Differential Expression of Thymus and Activation Regulated Chemokine and Its Receptor CCR4 in Nodal and Cutaneous Anaplastic Large-Cell Lymphomas and Hodgkin's Disease

M. H. Vermeer, D. F. Dukers, R. L. ten Berge, E. Bloemena, L. Wu, W. Vos, E. de Vries, C. P. Tensen, C. J. L. M. Meijer, M.D., Ph.D., R. Willemze

Departments of Dermatology (MHV, EdV, CPT, RW) and Pathology (DFD, RLtB, EB, WV, CJLMM), Vrije Universiteit Amsterdam, the Netherlands; and Millennium Pharmaceuticals Inc. (LW), Cambridge, Massachusetts

Recent studies demonstrated that Hodgkin/Reed-Sternberg (H/RS) cells in Hodgkin's disease (HD) express thymus and activation-regulated chemokine (TARC), whereas reactive lymphocytes surrounding H/RS cells express its ligand, CC-chemokine receptor 4 (CCR4). Because *in vitro* studies showed that CCR4 expression is a marker for lymphocytes bearing a T-helper 2 (Th2) phenotype, it was suggested that expression of TARC is a new immune escape mechanism in HD. To find out whether this mechanism might also be operative in CD30+ malignant lymphomas other than HD, TARC and CCR4 expression was investigated by immunohistochemistry on paraffin and frozen-tissue sections of 39 nodal CD30+ anaplastic large cell lymphomas (ALCL), including 27 ALK-negative and 12 ALK-positive ALCL, 25 primary cutaneous CD30+ ALCL, including 11 patients with lymphomatoid papulosis, and 31 cases of HD. TARC was expressed by the neoplastic cells in 12/27 (44%) nodal ALK-negative ALCL and all cases of classic HD, but not in nodal ALK-positive ALCL (0/12) and only rarely in primary cutaneous CD30+ ALCL (3/25). In contrast, CCR4 was expressed by the neoplastic cells in 9/9 cutaneous CD30+ ALCL, and in 9/15 (60%) nodal ALK-negative ALCL, but only in 1/4 (25%) nodal ALK-positive ALCL and not by the H/RS cells in HD (0/8). Apart from three cases of HD showing 10 to 15% CCR4-positive lymphocytes surrounding TARC-positive H/RS cells, CCR4-positive

reactive T cells were few (<5%) in all other cases studied. Our results demonstrate a differential expression of TARC and CCR4 in different types of CD30+ malignant lymphomas. The small number of CCR4-positive reactive T cells in most cases studied argues against an important role of TARC expression in the evasion of antitumor responses.

KEY WORDS: ALCL, CCR4, Chemokine, TARC.

Mod Pathol 2002;15(8):838-844

Chemokines are members of a family of small, secreted proteins that are potent activators and chemoattractants for leukocyte subpopulations. Chemokines act as ligands for a family of seven-transmembrane-spanning G-protein-coupled receptors (1, 2). Chemokine receptor expression on different cell types and their binding and response to specific chemokines are highly variable (2). By virtue of this diversity, chemokines can direct the selective migration of lymphocyte subsets from intravascular to extravascular compartments, trafficking of lymphocyte subsets within lymph node and thymus and/or recirculation of lymphocytes from tissues to lymphatics (3).

Recent studies have started to investigate the expression of chemokines and chemokine receptors in Hodgkin's disease (HD) and non-Hodgkin lymphomas (NHL; 4-6). Chemokines are considered to play an important role both in the selective homing of neoplastic B and T cells, which may contribute to the preferential localization of some types of lymphomas at certain sites, and in the recruitment of inflammatory cells, which may modulate antitumor responses. In a recent study, Van den Berg *et al.* (7) reported expression of the chemokine TARC (thymus and activation-regulated chemokine) by Reed-Sternberg cells in all cases of mixed cellularity (mc) and nodular sclerosing (ns) Hodgkin's disease. TARC is considered to play a role in T-cell develop-

Copyright © 2002 by The United States and Canadian Academy of Pathology, Inc.

