* signals indeed derive from the R-S cells.

In situ hybridization for *TARC* on paraffin tissue sections involved by Hodgkin's lymphoma demonstrated a strong specific signal with the antisense probe diffusely in the cytoplasm of R-S cells (Figure 4A). Other cell types were negative, and no signal was obtained with the control sense probe. Immunohistochemical staining for the *TARC* protein on paraffin sections resulted in a very strong signal in the cytoplasm of R-S cells with a diffuse or a distinct paranuclear dot-like staining pattern (Figure 4C). The 12 NS-type cases stained stronger than the 4 MC-type cases, whereas all 6 cases of NLP type were negative. The only other staining for *TARC* was found in interdigitating cells in reactive lymph nodes and was much weaker.

Figure 1. RT-PCR analysis for *TARC* and *CCR4* on Hodgkin's and non-Hodgkin's lymphoma cell lines. **A:** RT-PCR analysis of *TARC* (33 cycles); **B:** RT-PCR analysis of *CCR4* (33 cycles); **C:** RT-PCR analysis of *GAPDH* (33 cycles). The samples analyzed are L428 (Hodgkin), L540 (Hodgkin), L591 (Hodgkin), L1236 (Hodgkin), Rose (non-Hodgkin), Ver (non-Hodgkin), Karpas (anaplastic), RAY (EBV-transformed B-cell), and Pop (EBV-transformed B-cell). Positive *TARC* signals are seen only in the Hodgkin cell lines.

The six samples involved by NLP Hodgkin's disease were negative for *TARC* by RT-PCR as well as by immunohistochemistry. ALCL of T/O phenotype as well as large B-cell lymphomas with CD30 positivity also had no expression of the *TARC* gene and protein. One of five T-cell-rich B-cell lymphomas studied was positive for *TARC* by RT-PCR, and by *in situ* hybridization on paraffin tissue sections an additional case of TCRBCL showed a specific signal for the antisense *TARC* probe in the tumor cells. Positive staining for *TARC* could be confirmed in both cases with immunohistochemical staining. Other common B-cell non-Hodgkin lymphomas, including small lymphocytic B-cell lymphomas (five cases), follicular lymphomas (five cases), mantle cell lymphomas (five cases), and diffuse large B-cell lymphomas (five cases), were all negative for *TARC* by immunohistochemistry.

We also analyzed the samples for expression of *CCR4* and *CCR8*,¹²⁻¹⁴ which are known to be specific receptors

Table 4. RT-PCR Analyses for *TARC*, *CCR4*, and *CCR8* on Hodgkin and Non-Hodgkin Cell Lines

Cell line	Origin	<i>TARC</i>	<i>CCR4</i>	<i>CCR8</i>
L428	Hodgkin	+	+	±
L540	Hodgkin	±	±	±
L591	Hodgkin EBV+	±	±	±
L1236	Hodgkin	+	+	±
Rose	Non-Hodgkin	—	±	+
Ver	Non-Hodgkin	—	±	+
Karpas	Anaplastic	—	+	+
RAY	B-cell	—	±	+
Pop	B-cell	—	±	+

+, a strong positive result was obtained for the RT-PCR analysis; ±, a moderate signal was obtained for the RT-PCR analysis; —, no PCR product could be detected in the RT-PCR analysis.

Figure 2. RT-PCR analysis for *TARC* and *CCR4* on Hodgkin and non-Hodgkin tumor tissues and cell suspensions. **A:** RT-PCR analysis of *TARC* (33 cycles); **B:** RT-PCR analysis of *CCR4* (33 cycles); **C:** RT-PCR analysis of *GAPDH* (33 cycles). The samples analyzed are MC (mixed cellularity Hodgkin), NS (nodular sclerosis Hodgkin), NLP (nodular lymphocyte predominance Hodgkin), B-ANA (B-cell anaplastic large cell lymphoma), T/0-ANA (T or null cell anaplastic large-cell lymphoma), TCRBCL (T-cell-rich B-cell lymphoma). Positive *TARC* signals are seen only in the NS and MC Hodgkin samples and one of the TCRBCL samples. Positive *CCR4* signals are present in all samples.

