Hodgkin’s lymphoma (HL) is categorised into two distinct clinicopathological entities: classical HL (CHL) and nodular lymphocyte predominance type of HL (NLPHL). Both types of HL are characterised by a minority of neoplastic cells and a vast majority of reactive infiltrating cells. However, these two HL entities differ in features like immunophenotype and genetic characteristics of the Hodgkin and Reed–Sternberg (HRS) cells, distribution of these cells within the involved lymph node, and composition of the reactive cell population. The neoplastic cells of NLPHL, the lymphocytic and/or histiocytic (L&H) cells, generally reside within follicular structures containing follicular dendritic cells, T-cells and B-cells, whereas the neoplastic cells of CHL, the HRS cells, are predominantly found in the interfollicular zone. The reasons for the confinement of the tumour cells in distinct lymphoid compartments are not known.

The reactive background cells in HL tissues are mostly CD4 positive T-helper (\(T_H\)) cells,\(^2\) HL associated \(T_H\) cells may represent an influx of \(T_H\) cells from the circulation into the lymphoid tissues. In both types of HL, the T-cells rosetting around the neoplastic cells have a helper/inducer memory phenotype (CD4\(^+\)/CD45RO\(^+\)), express CD28 and display an activated phenotype with expression of CD69.\(^3\) Recently, the presence of CD4\(^+\)/CD25\(^+\) and FOXP3\(^+\) T-regulatory cells among the infiltrating lymphocytes of HL was described.\(^4,5\) The rosetting T-cells around the L&H cells co-express CD57\(^+\) and BCL6,\(^6\) a \(T_H\) cell immunophenotype exclusively present in germinal centre (GC) T-cells.\(^7\) The biological significance of these T-cell rosettes in NLPHL has not been elucidated. CD4\(^+\)/CD57\(^+\) T-cells of NLPHL are not only found as rosette formations around the L&H cells but are also more widely distributed throughout the nodules of NLPHL.

Progressively transformed germinal centres (PTGC) have been shown to be associated with NLPHL.\(^8,9\) PTGC are enlarged disrupted GCs with a predominance of small B-lymphocytes and dispersed centroblasts that may somewhat resemble the L&H cells cytologically, but only occasionally are surrounded by CD4\(^+\)/CD57\(^+\) T-cells. Moreover, PTGC can be observed in 15% of patients with NLPHL before, during or after NLPHL, while PTGC occur in only 2% of patients with CHL.\(^10\)

\(T_H\) cell differentiation is the central process defining the nature of developing immune responses.\(^11,12\) GATA3, T-bet (also known as TBX21) and c-Maf (also known as MAF) are the key regulator transcription factors (TFs) that dictate the development of \(T_H\) subsets.\(^12\) Although the mechanisms of action of the above-mentioned T-cell TFs have been extensively studied,\(^13,14\) there have been very few\(^14\) or no reports of their pattern of distribution in normal lymphoid tissues as revealed by immunohistological staining. Previously, we investigated expression of T-cell TFs in HL cell lines and tissues involved by HL and found frequent expression of GATA3 and T-bet in the neoplastic cells of HLs,\(^15\) a finding confirmed by Dorfman et al.\(^15\) The aim of the present study was to gain further insight into the nature of the \(T_H\) immune response in HL tissues and to investigate the T-cell TF expression profile of the normal lymphoid tissues. We performed an immunohistochemical profiling of the reactive T-cells in HL and reactive lymphoid tissues for the three aforementioned T-cell TFs. We extended our analysis by including PTGC cases to investigate the potential relation between PTGC and NLPHL and cases of “paragranuloma type” T-cell/histiocyte rich B-cell lymphoma (T/HRBC) that morphologically resemble NLPHL.\(^16\) The expression pattern was evaluated by considering frequency, staining intensity and localisation of positive cells with respect to lymph node architecture and tumour cells.