VOL. 15, NO. 8, P. 838, 2002 Printed in the U.S.A.

Date of acceptance: April 19, 2002.

Authors Vermeer and Dukers contributed equally to this study.

Address reprint requests to: C.J.L.M. Meijer M.D., Ph.D., Department of Pathology, VU Medical Center, De Boelelaan 1117 1816 TH Amsterdam, The Netherlands; e-mail: cjlmm@vumc.nl; fax: 31-20-444-2964.

DOI: 10.1097/01.MP.0000021006.53593.B0

ment in the thymus and in trafficking and activation of mature T cells (8). TARC-positive Reed-Sternberg cells were surrounded by T cells expressing CC-chemokine receptor 4 (CCR4), one of the receptors of TARC, suggesting that TARC production by Hodgkin/Reed-Sternberg (H/RS) cells could play a role in the influx of reactive T-lymphocyte subsets (7). Because CCR4 is preferentially expressed by Th2-polarized T cells expressing IL-4, IL-5, and IL-10 (9, 10), it was postulated that the preferential attraction of CCR4-bearing Th2 lymphocytes may cause a shift towards a Th2-dominated cytokine microenvironment, thereby hampering the cytotoxic immune response and providing a mechanism by which neoplastic cells are able to escape from the immune system. Whether such a mechanism might also be operative in CD30-positive malignant lymphomas other than HD is as yet unknown, because data on the expression of TARC and CCR4 in these conditions are scarce.

In the present study, we therefore investigated expression patterns of TARC and CCR4 in a large group of 64 CD30-positive NHL, including ALK-positive and ALK-negative nodal anaplastic large cell lymphomas (ALCL), CD30-positive primary cutaneous ALCL, and lymphomatoid papulosis (LyP); in addition, 31 cases of HD were investigated.

MATERIALS AND METHODS

Patients

Paraffin-embedded material of the following lymphoma types, classified according to the World Health Organization (WHO) criteria (11), were retrieved from the archives of the Department of Pathology of the Vrije Universiteit Medical Center, Amsterdam, the Netherlands: nsHD ($n = 18$), mcHD ($n = 6$), and lymphocyte predominant (lp) HD ($n = 7$) and nodal ALCL ($n = 39$), including 12 ALK-positive and 27 ALK-negative ALCL, as assessed by staining with monoclonal antibody ALK-1 (12, 13). Frozen material of these lymphomas was available in 8 cases of nsHD and in 19 nodal ALCL.

Primary cutaneous lymphomas were classified according to the WHO criteria and also according to the classification of primary cutaneous lymphomas of the European Organization for the Research and Treatment of Cancer (11, 14) and included 11 primary cutaneous CD30-positive ALCL and 15 cases of lymphomatoid papulosis (LyP). Frozen material was available of 5 primary cutaneous ALCL and 4 cases of LyP.

All cases were reviewed by an expert hematopathologist (CJLMM) and dermatologist (RW). Furthermore, all lymphoma diagnoses were confirmed by a panel of expert hematopathologists from the

Comprehensive Cancer Center Amsterdam. Tonsil ($n = 2$), hyperplastic lymph nodes ($n = 2$), thymus ($n = 4$), and skin biopsies from lichen planus ($n = 4$) were included as positive control for the expression of CCR4 and TARC, as described elsewhere(3).

Immunohistochemistry

Lymphoma Phenotyping

Lymphomas were extensively immunophenotyped using monoclonal antibodies against CD3, CD4, CD8, CD45RO, CD15, CD20, CD23 and TIA-1, GrB, CD30, and ALK-1, as described elsewhere (15, 16). In all cases of classical HD, the neoplastic H/RS cells stained with the CD30 antibody. Expression of CD15 was detected in 17/24 cases, and a variable number of neoplastic cells stained positive for CD20 in 6/24 cases. No expression of T-cell markers was found. The tumor cells in all lpHD cases ($n = 7$) were negative for CD30, CD15, and T-cell-associated antigens but were positive for CD20, as expected. The presence of Epstein-Barr virus was detected in 8/24 cases of classical HD by EBER1/2 RNA *in situ* hybridization and LMP1 immunohistochemistry (17).