for TARC. All Hodgkin's and non-Hodgkin's lymphoma cell line and tissue samples were found to be positive by RT-PCR (Figures 1 and 2; Tables 4 and 5). In normal peripheral blood cells, expression of *CCR4* could be detected only after a 24-hour stimulation with PHA. *In situ* hybridization with an antisense probe for *CCR4* showed a small dot-like signal in the cytoplasm of a high proportion of the lymphocytes surrounding the R-S cells (Figure 4B).

Table 5. RT-PCR Analysis for *TARC*, *CCR4*, and *CCR8* on Hodgkin and Non-Hodgkin Tumor Samples

Tissue	Number of cases	<i>TARC</i>	<i>CCR4</i>	<i>CCR8</i>
Lymph node*	1	+	±	+
Lymph node†	1	—	+	+
MC Hodgkin	4	+	+	+
NS Hodgkin	8	+	+	+
NLP Hodgkin	6	—	+	+
PKC‡	1	—	+	+
Anaplastic B-cell	6	—	+	+
Anaplastic T/0-cell	4	—	+	+
TCRBCL	3	—	+	+
TCRBCL§	2	+	+	+

*Lymph node with dermatopathic lymphadenopathy.

†Lymph node with follicular and diffuse hyperplasia.

‡Progressively transformed germinal centers and follicular hyperplasia.

§For one of the two cases expression of *TARC* was determined only by *in situ* hybridization because no frozen tissue was available.

Figure 3. RT-PCR analysis of *TARC* in a suspension of a NS Hodgkin's lymphoma involved lymph node, comparing the signals in the original cell suspension with those in the CD30-positive cell-enriched fraction and in the depleted fraction. The enriched fraction shows an enhanced signal for *TARC* (33 cycles) (row A) despite the fact that the *GAPDH* signal (33 cycles) is clearly weaker due to the much lower number of cells (row B).

Figure 4. RNA *in situ* hybridization of *TARC* and *CCR4*. **A:** *In situ* hybridization with an antisense *TARC* probe on a NS Hodgkin's lymphoma shows cytoplasmic staining in lacunar R-S cells. **B:** *In situ* hybridization with an antisense *CCR4* probe on the same Hodgkin case shows dot-like staining in many of the lymphocytes. **C:** Immunohistochemical staining of a representative case of NS Hodgkin's lymphoma for TARC protein. A clear diffuse or dot-like staining can be found in the R-S cells present in the Hodgkin's lymphoma tissue.

No *CCR4* signal was found in the R-S cells in these tissue sections, and the sense probe also showed no positive signal.

Discussion

Our results demonstrate consistently high expression of the CC chemokine *TARC* by RT-PCR in Hodgkin's lymphoma cell lines and in tissues involved by NS and MC subtypes of Hodgkin's lymphoma. The presence in enriched R-S cells and the specific localization by *in situ* hybridization confirm that *TARC* mRNA indeed is present

in R-S cells of these subtypes. Moreover, TARC protein was demonstrated by immunohistochemical analysis. Expression of TARC has been reported constitutively in the thymus and in activated peripheral blood monocytes.¹¹ TARC may play a role in T-cell development in the thymus and in trafficking and activation of mature T cells.¹¹

Binding assays of the known CC chemokine receptors (CCR 1 to 5) have shown that TARC binds to the CCR4 receptor with high affinity, and expression of CCR4 has been reported for T-cell lines and activated peripheral blood T cells.¹² Analysis of T-cell clones and polarized Th1 and Th2 cells has indicated that expression of CCR4 is specific for activated Th2 cells whereas CCR5 is expressed on Th1 cells.^{13–15} More recently, TARC was also shown to bind to CCR8.¹⁶ Expression of CCR8 has been reported for interleukin-2-treated T lymphocytes.¹⁷ RT-PCR analysis revealed the presence of CCR4 mRNA in all Hodgkin's lymphoma samples and also in the Hodgkin's lymphoma cell lines analyzed. By RNA *in situ* hybridization, CCR4 mRNA was found only in the T cells surrounding the R-S cells and not in the R-S cells. The presence of CCR4 in the Hodgkin's and non-Hodgkin's lymphoma cell lines may be an artifact as the growth of these cells *in vitro* is an unusual event that may require or induce expression of certain genes.

