

The CD4 + CD26– T-cell population in classical Hodgkin's lymphoma displays a distinctive regulatory T-cell profile

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Little is known about the gene expression profile and significance of the rosetting CD4 + CD26– T cells in classical Hodgkin's lymphoma (cHL). To characterize these T cells, CD4 + CD26– and CD4 + CD26 + T-cell populations were sorted from lymph node (LN) cell suspensions from nodular sclerosis HL (NSHL) and reactive LNs. mRNA profiles of stimulated and resting cell subsets were evaluated with quantitative RT-PCR for 46 genes. We observed a higher percentage of CD4 + CD26– T cells in NSHL than in reactive LNs. The resting CD4 + CD26– T cells in NSHL showed higher mRNA levels of CD25, CTLA4, OX40 and CCR4 compared with in LNs, supporting a regulatory T-cell (Treg) type, and this was validated by immunohistochemistry. Moreover, these cells showed low or no expression of the Th1- or Th2-related cytokines IL-2, IFN- γ , IL-13, IL-12B, IL-4, and IL-5, and the chemoattractant receptor CRTH2. Besides Tregs, Th17 cells may exist in NSHL based on the significantly higher IL-17 mRNA level for both T-cell populations in NSHL. Upon stimulation *in vitro*, lack of upregulation of mRNA levels of most cytokine genes indicated an anergic character for the CD4 + CD26– T-cell subset. Anergy fits with the Treg profile of these cells, probably explaining the immunosuppressive mechanism involved in NSHL.

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The characteristic morphological appearance of Hodgkin's lymphoma (HL) is a minority of neoplastic Hodgkin and Reed–Sternberg (HRS) cells surrounded by a vast majority of reactive infiltrating cells. The infiltrating cells consist of CD4 + T cells, B cells, eosinophils, neutrophils, plasma cells, histiocytes and fibroblasts. The histological appearance, phenotype and genotype of the neoplastic cells, and the composition of the infiltrating cells, distinguish the most commonly observed classical HL (cHL) from the nodular lymphocyte predominant HL (NLPHL) subtype.¹ Several lines of evidence suggest that the reactive cells are attracted by chemokines produced by the HRS cells.^{2–5} In return, these infiltrating cells produce cytokines that may contribute to proliferation and survival of the HRS cells.^{6,7}

The CD4 + T cells surrounding the neoplastic cells in both cHL and NLPHL are CD45RO + /CD45RA–/CD45RB^{dim}, suggesting a memory Th2 phenotype.⁸ Expression of several activation-associated surface markers, including CD38, CD69 and HLA class II, indicates that these CD4 + T cells

are activated.⁹ However, they do not express another activation molecule DPP4-dipeptidyl peptidase IV (CD26).¹⁰ CD26 is a proteolytic enzyme associated with adenosine deaminase and CD45RO, which is involved in co-stimulation of T cells.¹¹ Normal CD26– T cells become CD26 + after stimulation under physiological conditions,^{12,13} but the CD26– T cells from cHL cases remain negative after stimulation.⁹ This indicates that absence of CD26 is potentially relevant to the impaired immune response observed in cHL.¹⁴

The mechanism of the ineffective immune response against the tumor cells in cHL remains unclear. Recently, regulatory T cells (Treg) have been found to be enriched among the infiltrating cells of cHL in affected lymph nodes (LNs),^{15,16} as well as in the peripheral blood of cHL patients.¹⁷ Treg cells can suppress pathological and physiological immune responses, thereby contributing to maintenance of immunological self-tolerance¹⁸ and in the case of cHL, probably to the failing antitumor response.

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To date, some studies have reported about the expression of T-cell-specific cytokines mainly with the objective to study production by HRS cells.^{19,20} Atayar *et al*⁷ demonstrated a distinct cytokine gene expression profile for the CD4 + CD57 + T cells of NLPHL, indicating a Tr1 immune response in NLPHL. A recent study showed expression of several transcription factors, including T-bet and GATA-3, that respectively direct Th1 and Th2 type cytokine production, by the T cells in cHL.²¹

In this study, we investigated the gene expression pattern of CD4 + CD26⁻ and CD4 + CD26 + T-cell subsets isolated from cell suspensions from nodular sclerosis HL (NSHL) and reactive LNs without or with *in vitro* stimulation, to further characterize the distinctive T-lymphocyte population in cHL.

MATERIALS AND METHODS

Immunohistochemistry

Frozen sections of previously diagnosed cHL cases were stained with anti-CD26 (undiluted, 2A6²²), CD69 (1/10), CTLA4 (1/10), IL-2R (CD25, 1/10) (BD Biosciences, San Jose, CA, USA) and IL-17 (1/2) (R&D Systems, Minneapolis, MN, USA) according to standard procedures. Positive staining was visualized using an HRP-labelled second step and 3-amino-9-ethylcarbazole (Sigma-Aldrich). Paraffin sections of the same cases were stained with antibodies to CCR4 (1/50; BD Biosciences) GITR (1/5; R&D Systems) and FoxP3 (1/100; Abcam, Cambridge, UK) according to standard procedures, using HRP-labelled secondary antibodies and 3,3'-diaminobenzidine. Appropriate positive and negative controls were performed.