All cases of ALCL ($n = 39$) were subclassified according to the WHO classification (11). All ALCL showed expression of CD30; EMA was observed in 14/39 cases, and the majority of ALCL expressed T-cell markers (21/27). Expression of B-cell markers CD20 and CD79a was not observed (Table 1).

In primary cutaneous CD30-positive ALCL, expression of CD30 was found in $\geq 75\%$ of the tumor cells. In nearly all cases, the neoplastic cells showed a CD3⁺/CD4⁺/CD8[−] phenotype. The great majority of neoplastic T-cells in LyP expressed CD3, CD4, and CD30; CD8 was never detected.

TARC Detection

Paraffin sections were incubated with TARC polyclonal antibody 1:200 (goat-anti-TARC; R&D Systems, Minneapolis, MN) followed by biotinylated rabbit anti-goat IgG (1:250; Vector, Burlingame, CA). TARC detection on frozen tissues was performed using a mouse monoclonal antibody (moab) 2D8 1:1000 (Leukosite Inc., Boston, MA) and biotinylated rabbit anti-mouse antibodies. In both frozen and paraffin-embedded material, the biotinylated antibodies were detected with horseradish peroxidase-conjugated streptavidin-biotin complex (sABC, DAKO, Glostrup, Denmark) and visualized with diaminobenzidine (3,3'-diaminobenzidine).

Tumors were considered positive if $>5\%$ of tumor cells stained for TARC.

CCR4 Detection

Immunohistochemical detection of CCR4 was performed on frozen material using mouse moab 1G1

TABLE 1. Immunophenotypical Analysis of ALK-Positive and ALK-Negative Cases of Anaplastic Large-Cell Lymphomas

Case No.	CD30	CD15	EMA	B ^a	T ^b	Cyt Phenotype
ALK-positive cases						
1	+	-	-	-	+	+
2	+	-	+	-	+	+
3	+	-	+	-	+	-
4	+	-	+	-	-	+
5	+	-	+	-	+	+
6	+	-	ND	-	+	+
7	+	-	+	-	+	+
8	+	-	+	-	+	+
9	+	-	+	-	+	+
10	+	-	+	-	-	+
11	+	-	+	-	-	+
12	+	-	+	-	+	+
ALK-negative cases						
13	+	-	+	-	+	-
14	+	ND	-	-	+	-
15	+	-	+	-	+	+
16	+	ND	-	-	+	-
17	+	+	-	-	+	-
18	+	ND	-	-	+	+
19	+	+	-	-	+	+
20	+	-	-	-	+	+
21	+	+	-	-	-	-
22	+	-	-	-	+	-
23	+	-	-	-	-	-
24	+	-	ND	-	+	-
25	+	-	-	-	-	-
26	+	ND	-	-	+	-
27	+	-	+	-	+	+
28	+	ND	ND	-	+	+
29	+	-	-	-	+	-
30	+	-	-	-	+	-
31	+	+	ND	-	+	-
32	+	ND	-	-	-	+
33	+	-	-	-	+	+
34	+	-	-	-	+	+
35	+	ND	-	-	+	+
36	+	-	-	-	+	-
37	+	+	-	-	-	-
38	+	ND	-	-	+	-
39	+	-	+	-	+	-

ND, not determined; cyt phenotype; cytotoxic phenotype of the tumor cells.

^aB-cell phenotype as determined by expression of CD20 and/or CD79a.

^bT-cell phenotype as determined by expression of CD3, CD4, CD8, and CD45.

1:100 (Leukosite Inc.). Visualization of the primary antibody was performed using a standard three-step sABC method and 3,3'-diaminobenzidine.

Tumors were considered positive if >5% of tumor cells stained for CCR4.