**Abbreviations:** CHL, classical Hodgkin’s lymphoma; GC, germinal centre; HL, Hodgkin’s lymphoma; HRBCL, histioocyte rich B-cell lymphoma; HRS, Hodgkin and Reed–Sternberg; L&H, lymphocytic and/or histiocytic; NLPHL, nodular lymphocyte predominance type of Hodgkin’s lymphoma; PTGC, progressively transformed germinal centre; TF, transcription factor; \(T_H\), T-helper
and for an additional 30 min with the tertiary antibody.

for c-Maf and in 10 mmol/l citrate (Merck) buffer (pH 6.0) for
Germany)–EDTA (Serva, Heidelberg, Germany) buffer (pH 8.0)
times for 15 min at 300W) in 1 mmol/l Tris (Merck, Darmstadt,
9
labelled rabbit anti-goat Ig (DAKO). Antibody binding was
The second step for c-Maf and T-bet was performed with

RS591SS, Salm

et al
HRBCL that were previously described by Rudiger
cases, 10–25% of the follicles per tissue section were
usually less than 10–20% of the involved lymph node area. Two
cases of PTGC had a HL history and 2 cases were de novo cases.
In the PTGC cases, 10–25% of the follicles per tissue section were
involved. We also studied 6 cases of “paragranuloma type” T/
HRBCL that were previously described by Rudiger et al.16 Tissue
blocks of 3 reactive tonsils, 5 reactive lymph nodes and 3 normal
ad thymus were used as control tissues. All samples were
anonymised; full ethical approval in accordance with local policy
was obtained by the institution’s review board for human subject
research for obtaining and using these tissue samples.

Immunohistochemistry
Paraffin-embedded tissue sections (4 μm thick) were cut,
mounted on aminopropyltriethoxysilane-coated slides (Lo-
laboroptik GmbH, Friedrichsdorf, Germany), dried, deparaffi-
nised in xylene and rehydrated in graded alcohols. Antigen
retention was carried out in a microwave oven (Amana
RS591SS, Salm & Kipp, Breukelen, The Netherlands) (three
times for 15 min at 300W) in 1 mmol/l Tris (Merck, Darmstadt,
Germany)–EDTA (Serva, Heidelberg, Germany) buffer (pH 8.0)
for c-Maf and in 10 mmol/l citrate (Merck) buffer (pH 6.0) for
T-bet. After antigen retrieval the slides were incubated for 1
hour at room temperature with the primary antibodies (table 1),
followed by 30 min incubation with the secondary antibody
and for an additional 30 min with the tertiary antibody.

All antibody dilutions were prepared with phosphate
buffered saline, pH 7.4 containing 1% bovine serum albumin.
The second step for c-Maf and T-bet was performed with
horseradish peroxidase (HRP) labelled goat-anti-rabbit Ig
(DAKO, Copenhagen, Denmark) and the third step by HRP
labelled rabbit anti-goat Ig (DAKO). Antibody binding was
detected by 3,3’-diaminobenzidine (Sigma–Aldrich, Saint
Louis, MO, USA) staining. For GATA3 staining 4 μm-thick
sections from frozen reactive tonsils and lymph nodes were air-
dried, fixed in acetone, and incubated for 1 hour with primary
antibody, followed by application of HRP labelled rabbit-anti-
mouse Ig and goat-anti-rabbit Ig (DAKO) incubation steps for
30 min each. 3-Amino-9-ethylcarbazole (Sigma–Aldrich) was
used to visualise the positive cells. Positive control tissues as
well as negative controls, sections stained in parallel without
primary antibody, were used in all instances. The pattern and
intensity (+, weak; ++, moderate; or ++++, strong) of the staining
was also recorded. PTGC findings reported are the findings
observed in the transformed follicles of PTGC cases. Reactive
follicles and paracortical areas surrounding the transformed
follicles had the same staining pattern as reactive/normal
lymphoid tissues.