Patients and Single-Cell Suspensions

Cryopreserved single-cell suspensions from primary diagnostic LNs of 7 cHL patients (3 female, 4 male; age 11–32 (mean 23 years, median 24 years)) were retrieved from the cell bank of the Department of Pathology and Laboratory Medicine, University Medical Center Groningen, Groningen, The Netherlands. The histological type of all cases was NS grade 1. They were all EBV negative as indicated by *in situ* hybridization of EBV-encoded small RNA. Single-cell suspensions from five reactive LNs (1 female, 4 male; age 13–43 (mean 35 years, median 39 years)) were used as controls. Four cases (LN1–LN4) were diagnosed as reactive follicular hyperplasia; LN4 was diagnosed with cHL 15 years earlier, with no relapse consecutive. LN5 was diagnosed as lymphoid hyperplasia, Castleman disease. The protocols for obtaining and studying human tissues were approved by the institution's review board for human subject research.

Flow Cytometry

Cell suspensions were immunostained simultaneously for the following monoclonal antibodies: fluorescein-conjugated anti-CD4 (clone Edu-2; IQ Products, Groningen, Netherlands) and anti-CD26 (2A6)²² combined with phycoerythrin-conjugated anti-IgG (Southern Biotechnology Associates).

CD4 + CD26 + and CD4 + CD26⁻ T-cell populations were sorted on a MoFlo cytometer (DakoCytomation, Fort Collins, CO, USA). Purity of sorted populations was checked by flow cytometry and populations with purity of more than 95% were used for expression profiling as resting cell subsets. Due to variations in the percentage of T-cell subsets and limitations of the available cell suspensions, the amount of sorted cells varied between 0.5 and 10 million. To allow analysis of a large number of individual genes on the limited amounts of T-cell subsets, we performed qRT-PCR to determine mRNA levels for selected genes.

Cell Stimulation

Half of the sorted cells from three LNs (LN1, LN2, LN4) and five cHL cases (cHL1–cHL5) were resuspended in RPMI 1640 + 10% FCS and stimulated with phorbol 12-myristate 13-acetate (PMA, 5 ng/ml) (Sigma Aldrich, Saint Louis, MO, USA) and ionomycin (1.5 μ M/ml) (Sigma) for 6 h at 37°C and 5% CO₂.

Quantitative RT-PCR

Total RNA extraction, which includes a DNase treatment step, was performed using the Absolutely RNA Miniprep kit (Stratagene, La Jolla, CA, USA). The integrity of the RNA was checked and cDNA was synthesized from 48 to 200 ng of total cellular RNA using the First-Strand cDNA Synthesis System, using Superscript II RT (Invitrogen Carlsbad, CA, USA) with random hexamers. Real-time PCR was performed with Taqman[®] low-density arrays on Applied Biosystems 7900HT sequence detection system (Applied Biosystems, Foster City, CA, USA). TaqMan low-density arrays are 384-well microfluidic cards (MFCs), pre-loaded with chosen TaqMan[®] gene expression assays, which were obtained from Applied Biosystems as Assay-on-demand[™] (AOD) gene expression products. The gene IDs are listed in Supplementary Table 1. A 50- μ l volume of cDNA was mixed with TaqMan[®] Universal PCR Master Mix (Applied Biosystems) and loaded into the MFC, according to the manufacturer's protocol, at a final amount of 2 ng cDNA/well or at a lower concentration when the total RNA amount was not sufficient. All assays were performed in duplicate. Expression of RNA polymerase II (POLR2A; RPII) and GAPDH was included as housekeeping genes. For RPII, an average C_t value of 24.9 \pm 1.4 (range 22.5–27.9) and for GAPDH an average C_t value of 21.3 \pm 1.5 (range 19.3–25.4) was observed for the 25 T-cell subsets with an input of 2 ng cDNA/well. Based on the lower s.d. and a C_t value in the same range as observed for the other genes, RPII was selected as the housekeeping gene for normalization of the data. Five of the 40 sorted T-cell subsets had C_t value > 31 for RPII; these five T-cell subsets were excluded from the analysis. T-cell subsets with a C_t value > 37 for a specific AOD were considered as negative for the corresponding gene. The relative expression level was deduced by calculating the difference in C_t from the C_t of the reference

gene RPII (ΔC_t). Results were expressed as $2^{-\Delta C_t} \times 10^4$ to indicate the relative mRNA level in each T-cell subset.

Statistics

The data were compiled with the software package SPSS, version 12.0.2. Results between different T-cell subsets were compared to determine a possible significant increase or decrease among the groups. Due to skewed distribution of the relative mRNA values for most T-cell subsets, a non-parametric method was used (Mann-Whitney *U* test). *P*-values < 0.05 were considered statistically significant.