Double Staining

Double staining of TARC and CCR4 was performed to illustrate the topographical distribution of CCR4-positive infiltrating cells in relation to TARC-expressing neoplastic cells. Goat anti-TARC and 1G1 were applied simultaneously, followed by simultaneous incubation of biotinylated donkey anti-goat and rabbit anti-mouse antibody. Subsequently, biotin was detected by sABC, whereas a double alkaline phosphatase anti-alkaline phos-

phatase was used to detect CCR4. TARC was visualized by 3,3'-diaminobenzidine and CCR4 by new fuchsin.

RESULTS

Hodgkin's Disease

TARC expression was found in paraffin sections of all cases of nsHD ($n = 18$) and mcHD ($n = 6$), showing a strong cytoplasmic staining in the neoplastic H/RS cells (Fig. 1, Table 2). Virtually all H/RS cells showed expression of TARC, whereas no expression was found in small or intermediate-sized cells in the reactive infiltrate. Expression of TARC was also detected in 3/7 cases (43%) of lPHD. In eight cases of nsHD from which frozen material was available, an identical staining pattern for TARC was observed (Fig. 2A). Evaluation of CCR4 expression in these sections showed that H/RS cells were negative for CCR4 in all cases (Tables 2 and 3 and Fig. 2B).

Expression of TARC was also detected on dendritic cells and on endothelial cells, especially in high endothelial venules. Staining for CCR4 was detected as a membranous staining on small numbers (<5%) of reactive T cells in 5 of 8 cases and on higher numbers (10–15%), mainly surrounding the H/RS cells, in 3 of 8 cases. This was confirmed by immunohistochemical double staining as depicted in Figure 2C.

Nodal ALCL

Paraffin sections of 39 cases of ALCL were investigated for the expression of TARC. In 12/39 (31%)

FIGURE 1. Expression of thymus and activation-regulated chemokine (TARC; **brown**) in a case of nodular-sclerosing Hodgkin's disease. TARC staining is seen as a **brown** cytoplasmic or perinuclear staining in neoplastic cells. No staining is observed in small or intermediate-sized cells. Inset, a classical Hodgkin/Reed-Sternberg (H/RS) cell showing strong cytoplasmic expression of TARC.

TABLE 2. Expression of Thymus and Activation–Regulated Cytokine (TARC) and CCR4 by Neoplastic Cells in Hodgkin's Disease and Non-Hodgkin Lymphoma and Expression of CCR4 by the Reactive Infiltrate

Diagnosis	TARC		CCR4	
	Paraffin	Frozen	Neoplastic cells	Reactive Infiltrate
Hodgkin's disease (HD)				
HD, ns	18/18	8/8	0/8	8/8
HD, mc	6/6	ND	ND	ND
HD, lp	3/7	ND	ND	ND
CD30+ T non-Hodgkin lymphomas				
Nodal ALCL ALK positive	0/12	0/4	1/4	4/4
Nodal ALCL ALK negative	12/27	9/15	9/15	15/15
Cutaneous CD30+ ALCL	1/11	0/5	5/5	5/5
Lymphomatoid papulosis	2/15	1/4	4/4	4/4

Cases were considered positive if >5% of the tumor cells expressed TARC or CCR4. ALCL, anaplastic large cell lymphoma; ns, nodular sclerosing; mc, mixed cellularity; lp, lymphocyte predominant; ND, not determined.

cases a clear, dotlike, cytoplasmic staining was observed in the neoplastic cells (Fig. 3) In all TARC-positive cases, >90% of the tumor cells stained positive. TARC expression was observed in the neoplastic T cells of 12/27 (44%) ALK-negative nodal ALCL, but not in any of the 12 ALK-positive cases. Similarly, on frozen tissue, 9/15 (60%) ALK-negative ALCL, but 0/4 ALK-positive ALCL, expressed TARC (see Table 2). The expression of TARC by the neoplastic cells on frozen tissue was identical to paraffin sections in all cases.