The characteristics of the T cells directly surrounding the R-S cells are consistent with an activated Th2-like phenotype.^{2,3} The high expression of TARC in the R-S cells and the expression of CCR4 in the surrounding T cells suggest that the CC chemokine TARC may be responsible for the attraction of Th2 lymphocytes into the tissues involved by Hodgkin's lymphoma. Migration and binding of lymphocytes to tumor cells *in vivo* and *in vitro*, the so-called lymphocyte rosetting phenomenon, occurs around R-S cells and Hodgkin's but not non-Hodgkin's lymphoma cell lines and correlates with TARC expression.

The lymphocytic & histiocytic (L&H)-type R-S cells of the NLP subtype of Hodgkin's lymphoma do not express the TARC chemokine. This presents yet another distinction from the other subtypes. The L&H cells have a more typical germinal center B-cell phenotype with somatic hypermutations but no crippling mutations^{1,18,19} and frequently express membrane or cytoplasmic immunoglobulin.²⁰ The lymphocytic infiltrate in NLP Hodgkin's lymphoma also differs and consists of predominantly small B lymphocytes with rosettes around the L&H cells of CD57-positive CD4 cells that are normally found in the light zones of germinal centers.²¹ The presence of a similar lymphocyte population in progressively transformed germinal centers, a potential precursor lesion of NLP Hodgkin's lymphoma in which L&H cells are lacking, suggests that the L&H cells are not responsible for the influx of the T lymphocytes in this subset.

The tumor cells of ALCL have several morphological and immunophenotypic similarities to R-S cells, but all cases of T/0 type, whether ALK positive or negative, were found to be TARC negative. Cases of large B-cell lymphoma with anaplastic morphology and/or CD30 positivity were also negative. It will be of interest to see how so-called cases of Hodgkin's-like ALCL²² will segregate

and whether expression of TARC or other dendritic cell characteristics is clinically relevant.

Another lymphoma with similarities to Hodgkin's lymphoma is TCRBCL, which comprises a group of non-Hodgkin lymphomas characterized by neoplastic large B cells and a high content of T lymphocytes. In two of five cases, expression of TARC was detected. This indicates that in some cases of this type of non-Hodgkin's lymphoma, TARC may be involved in the lymphocyte attraction in a similar way as in Hodgkin's lymphoma and suggests that also other chemokines may be involved.

TARC expression is normally restricted to cells that have antigen-presenting functions, such as the interdigitating cells in the paracortex of lymph nodes. These cells have much weaker expression than the R-S cells. The mechanisms that lead to the very high expression of TARC in R-S cells are not known. Many cytokines, including interleukin (IL)-1, IL-5, IL-6, IL-9, IL-10, and transforming growth factor- β are produced by R-S cells,⁴ and it is suspected that constitutive nuclear expression of nuclear factor (NF)- κ B is responsible for this phenomenon.^{23,24} NF- κ B activation caused by the HTLV-1-encoded transactivator Tax can lead to the expression of several chemokines.²⁵ It is therefore possible that the up-regulation of TARC also results from NF- κ B activation.

The significance of a high expression of TARC by R-S cells is that TARC may cause an influx of activated Th2-type lymphocytes and thereby contribute to the characteristic lymphocytic infiltrate of Hodgkin's lymphoma. The upside of this phenomenon is that it may provide an early warning of the disease by leading to an exaggerated swelling of the node while still relatively few tumor cells are present. The downside is that a Th2-type response in combination with immunosuppressive factors such as IL-10 and transforming growth factor- β may prevent an effective immune response against the R-S cells.

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