RESULTS

Activation Marker Expression in cHL Tissue

Immunohistochemical staining showed that the majority of lymphocytes surrounding HRS cells were positive for activation marker CD69, but did not express CD26 (Figure 1).

Sorting of T Cell Subsets

The mean percentage of CD4+ CD26- T cells was 67% (range, 38–89%) in NSHL and 44% (range, 17–67%) in reactive LNs (Table 1). In five out of seven NSHL cases, and only one out of five reactive LN cases, the percentage of CD4+ CD26- T cells was at least twofold higher compared with CD4+ CD26+ T cells.

Gene Expression Results

As an extra control for FACS, we first analyzed CD26 mRNA levels in the CD4+ CD26+ and CD4+ CD26- T-cell populations from both NSHL and reactive LNs. This demonstrated a significant difference between the CD26+ and CD26- T-cell population (*P* = 0.001), with a median mRNA level of 78 for CD4+ CD26- T cells and 4161 for CD4+ CD26+ T-cell subsets. In summary, we found for 14 genes (CCR4, CTLA4, IL-7R, IL-10RA, CCR7, TGFβ1

(TGFβ1), ICOS, CCL5, FOXP3, GATA3, CD69, IL-4R, IL-5RA and TNFRSF4 (OX40)) high mRNA levels in all four CD4+ T-cell subsets in both NSHL and LNs (mean ($2^{-\Delta C_t} \times 10^4$) > 10 000). For 22 genes (PDCD1, MAF, TNF, IL-2RA (CD25), IL-12RB2, IL-21, IL-5, CCR2, CXCR3, TGFβR1, CD26, CCR5, IL-10, IFNG (IFNγ), CCR8, TBX21 (T-bet), IL-8, IL-1R1, IL-17, GPR44 (G protein-coupled receptor-44; CRTH2), TNFRSF18 (glucocorticoid-induced TNFR; GITR) and CCR3) an intermediate mRNA level was observed in all T-cell subsets ($100 < \text{mean} (2^{-\Delta C_t} \times 10^4 < 10\,000)$). Ten genes (IL-2, IL-8RB, IL-13, IL-9R, IL-12A, IL-8RA, IL-4, CCR10, IL-12B and IL-13RA2) showed very low level or no expression (mean ($2^{-\Delta C_t} \times 10^4 < 100$)) (Supplementary Tables 2 and 3).

Comparison between T-Cell Subsets Isolated from cHL and Reactive LNs

Significant differences in mRNA levels were observed for 10 genes between T-cell subsets from reactive LNs and NSHL (Table 2; Figure 2). Eight genes were upregulated and two genes were downregulated in cHL compared with reactive LNs. CTLA4, CCR4, OX40, CD25 and IL-12A showed significantly higher mRNA levels in CD4+ CD26- T cells of NSHL than of LNs, whereas no significant difference was observed between CD4+ CD26+ T-cell subsets. Both CD4+ CD26+ and CD4+ CD26- T-cell populations from NSHL demonstrated a significantly higher mRNA level of IL-12RB2 and IL-17 than their counterparts from reactive LNs. CCR3, on the contrary, only exhibited significantly elevated mRNA level in CD4+ CD26+ T cells from NSHL as compared with reactive LNs. IL-2 and CRTH2 showed significantly lower mRNA levels in CD4+ CD26+ T cells of NSHL than of LNs.