Double immunostaining
Double immunostaining was carried out on paraffin sections of
reactive tonsils to confirm that T-bet and c-Maf was expressed
by T-cells. To detect T-bet and c-Maf, the above mentioned
protocol was followed; subsequently the same protocol was
repeated using CD3 and CD57 (table 1) which was developed
with rabbit-anti-mouse Ig and goat-anti-rabbit Ig conjugated to
alkaline phosphatase (DAKO) and Fast Blue. No counter stain
was used. Single staining for T-bet, c-Maf, CD3, and CD57 was
also performed by applying all the other steps used for the
double staining to exclude cross-reaction of the different
antibodies. Negative control staining (incubation without
primary antibody) was performed routinely.

RESULTS
Strong expression of T-bet in reactive infiltrate of CHL
cases
In thymic cortex and medulla only a few cells exhibited
moderate to strong T-bet positivity (fig 1A, table 2).
In GGs of tonsil or reactive lymph node, virtually no T-bet
staining was observed (fig 1B). Staining was restricted to the
interfollicular compartments as well as the subepithelial area in
the tonsil. In reactive lymph nodes, interfollicular lymphocytes
as well as marginal sinus lymphocytes stained intensely for
T-bet and were especially prominent in vascular and perivascular
areas. In addition to the mature lymphocytes, some macro-
phages also showed reactivity with T-bet antibody in moderate
intensity. In PTGC cases strongly stained cells were present in
the interfollicular area similar to reactive lymph nodes.
Additionally, a few scattered moderately or strongly stained
T-bet+ cells were observed in broken up GCs as well as in the
enlarged mantle zone (fig 1C).

NLPHL cases showed weak (fig 1D) to moderate staining in
5–10% of the non-neoplastic lymphocytes which were prefer-
entially located in perivascular areas. One case in which we
observed strong T-bet immunoreactivity in 25–50% of L&H
cells14 also exhibited intense T-bet reactivity in reactive
lymphocytes. In 14 CHL cases the majority of T-lymphocytes
showed moderate to strong (fig 1E) T-bet reactivity (table 3).
In two cases T-bet expression was observed in less than 10% of
the T-lymphocytes. Remarkably, the HRS cells of these two
cases were also negative for T-bet staining, whereas in the rest
of the cases HRS cells also expressed T-bet. Double immunos-
taining with CD3 on tonsil sections revealed that ~20% of the
CD3+ T-cells were T-bet+. These T-bet+ cells were exclusively
located in the interfollicular area. On the other hand, ~50% of
the T-bet+ cells were expressing CD3 in tonsil. On CHL sections

Table 1 Antibodies used in the present study

<table>
<thead>
<tr>
<th>Transcription factor</th>
<th>Antibody</th>
<th>Source</th>
<th>Dilution</th>
</tr>
</thead>
<tbody>
<tr>
<td>T-bet</td>
<td>4B10 (monoclonal, IgG1)</td>
<td>Prof LH Glimcher, Harvard Medical School, Boston, MA, USA</td>
<td>1/50</td>
</tr>
<tr>
<td></td>
<td>52-3567 (polyclonal)</td>
<td>Zymed Laboratories Inc., San Francisco, CA, USA</td>
<td>1/50</td>
</tr>
<tr>
<td>GATA3</td>
<td>HG3-31 (monoclonal, IgG1)</td>
<td>Santa Cruz Biotechnology, Santa Cruz, CA, USA</td>
<td>1/50</td>
</tr>
<tr>
<td>c-Maf</td>
<td>sc 7866 (polyclonal)</td>
<td>Santa Cruz Biotechnology, Santa Cruz, CA, USA</td>
<td>1/50</td>
</tr>
<tr>
<td>CD3</td>
<td>P31 (monoclonal, IgG2a)</td>
<td>Monosan, Uden, The Netherlands</td>
<td>prediluted</td>
</tr>
<tr>
<td>CD57</td>
<td>NK1 (monoclonal, IgM)</td>
<td>Monosan, Uden, The Netherlands</td>
<td>1/40</td>
</tr>
</tbody>
</table>
80–90% of the CD3+ cells were also T-bet+ and more than 90% of the T-bet+ cells were CD3+.