Expression of CCR4 by the large anaplastic cells was found in 9/15 (60%) ALK-negative ALCL and 1/4 (25%) ALK-positive ALCL (see Tables 2 and 3). Coexpression of TARC and CCR4 was found in 5/15 (33%) ALK-negative ALCL.

Expression of TARC was also detected on high endothelial venules in all cases. Stainings for CCR4 demonstrated that CCR4 was expressed by a small minority (<5%) of reactive T cells in all cases of nodal ALCL (see Table 2).

Primary Cutaneous CD30-Positive Lymphomas

Staining of paraffin sections showed TARC expression by a minority of the neoplastic CD30-positive cells in 1/11 cases of primary cutaneous ALCL and 2/15 cases of LyP. Expression of CCR4 by neoplastic cells was observed in 5/5 primary cutaneous ALCL and in 4/4 of LyP. In all cases, a small minority (<5%) of reactive lymphocytes expressed CCR4 (Table 2).

Control Tissue

In thymus, TARC was detected as a strong cytoplasmic staining in cells with a dendritic morphology residing in the medulla. In tonsils and inflamed lymph nodes, expression of TARC was found in high endothelial venules. Endothelial cells in lichen planus did, however, not express TARC. Expression of CCR4 was found on 10–20% of reactive T lymphocytes and by dendritic cells in the dermis in skin

biopsies from lichen planus, as described elsewhere (3).

DISCUSSION

In this study, major differences in the expression of the CC-chemokine TARC and its ligand CCR4 were demonstrated in different types of CD30+ lymphomas. TARC was expressed by the neoplastic cells in 27 of 31 cases of HD, including 18/18 nsHD, 6/6 mcHD, and 3/7 lpHD; and in 12/27 CD30+ ALK-negative ALCL but not in CD30+ ALK-positive ALCL; and in only 3/27 primary cutaneous CD30+ lymphoproliferative disorders, including 1/11 primary cutaneous CD30-positive ALCL and 2/15 cases of LyP.

The consistent expression of TARC by H/R-S cells in cases of mcHD and nsHD is in accordance with the results of previous studies (7). In contrast to Van den Berg *et al.* (7), TARC expression was also found in 3/7 cases of lpHD. These findings suggest that TARC expression is a constant feature of HD. However, the expression of TARC in approximately half of the cases of nodal ALK-negative ALCL indicates that TARC expression is not specific for HD, and thus cannot be used as a diagnostic criterion in the differential diagnosis between HD and ALCL.

The differential expression of TARC between ALK-negative (12/27, 44%) and ALK-positive (0/12) nodal ALCL is a new finding providing yet another biological difference between these two subtypes of ALCL. Earlier studies of TARC expression in ALCL are limited to one recent study in which expression of TARC was not found on three ALK-negative ALCL, whereas ALK-positive ALCL were not investigated (7). In addition, we found that TARC expression was not specific for CD30-positive lymphomas because in a minority of CD30-negative lymphomas, of either nodal or cutaneous origin, TARC expression was detected in the neoplastic cells (1/11 follicular lymphomas, 1/12 diffuse large B-cell lymphomas (DLBCL), 1/2 cutaneous DLBCL, and

TABLE 3. Coexpression of TARC and CCR4 by Neoplastic Cells in HD, Nodal ALCL, and Cutaneous CD30+ Lymphomas

Diagnosis	N	TARC+, CCR4+	TARC+, CCR4−	TARC−, CCR4+	TARC−, CCR4−
HD ns	8	0	8	0	0
Nodal ALCL	19	5	4	5	5
ALK positive	4	0	0	1	3
ALK negative	15	5	4	4	2
Cutaneous CD30+ NHL ^a	9	0	0	9	0

^a Cutaneous CD30+ ALCL (*n* = 5) and lymphomatoid papulosis (*n* = 4), as found in double stainings.