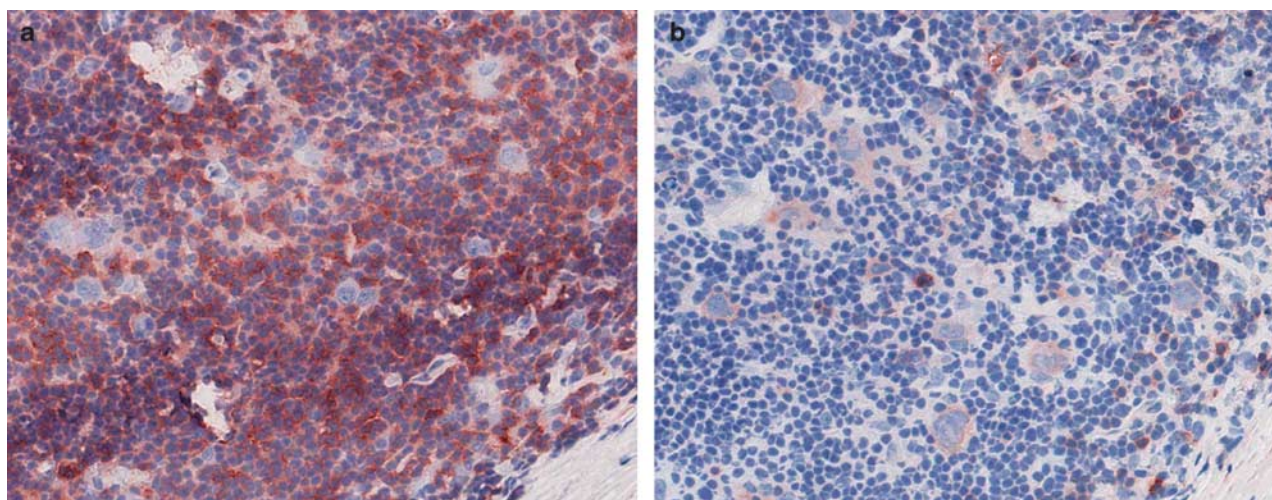


Figure 1 Immunohistochemistry for activation markers CD69 and CD26. An example of a NSHL case with typical patterns for CD69, showing positive T cells surrounding the HRS cells, which were negative (a); CD26, showing absence of positive lymphocytes in the same area (b). HRS cells were stained positively.

Table 1 Overview of percentage of CD4+ T cells in total suspension and CD26+/- T cells in the CD4+ population in seven NSHL (cHL) and five reactive LN cases

Sample	% CD4+ cells in total	% In CD4+ cells	
		CD26+	CD26–
LN1	44	69	31
LN2	26	49	51
LN3	55	83	17
LN4	31	33	67
LN5	36	46	54
cHL1	16	35	65
cHL2	45	58	42
cHL3	50	24	76
cHL4	33	62	38
cHL5	73	14	86
cHL6	57	26	74
cHL7	45	11	89

Comparison between Resting and Stimulated CD4 + T-Cell Subsets

Upon stimulation, eight cytokine genes (IFN- γ , IL-2, IL-8, IL-21, IL-17, IL-13, IL-12A and IL-4) plus the transcription factor T-bet exhibited a different expression level between the CD4 + CD26– T-cell populations in NSHL and LNs. In the CD4 + CD26– T-cell subset from NSHL, these genes could not be induced upon PMA/ionomycin stimulation, whereas they showed elevated expressions in the stimulated CD4 + CD26– T-cell subset in the LNs (Figure 3). The CD4 + CD26+ T-cell subset in NSHL could be induced for some of the genes, but not or to a very low degree in IFN- γ , IL-2, IL-4 and IL-12A. Six genes, CTLA4, ICOS, OX40, CD25, IL-12RB2 and IL-1R1, with high mRNA levels in resting CD4 + CD26– NSHL T cells could not be upregulated or only to a low degree upon stimulation. For the remaining 31 genes, no induction or no clear differences were observed for all T-cell subsets upon PMA/ionomycin stimulation (Supplementary Tables 2 and 3).

Validation of qRT-PCR by Immunohistochemistry

The regulatory phenotype of the cells surrounding the HRS cells was validated by immunohistochemistry and representative stainings are shown in Figure 4. Cells immediately surrounding the HRS cells are CCR4-, GTR- and CD25-positive. In the area containing CD26– T cells, CTLA4-, FOXP3- and CD25-positive cells are found interspersed between the HRS cells. IL-17-positive cells are found as single cells interspersed throughout the whole LN (Figure 4).

Table 2 Relative mRNA levels of the significantly differentially expressed genes in resting T-cell subsets from NSHL compared with reactive LNs

Gene	CD4+CD26– NSHLs		CD4+CD26– reactive LNs		P-value
	Median	Range	Median	Range	
CTLA4	53 238	27 447–65 227	23 040	2032–29 351	0.016
CCR4	38 918	12 518–150 986	8950	4020–17 114	0.032
OX40	16 995	15 395–37 767	11 096	2374–14 449	0.008
IL-12RB2	13 183	3466–30 811	508	148–6427	0.016
CD25	11 579	6766–13 065	2614	1822–4137	0.008
IL-17	325	132–917	5	0–186	0.016
IL-12A	58	37–67	30	13–47	0.032
	CD4+CD26+ NSHLs		CD4+CD26+ reactive LNs		P-value
	Median	Range	Median	Range	
IL-12RB2	2481	1182–10 946	639	185–2389	0.030
IL-17	213	22–3249	5	0–80	0.017
CCR3	118	40–250	45	22–65	0.030
CRTH2	104	61–271	403	158–732	0.017
IL-2	42	9–58	126	41–352	0.030

The results are expressed as $2_t^{-\Delta C} \times 10^4$, normalized to RP II.

DISCUSSION

This study provides gene expression profiles of CD4 + CD26+ and CD4 + CD26– T-cell subsets from NSHL and reactive LN. Our data fit with the general notion that HL is associated with disturbed cytokine production.^{23–25} The CD4 + CD26– T cells from NSHL cases express a distinct set of genes compared with their counterparts in reactive LNs.