Low levels of GATA3 expression in T-cell infiltrates of HL

In thymus most cortical thymocytes had cytoplasmic GATA3 and only some had nuclear GATA3, while medullary lymphocytes had exclusively nuclear GATA3 staining (fig 1F). The staining in thymus was generally moderate to strong in intensity. In tonsil and reactive lymph nodes strong nuclear GATA3 immunoreactivity was seen in scattered extracellular lymphocytes (table 2). Eosinophils, with strong cytoplasmic GATA3 staining, were also detected in interfollicular areas. In the light and dark zones of GCs a staining of weak to moderate intensity was observed, especially in larger lymphocytes. In the mantle zone some of the lymphocytes showed weak GATA3 immunoreactivity (fig 1G). In PTGC both transformed follicles and enlarged mantle zone displayed moderately or weakly interspersed positive cells, whereas in interfollicular areas strongly GATA3+ lymphocytes were observed similar to the reactive lymph node (fig 1H).
GATA3 immunostaining was observed at very low numbers of NLPHL associated T-cells (fig 1I). The positive T-lymphocytes within the nodules were distributed randomly with no spatial relation to tumour cells. In most CHL cases 10–25% of the reactive infiltrate showed a moderate staining for GATA3. The positive T-lymphocytes appeared to be distributed randomly with no specific relation to HRS cells (fig 1J).

Striking expression of c-Maf in NLPHL and PTGC, but not in "paragranuloma type" T/HRBCL

In thymus, scattered larger thymocytes in the cortex stained strongly with c-Maf, whereas in the medulla large cells as well as interspersed smaller cells were stained with weak to moderate intensity (fig 1K, table 2). c-Maf+ T-cells were predominantly detected in the light zone of GCs and just beneath the follicular mantle, even though they were also observed in the other compartments including the mantle zone and interfollicular area (fig 1L). Some of the GC macrophages were also stained in moderate intensity. When we performed double immunostaining on tonsil sections with CD3, 90–95% of the c-Maf+ cells were CD3+. These cells were predominantly located in GCs. Moreover, 5–10% of the CD3+ T-cells were c-Maf co-positive. Double immunostaining with CD57 on tonsil sections revealed that 60–70% of the c-Maf+ cells were co-expressing CD57 and these cells were exclusively located within the GCs. In total more than 90% of the CD57+ T-cells were c-Maf co-positive. In the interfollicular areas, intensely stained cells were interspersed. Strongly c-Maf+ cells were highly increased in numbers in transformed follicles of PTGC cases (fig 1M). In contrast to normal GC, there was no regional distribution of c-Maf+ cells in PTGC. Although few in number, some c-Maf+ collarettes with strong staining intensity were observed around large lymphocytes in PTGC (fig 1M, inset). These scattered rosette like formations were found especially in the enlarged mantle zone. Single cells that were c-Maf+ were also observed in the expanded mantle zone.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>% Positive cells</th>
<th>Intensity</th>
<th>% Positive cells</th>
<th>Intensity</th>
<th>% Positive cells</th>
<th>Intensity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thymus</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cortex</td>
<td>1–2</td>
<td>++</td>
<td>50–75</td>
<td>+++</td>
<td>1–5</td>
<td>+++</td>
</tr>
<tr>
<td>Medulla</td>
<td>1–3</td>
<td>++</td>
<td>10–25</td>
<td>++</td>
<td>5–10</td>
<td>++</td>
</tr>
<tr>
<td>Reactive lymphoid tissue</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GCs</td>
<td>&lt;1</td>
<td>++</td>
<td>1–5</td>
<td>++</td>
<td>5–10</td>
<td>+++†</td>
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<td>Extracellular compartment</td>
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<td>++ ‡</td>
<td>5–10</td>
<td>+++</td>
<td>3–5</td>
<td>++</td>
</tr>
<tr>
<td>PTGC+</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Transformed GCs</td>
<td>&lt;1</td>
<td>++</td>
<td>1–5</td>
<td>++</td>
<td>10–25</td>
<td>+++</td>
</tr>
<tr>
<td>Expanded mantle zone</td>
<td>1–2</td>
<td>+++</td>
<td>1–5</td>
<td>++</td>
<td>2–3</td>
<td>+++</td>
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<tr>
<td>Extracellular compartment</td>
<td>3–5</td>
<td>++</td>
<td>5–10</td>
<td>+++</td>
<td>3–5</td>
<td>++</td>
</tr>
</tbody>
</table>

GC, germinal centre.