TARC, thymus and activation-regulated cytokine; HD, Hodgkin's disease; ns, nodular sclerosing; ALCL, anaplastic large-cell lymphoma; NHL, non-Hodgkin's lymphoma.

FIGURE 2. Thymus and activation-regulated chemokine (TARC) expression in a frozen section of nodular-sclerosing Hodgkin's disease displaying a cytoplasmic staining in the neoplastic cells. No small or intermediate-sized cells stain positive. **A**, expression of CCR4 detected in the same case showing CC-chemokine receptor 4 (CCR4)-positive reactive lymphocytes surrounding the neoplastic Hodgkin/Reed-Sternberg (H/RS) cells. **B**, immunohistochemical double staining between TARC (brown) and CCR4 (red) clearly show CCR4-positive cells surrounding the TARC-expressing neoplastic cells.

FIGURE 3. TARC expression in a case of anaplastic large-cell lymphoma showing a clear cytoplasmic staining for TARC (brown) in virtually all neoplastic cells.

3/11 T-NHL not otherwise specified [data not shown]).

In contrast, CCR4 was expressed most strikingly and consistently by the neoplastic cells in primary cutaneous CD30+ lymphoproliferative disorders (9/9) and to a lesser extent by CD30+ ALK-negative ALCL (9/15, 60%), but not by HD and in only one case of ALK-positive ALCL. The expression of CCR4 on neoplastic cells in half of the cases of ALK-negative ALCL is in line with the study by Jones *et al.* (18) who demonstrated CCR4 expression in 3/6 ALK-negative ALCL. In contrast to our study, expression of CCR4 was found in all cases of ALK-positive ALCL (5/5; 18). This difference observed in the expression of CCR4 by ALK-positive ALCL is as yet unexplained.

The expression of CCR4 in all cases of cutaneous ALCL and LyP is consistent with the hypothesis that CTCL are characterized by a Th2-like cytokine profile (19, 20). With respect to cutaneous CD30+ lymphoproliferations, one study indeed showed increased expression of Th2 cytokines by transcription analysis (21).

Examination of serial sections and double-staining experiments indicate a TARC+/CCR4− phenotype in all cases of nsHD, a TARC−/CCR4+ phenotype in all cases of primary cutaneous CD30+ lymphoproliferative disorders, and a TARC−/CCR4− phenotype in 3/4 ALK-positive ALCL, whereas the phenotype of ALK-negative ALCL showed considerable variation (see Table 3).

Previous studies demonstrated TARC expression by H/RS cells in nsHD and mcHD and CCR4 expression by reactive T cells surrounding these TARC-positive H/RS cells. Because CCR4 is preferentially expressed by Th2-cells expressing IL-4, IL-5, and IL-10 (9, 10), it was postulated that production of TARC by H/RS cells may play a role in the evasion of the antitumor immune response in HD (22). In the present study, the expression of TARC by H/RS cells was confirmed. However, in contrast to the results of Van den Berg *et al.* (7), considerable numbers of CCR4+ reactive T lymphocytes surrounding the H/RS cells were only found in 3/8 cases, whereas in the other 5 cases, very few CCR4+ T lymphocytes were found. This difference in the expression of CCR4 may be explained by differences in the techniques used to detect CCR4. We used immunohistochemistry to detect CCR4 at the protein level, whereas Van den Berg *et al.* (7) used *in situ* hybridization to detect CCR4 mRNA, indicating either posttranscriptional modification of CCR4 mRNA or alternatively, a less sensitive immunohistochemical method to detect CCR4 protein. However, staining for CCR4 observed in neoplastic cells in nodal ALCL, and reactive T lymphocytes in skin biopsies of lichen planus is in accordance with earlier studies (3) and argues against this latter possibility. Not only in most cases of nsHD, but also in all nodal and cutaneous CD30-positive lymphomas, only few scattered CCR4+ reactive T cells were observed. This observation argues against an important role for TARC in modulating the cytokine microenvironment by attracting CCR4-positive T lymphocytes. Nevertheless, the presence of considerable proportions of CCR4-positive reactive lymphocytes, often in close vicinity of H/RS cells in 3/8 cases of nsHD, may suggest that evasion of the antitumor immune response by production of TARC might still be a mechanism operative in a minority of HD cases.