In HL, chemokines produced by HRS cells largely account for the attraction of inflammatory cells. Chemokine receptors expressed on different types of immune cells contribute to the large variety of cells in the infiltrate.^{2,3,26} Buri *et al*²⁷ reported strong CCR3 expression in half of the infiltrating cells of LNs involved by cHL, but not in reactive LNs. Our results indicate a similar CCR3 expression pattern at the mRNA level: CD4 + CD26+ T cells showed significantly higher expression in the cells of NSHL than of reactive LNs, whereas CD4 + CD26– T cells also showed higher expression levels, which were not significant. CCR3 binds CCL5 (RANTES) and CCL11 (eotaxin),^{28,29} both of which are expressed in cHL tissue.^{30,31} This suggests that chemokines contribute to recruitment of the CCR3 + CD4 + T cells surrounding HRS cells. CCR4 is the specific receptor for CCL17 (TARC) and CCL22 (MDC). Our data showed higher

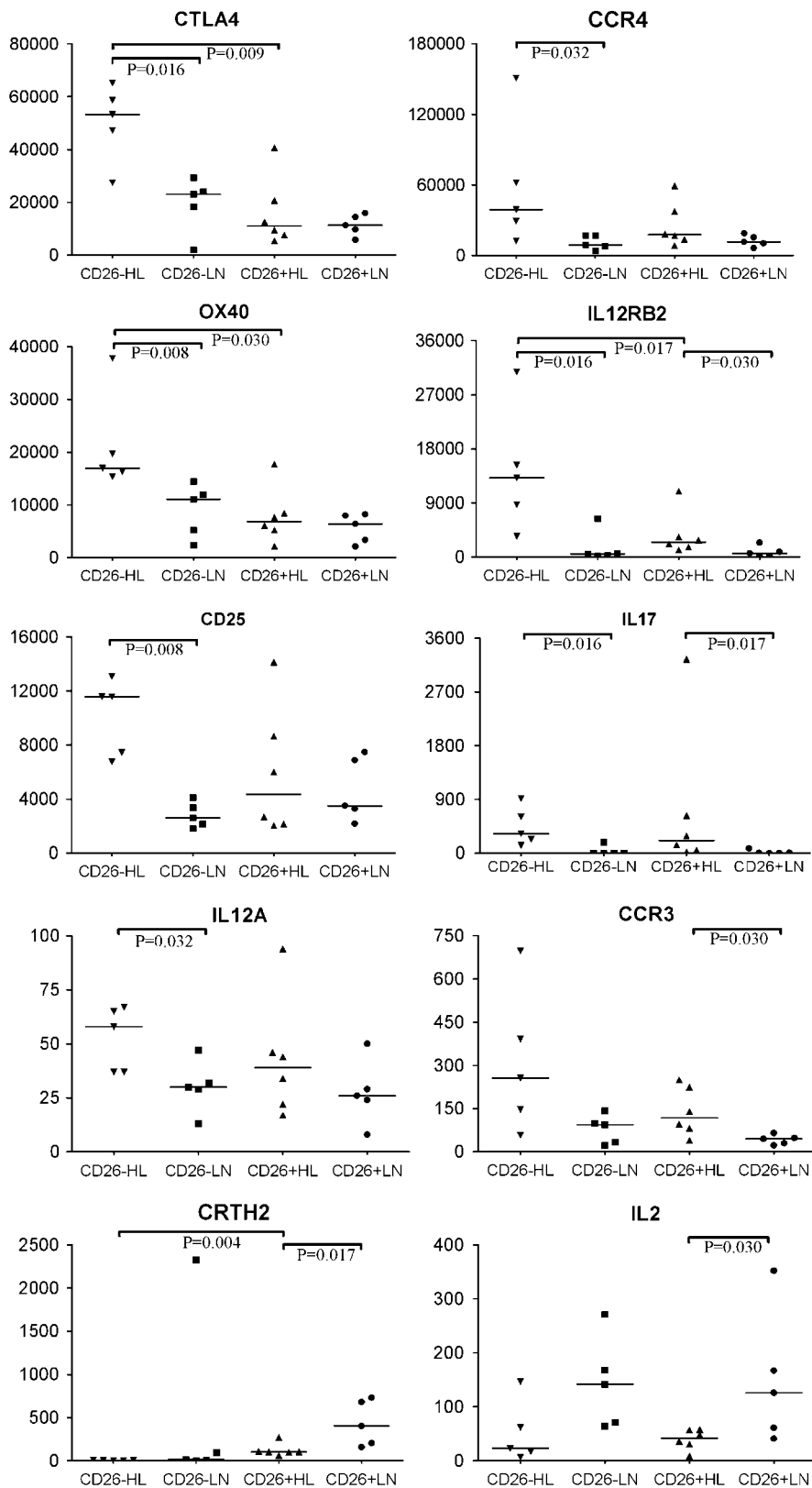


Figure 2 Relative mRNA levels of the significantly differentially expressed genes in resting CD4+ T-cell subsets from NSHL compared with reactive LNs. The results are expressed as $2^{-\Delta Ct} \times 10^4$, normalized to RPII. P-value is illustrated if the difference between T-cell subsets is significant.