Strikingly, c-Maf staining was observed in reactive lymphoid tissues and progressively transformed GCs (PTGC). In two cases, 25–50% of the reactive infiltrate showed a moderate staining for c-Maf. Double immunostaining on tonsil sections revealed that 60–70% of the c-Maf+ cells were co-expressing CD57 and these cells were exclusively located within the GCs. In total more than 90% of the CD57+ T-cells were c-Maf co-positive. In the interfollicular areas, intensely stained cells were interspersed. Strongly c-Maf+ cells were highly increased in numbers in transformed follicles of PTGC cases (fig 1M). In contrast to normal GC, there was no regional distribution of c-Maf+ cells in PTGC. Although few in number, some c-Maf+ collarettes with strong staining intensity were observed around large lymphocytes in PTGC (fig 1M, inset). These scattered rosette like formations were found especially in the enlarged mantle zone. Single cells that were c-Maf+ were also observed in the expanded mantle zone.

Table 2: Immunohistochemical expression pattern of T-cell transcription factors (TFs) in thymus, reactive lymphoid tissues and progressively transformed germinal centres (PTGC)

<table>
<thead>
<tr>
<th>Tissue</th>
<th>% Positive cells</th>
<th>Intensity</th>
<th>% Positive cells</th>
<th>Intensity</th>
<th>% Positive cells</th>
<th>Intensity</th>
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<td>+++</td>
<td>1–5</td>
<td>+++</td>
</tr>
<tr>
<td>Medulla</td>
<td>1–3</td>
<td>++</td>
<td>10–25</td>
<td>++</td>
<td>5–10</td>
<td>++</td>
</tr>
<tr>
<td>Reactive lymphoid tissue</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GCs</td>
<td>&lt;1</td>
<td>++</td>
<td>1–5</td>
<td>++</td>
<td>5–10</td>
<td>+++†</td>
</tr>
<tr>
<td>Extracellular compartment</td>
<td>3–5</td>
<td>++ ‡</td>
<td>5–10</td>
<td>+++</td>
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<td>++</td>
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<tr>
<td>PTGC+</td>
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</tr>
<tr>
<td>Transformed GCs</td>
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<td>++</td>
<td>1–5</td>
<td>++</td>
<td>10–25</td>
<td>+++</td>
</tr>
<tr>
<td>Expanded mantle zone</td>
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<td>1–5</td>
<td>++</td>
<td>2–3</td>
<td>+++</td>
</tr>
<tr>
<td>Extracellular compartment</td>
<td>3–5</td>
<td>++</td>
<td>5–10</td>
<td>+++</td>
<td>3–5</td>
<td>++</td>
</tr>
</tbody>
</table>

GATA3 immunostaining was observed at very low numbers of NLPHL associated T-cells (fig 1I). The positive T-lymphocytes within the nodules were distributed randomly with no spatial relation to tumour cells. In most CHL cases 10–25% of the reactive infiltrate showed a moderate staining for GATA3. The positive T-lymphocytes appeared to be distributed randomly with no specific relation to HRS cells (fig 1J).