In addition to their potential role in modulating antitumor responses by recruitment of different types of inflammatory cells, chemokines are considered to play an important role in the selective homing and dissemination of both normal and malignant B and T cells (23, 24). Earlier studies demonstrating expression of TARC by endothelial cells in inflamed skin and expression of CCR4 by skin-homing (CLA+) T cells suggest that TARC and CCR4 are important in the homing of T cells to the skin

(3). The expression of CCR4 by neoplastic cells in all primary cutaneous CD30+ lymphomas and absence of CCR4 expression in HD is in line with this hypothesis. However, expression of CCR4 by neoplastic cells was also detected on 10/15 (67%) of nodal CD30+ ALCL. In all nodal lymphomas, a proportion of endothelial cells, especially in high endothelial venules, expressed TARC, whereas the endothelial cells in primary cutaneous CD30+ ALCL were consistently negative. Thus, our results do not support an important role for TARC-CCR4 interactions in the preferential homing of these primary cutaneous CD30+ ALCL in the skin.

In conclusion, we show that TARC expression is not specific for HD, but can also be found in half of the cases of nodal ALK-negative ALCL, but not in ALK-positive ALCL and only rarely in primary cutaneous ALCL. However, because expression of TARC is generally not associated with a considerable infiltration of CCR4-positive reactive T cells, the biologic significance of this differential expression requires further study. The expression of CCR4 by neoplastic cells of primary cutaneous CD30+ lymphoproliferative disorders and of 60% of nodal ALK-negative ALCL, in addition to the weak expression or even absence of TARC-positive endothelial cells in these lymphomas, does not support an important role in the spreading of these lymphomas.

REFERENCES

1. Rossi D, Zlotnik A. The biology of chemokines and their receptors. *Annu Rev Immunol* 2000;18:217–42.
2. Sallusto F, Mackay CR, Lanzavecchia A. The role of chemokine receptors in primary, effector, and memory immune responses. *Annu Rev Immunol* 2000;18:593–620.
3. Campbell JJ, Haraldsen G, Pan J, Rottman J, Qin S, Ponath P, *et al.* The chemokine receptor CCR4 in vascular recognition in cutaneous but not intestinal memory T cells. *Nature* 1999;400:776–80.
4. Foss HD, Herbst H, Gottstein S, Demel G, Araujo I, Stein H. Interleukin-8 in Hodgkin's disease. Preferential expression by reactive cells and association with neutrophil density. *Am J Pathol* 1996;148:1229–36.
5. Kapp U, Yeh WC, Patterson B, Elia AJ, Kagi D, Ho A, *et al.* Interleukin 13 is secreted by and stimulates the growth of Hodgkin and Reed-Sternberg cells. *J Exp Med* 1999;189:1939–46.
6. Jones D, Benjamin RJ, Shahsafaei A, Dorfman DM. The chemokine receptor CXCR3 is expressed in a subset of B-cell lymphomas and is a marker of B-cell chronic lymphocytic leukemia. *Blood* 2000;95:627–32.
7. Van den Berg A, Visser L, Poppema S. High expression of the CC chemokine in Reed-Sternberg cells. A possible explanation for the characteristic T-cell infiltrate in Hodgkin's lymphoma. *Am J Pathol* 1999;154:1685–91.
8. Imai T, Yoshida T, Baba M, Nishimura M, Kakizaki M, Yoshie O. Molecular cloning of a novel T cell-directed CC chemokine expressed in thymus by signal sequence trap using Epstein-Barr virus vector. *J Biol Chem* 1996;271:21514–21.