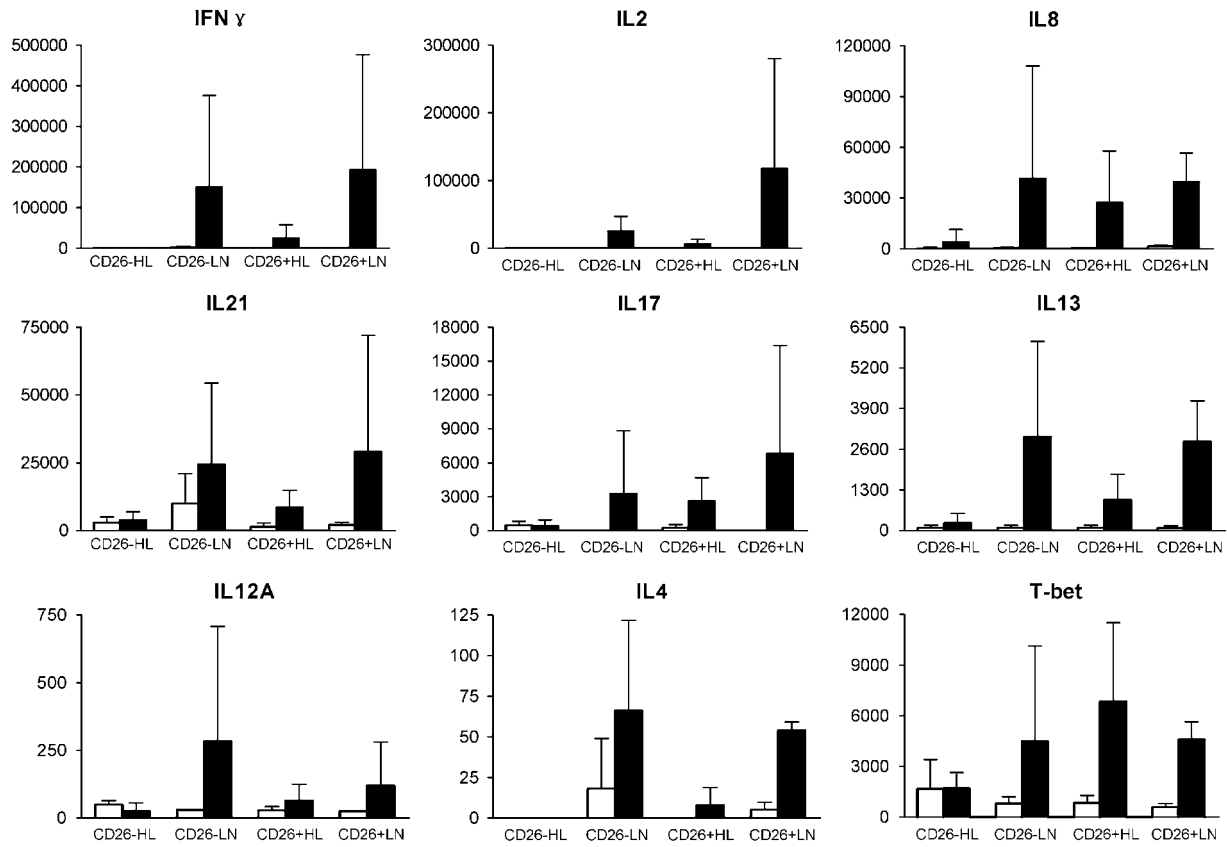


Figure 3 Average mRNA levels of resting (white bars) and stimulated (black bars) T-cell subsets showing a different expression pattern for CD4 + CD26⁻ T cells from other subsets upon PMA/ionomycin stimulation for 6 h. The results are expressed as $2^{-\Delta C_t} \times 10^4$, normalized to RPII.

CCR4 mRNA levels in CD4 + CD26⁻ T cells from NSHL than reactive LNs, which distinguishes the CD4 + CD26⁻ T cells surrounding HRS cells from those in reactive LNs. HRS cells produce high levels of TARC and MDC, which are chemoattractants for CCR4, and could play an active role in the influx of CCR4 + T lymphocytes.² Although some controversy exists about the presence of CCR4-positive cells immediately surrounding HRS cells in cHL,^{16,32} our results showed significantly higher mRNA level of CCR4 for CD4 + CD26⁻ T cells and CCR4-positive cells are indeed located in close vicinity to HRS cells. Since Mariani *et al*³³ showed rapid and efficient CCR4 internalization by MDC but not by TARC, the controversy is probably due to CCR4 internalization by binding to its ligand MDC, which is also highly expressed by HRS cells.^{5,30}