Table 3: Immunohistochemical expression of T-cell transcription factors (TFs) in reactive lymphoid cells in tissues involved by Hodgkin’s lymphoma (HL)

<table>
<thead>
<tr>
<th>HL subtype</th>
<th>% Positive cells</th>
<th>T-bet</th>
<th>GATA3</th>
<th>c-Maf</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Cases/total</td>
<td>Intensity</td>
<td>Cases/total</td>
<td>Intensity</td>
</tr>
<tr>
<td>NLPHL*</td>
<td>&lt;10</td>
<td>5/6+</td>
<td>7/7+</td>
<td>3/6+</td>
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<tr>
<td></td>
<td>10–25</td>
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<td>2/6+++</td>
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<tr>
<td></td>
<td>25–50</td>
<td>2/16+</td>
<td>5/12+</td>
<td>1/13+</td>
</tr>
<tr>
<td></td>
<td>&gt;75</td>
<td>3/16++</td>
<td>2/12+</td>
<td>8/13+</td>
</tr>
<tr>
<td>CHL†</td>
<td>&lt;10</td>
<td>7/16+++</td>
<td>4/16+</td>
<td>1/13+</td>
</tr>
<tr>
<td></td>
<td>10–25</td>
<td>3/16++</td>
<td>2/12+</td>
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<td></td>
<td>25–50</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>&gt;75</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

NLPHL, nodular lymphocyte predominance type of Hodgkin’s lymphoma; CHL, classical Hodgkin’s lymphoma.

*, weakly positive; ‡, moderately positive; †, strongly positive.

Percentages refer to the percentage of immunoreactive cells relative to all infiltrating cells.

* Most rosetting cells in NLPHL cases were moderately or strongly c-Maf+.
† There was no clear difference among different subsets of CHL according to number of positive cells, distribution or intensity of T-cell TF expression.
In the majority of NLPHL cases, 25–50% of the lymphocytes showed moderate to strong expression of c-Maf (table 3). High numbers of positive cells with a strong staining intensity for c-Maf were detected preferentially within the neoplastic follicles and in the lymphocytes, forming rosettes around the L&H cells (fig 1N and inset). A lower number of c-Maf+ cells were observed outside of the nodules of NLPHL. While strongly expressed on rosetting cells, a weak to moderate staining intensity was observed on non-rosetting lymphocytes. Double staining of NLPHL sections with c-Maf and CD57 revealed the abundant presence of c-Maf+/CD57+ phenotype T-cells as rosette formations around lymphocytic and/or histiocytic cells, or widely distributed in the NLPHL nodule. Original magnification x157.5.

DISCUSSION

In the present study, we analysed the expression pattern of three T-cell TFs, considered to be important in T<sub>1</sub> lineage commitment, in the reactive cells of HL. T-bet<sup>17</sup> and GATA3<sup>18,19</sup> were identified as master factors regulating induction of T<sub>H</sub>1 and T<sub>H</sub>2 pathways respectively. c-Maf is a basic leucine zipper transcription factor that is expressed in T<sub>H</sub>2 cells and has a selective function in regulation of interleukin 4 (IL4) transcription.<sup>20</sup> c-Maf expression has also been reported in multiple myeloma,<sup>21</sup> and T-bet expression has been found in normal B-cells<sup>22</sup> and some B-cell neoplasms.<sup>10,14</sup> To show the T-cell nature of the positive cells we performed double immunostainings with T-cell specific markers on control tonsil sections. These revealed that the vast majority of the c-Maf<sup>+</sup> and T-bet<sup>+</sup> cells were indeed of T-cell origin.

We have shown that the reactive T-cells in CHL cases are predominantly immunoreactive for T-bet. The T-cells in CHL include cells expressing IL2, IL10, IL13, IFNy, TGFβ1,2 and FOXP3<sup>3</sup> and can be regarded as having a T<sub>H</sub>2-like or immunoregulatory phenotype.<sup>4,5</sup> This cytokine profile is not consistent with the “master T<sub>H</sub>1 transcription regulator”—T-bet expression in the reactive T-cells of CHL. However, T-bet expression is not necessarily incompatible with T<sub>H</sub>2 cytokine production since retroviral expression of T-bet into T<sub>H</sub>2 cells does not extinguish expression of GATA3 or T<sub>H</sub>2 cytokines.<sup>24</sup> Moreover, T-bet immunoreactivity in the reactive T-cells of CHL does not indicate a T<sub>H</sub>1 type immune response per se; recently a new type of CD<sup>4</sup>/CD<sup>25</sup> adaptive T-regulatory cell has been identified that is phenotypically related to T<sub>H</sub>1 cells with expression of T-bet, FOXP3, ICOS, IL10 and IFNy.<sup>25</sup> Raised levels of FOXP3<sup>+</sup> T-cells were indeed described in CHL, albeit in a low percentage of cases.<sup>3</sup> Therefore, it is possible that the TF expression repertoire of the reactive T-lymphocytes of CHL indicates a specific subpopulation of T-regulatory cells.