9. Salusto F, Lanzavecchia A, Mackay CR. Chemokine and chemokine receptors in T cell priming and Th1/Th2-mediated responses. *Immunol Today* 1998;19:568–74.
10. D'Ambrosio D, Iellem A, Bonecchi R, Mazzeo D, Sozzani S, Mantovani A, *et al.* Selective upregulation of CCR4 and CCR8 upon activation of polarized human type 2 Th cells. *J Immunol* 1998;161:5111–5.
11. Jaffe ES, Harris NL, Stein H, Vardiman JW, editors. World Health Organisation classification of tumours. Pathology and genetics of tumours of haematopoietic and lymphoid tissues. Lyon, France: IARC Press; 2001.
12. Shiota M, Nakamura S, Ichinohasama R, Abe M, Akagi T, Takeshita M, *et al.* Anaplastic large cell lymphomas expressing the novel chimeric protein p80NPM/ALK: a distinct clinicopathologic entity. *Blood* 1995;86:1954–60.
13. Falini B, Pileri S, Zinzani PL, Carbone A, Zagonel V, Wolf-Peeters C, *et al.* ALK+ lymphoma: clinico-pathological findings and outcome. *Blood* 1999;93:2697–706.
14. Willemze R, Kerl H, Sterry W, Berti E, Cerroni L, Chimenti S, *et al.* EORTC classification for primary cutaneous lymphomas—a proposal from the Cutaneous Lymphoma Study Group of the European Organization for Research and Treatment of Cancer. *Blood* 1997;90:354–71.
15. Ten Berge RL, Dukers DF, Oudejans JJ, Pulford K, Ossenkoppele GJ, de Jong D, *et al.* Adverse effects of activated cytotoxic T lymphocytes on the clinical outcome of nodal anaplastic large cell lymphoma. *Blood* 1999;93:2688–96.
16. Dukers DF, Jaspars LH, Vos W, Oudejans JJ, Hayes D, Cillessen S, *et al.* Quantitative immunohistochemical analysis of cytokine profiles in Epstein-Barr virus positive and -negative cases of Hodgkin's disease. *J Pathol* 2000;190:143–9.
17. Jiwa NM, Kanavaros P, De Bruin PC, van der Valk P, Horstman A, Vos W, *et al.* Presence of Epstein-Barr virus harbouring small and intermediate-sized cells in Hodgkin's disease. Is there a relationship with Reed-Sternberg cells? *J Pathol* 1993;70:129–36.
18. Jones D, O'Hara C, Kraus MD, Perez-Atayde AR, Shahsafaei A, Wu L, *et al.* Expression pattern of T-cell-associated chemokine receptors and their chemokines correlates with specific subtypes of T-cell non-Hodgkin lymphoma. *Blood* 2000;96:685–90.
19. Vowels BR, Lessin SR, Cassin M, *et al.* Th2 cytokine mRNA expression in skin in cutaneous T-cell lymphoma. *J Invest Dermatol* 1994;103:669–73.
20. Rook AH, Vowels BR, Jaworsky C, Singh A, Lessin SR. The immunopathogenesis of cutaneous T-cell lymphoma. *Arch Dermatol* 1993;129:486–9.
21. Yagi H, Tokura Y, Furukawa F, Takigawa M. Th2 cytokine mRNA expression in primary cutaneous CD30-positive lymphoproliferative disorders: successful treatment with recombinant interferon- γ . *J Invest Dermatol* 1996;107:827–32.
22. Poppema S, Potters M, Visser L, van den Berg AM. Immune escape mechanisms in Hodgkin's disease. *Ann Oncol* 1998;9:21–4.
23. Drilenburg P, Pals ST. Cell adhesion receptors in lymphoma dissemination. *Blood* 2000;95:1900–10.
24. Sanz-Rodríguez F, Hidalgo A, Teixidó J. Chemokine stromal cell-derived factor-1 modulates VLA-4 integrin-mediated multiple myeloma cell adhesion to CS-1/fibronectin and VCAM-1. *Blood* 2001;97:346–51.

LIPPINCOTT
WILLIAMS & WILKINS

**Unauthorized Use
Prohibited**