Comparison of the cytokine expression pattern may provide clues for a Th1, Th2 or Treg nature of the CD4 + CD26⁻ subset in cHL. The expression level of the Th1 cytokine IL-2 was decreased in CD4 + CD26⁺ and CD4 + CD26⁻ T cells from NSHL compared with LN subsets, not supporting a Th1 cell type. However, Th1 cytokine IL-12A and the receptor IL-12RB2, known to allow lineage commitment toward a Th1 phenotype,³⁴ were increased in CD4 + CD26⁻ T cells. Due to lack of IL-12B on all subsets, it

is unlikely that significant biological activity of IL-12^{35,36} is achieved, despite significant increase of IL-12A mRNA. A low expression level of the Th1 cytokine IFN- γ in the CD4 + CD26⁻ T cells from NSHL also suggests that these cells do not have Th1 characteristics. Furthermore, these CD4 + CD26⁻ T cells exhibited a relatively high GATA3 and intermediate IL-10 level, which fits with the suspected Th2 differentiation.³⁷ However, no IL-4, IL-5, chemoattractant receptor CRTH2^{38,39} and only low IL-13 mRNA levels were found, which is inconsistent with a Th2 phenotype. A set of significantly higher expressed genes, including CTLA4, CCR4, OX40 and CD25, characterized the CD4 + CD26⁻ T cells in NSHL and indicated that these cells represent a Treg population. Tregs are a T-cell subset that attributes to T-cell-mediated regulation in immunological tolerance.^{40,41} They are characterized by a distinct phenotype of CD25,⁴² FOXP3^{43,44} and GITR expression.⁴⁵ We observed a significantly higher CD25 mRNA level in CD4 + CD26⁻ T-cell subset from NSHL than from reactive LNs. Although FOXP3 and GITR did not show significantly higher levels, a similar trend was observed for these genes. In this CD4 + CD26⁻ T-cell subset, increased mRNA levels were also observed for other costimulatory molecules, such as CTLA4 and OX40, which are constitutively expressed on Tregs.^{46,47} The higher

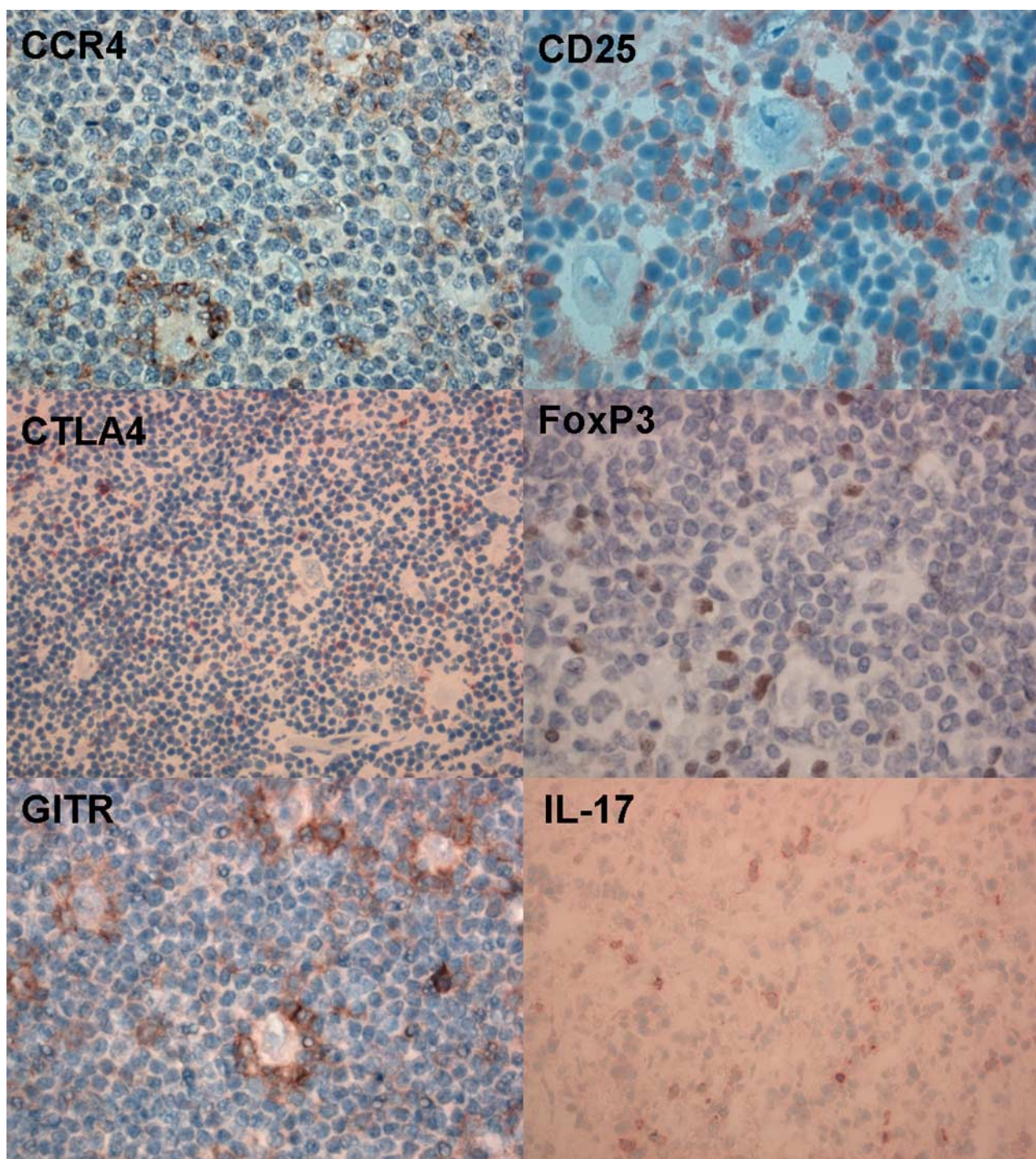


Figure 4 Immunohistochemistry for proteins associated with regulatory T and Th17 cells. Examples are shown of NSHL cases staining positive for CCR4 (a, $\times 400$), CD25 (b, $\times 200$), CTLA4 (c, $\times 400$), FOXP3 (d, $\times 400$), GITR (e, $\times 400$) and IL-17 (f, $\times 200$). CCR4, CD25 and GITR are positive on the lymphocytes immediately surrounding the HRS cells, whereas CTLA4, FOXP3 and additional CD25-positive cells are found interspersed in the larger area surrounding the tumor cells. IL-17 is found in single cells throughout the whole LN.

CCR4 levels may also be related to Tregs, since expression of CCR4 was also found on these cells.^{16,48,49} Compared with reactive LNs, the CD4+ CD26- T cells from NSHL represent a distinct phenotype and can be regarded as a Treg population.

Validated on NSHL tissue showed expression of CCR4, CD25, CTLA4, FOXP3 and GITR directly surrounding or in the close vicinity of the HRS cells, which overlaps with the location of the CD4+ CD26- T cells. Higher CD25 mRNA

levels in our data fits with a recent immunophenotyping study that showed the presence of CD4 + CD25 + Tregs in the cHL infiltrating lymphocytes.¹⁵ Some studies have reported increased Treg cell amounts in cancer, indicating their possible suppressive role in immune response against tumors.^{50,51} In cHL, these Tregs would be expected to exert local immunosuppressive effects, so that HRS cells can evade immunological surveillance and clearance.

Besides association with Tregs, the CD4 + CD26– T-cell subset in cHL could also be related to Th17 cells based on the significantly higher IL-17 mRNA levels than in reactive LNs. Several IL-17-positive cells were shown by immunohistochemistry to be present in NSHL. Th17 is a newly classified effector T-cell subset characterized by IL-17 production.^{52,53} A high TGF β 1 mRNA level, as observed in CD4 + CD26– T cells from NSHL, probably contributes to regulation of Th1 and Th17 cell differentiation, according to Li *et al.*⁵⁴ Moreover, the close relationship of IL-17 + cells and Tregs in tumor pathogenesis is suggested by their coexistence in tumor environment.⁵⁵ Whether the relatively high IL-17 mRNA level in T cells from NSHL indicates a Th17 subset, and the relationship with Tregs, is of interest for further study.

The stimulation data illustrate the association of the CD4 + CD26– T cells in NSHL with anergy. In our study, IL-4 mRNA was absent in CD4 + CD26– T cells from NSHL and could not be induced on PMA stimulation, confirming the results of Atayar *et al.*⁷ in CD4 + CD57 + T cells. Furthermore, the CD4 + CD26– T cells in NSHL behaved differently upon stimulation compared with all the other T-cell subsets. Eight cytokines could not be induced or only to a moderate level in the CD4 + CD26– T cells of NSHL when stimulated but exhibited markedly elevated mRNA levels in the three other T-cell populations. The absence of mRNA induction for most cytokines indicates a functional limitation of the CD4 + CD26– T cells in NSHL and supports that they are in a status of functional unresponsiveness. This unresponsiveness can inhibit generation of effective tumor immunity,^{56,57} and may also contribute to immune suppression in cHL. *In vitro*, Tregs have been shown to exist in an anergic state, which could partially be reversed by provision of IL-2.^{41,58} Absence of IL-2 in NSHL probably maintains the anergy of these cells. Another cytokine that may play a role is IL-10; its production is associated with the anergic state and may synergize to create a negative regulatory phenotype.^{59,60} CD4 + CD26– T cells from NSHL exhibited a relatively high IL-10 mRNA level, and presence of IL-10-secreting cells in cHL has been reported.¹⁵ The immunosuppressive properties of Tregs and their anergic state may cooperate to achieve an effective negative feedback regulatory mechanism for the immune response involved in cHL.

In conclusion, this study demonstrates clear differences between the gene expression profiles of CD4 + CD26– T cells from NSHL and reactive LNs. CD4 + CD26– T cells in

NSHL reveal a Tregs character that exists in an anergic state. Large numbers of Tregs together with the possible presence of Th17 T cells suggest their immunosuppressive effects and important regulatory activity in cHL.

Supplementary Information accompanies the paper on the Laboratory Investigation website (<http://www.laboratoryinvestigation.org>)

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