In reactive lymphoid tissues, T-bet<sup>+</sup> as well as FOXP3<sup>+</sup> T-cell populations were observed solely in the interfollicular compartment of the reactive lymphoid tissues; this is consistent with the presence of CHL in the interfollicular compartment of the lymph nodes.

In the CHL background population, we also detected GATA3<sup>+</sup> and c-Maf<sup>+</sup> cells at low numbers which did not exhibit a specific distribution pattern. The presence of T-cells with a different T-cell TF expression repertoire suggests various subpopulations with different specificities and functions. On the other hand, during the T-cell differentiation process, before achieving restricted expression in the fully polarised cell, persistent co-expression of both types of T-cell TFs has been reported in both T<sub>H</sub>1 subtypes.<sup>26</sup> Therefore, it is also plausible that part of the reactive T-cells of HL are not yet fully committed or have retained the ability to activate and express T<sub>H</sub>1 and T<sub>H</sub>2 type TFs together.

We have shown that c-Maf<sup>+</sup> T-cells are predominantly located within the GCs of the reactive lymphoid tissues. L&H cells of NLPHL have characteristics of GC B-cells, and the nodules of NLPHL are considered altered germinal centres.<sup>27</sup> Thus, L&H cells, surrounded by c-Maf<sup>+</sup> T-cells, proliferate in a GC-like environment. The CD57<sup>+</sup> T-cell rosetting pattern is a distinct and well known feature of NLPHL<sup>1</sup> but this is the first demonstration of c-Maf+/CD57<sup>+</sup> T-cell rosettes around L&H cells. Carbone et al<sup>28</sup> showed the presence of MUM1/IRF4<sup>+</sup> T-cells, which are located mainly in close proximity to L&H cells as rosettes. c-Maf is a specific TF for IL4<sup>20</sup> and MUM1/IRF4 synergises with NFATc2 and c-Maf, to augment IL4 promoter activity as well as to elicit significant levels of endogenous IL4 production.<sup>29</sup> However, we have recently reported that in contrast to their tonsillar counterparts, CD<sup>4</sup>/CD<sup>57</sup> T-cells of NLPHL do not express IL4 even after PMA–ionomycine activation.<sup>2</sup> This suggests that in NLPHL, c-Maf and MUM1/IRF4 induced expression of IL4 is blocked. c-Maf also has a transforming ability,<sup>30</sup> and has been shown to function as an oncogene.<sup>31</sup> The functional significance of c-Maf<sup>+</sup> T-cell rosettes in NLPHL cases remains to be elucidated.
T-cell transcription factors in Hodgkin’s lymphoma

NLPHL can histologically and immunophenotypically resemble a subset of T/HRBCL cases. To investigate whether the presence of c-Maf+ rosettes is of value in the differential diagnosis between T/HRBCL and NLPHL, we have stained a specific subtype of cases referred to as “paragranuloma type” of T/HRBCL for c-Maf antibody. None of the six paragranuloma type of T/HRBCL cases showed c-Maf+ rosettes but rather showed a “CHL pattern” of c-Maf staining. Therefore, the presence of c-Maf+ rosettes may help to distinguish NLPHL from paragranuloma type of T/HRBCL. In fact, three immunophenotypic features can be used to distinguish NLPHL from T/HRBCL, including two markers of the neoplastic B-cells (LSP-1, PU.1) and one differentially expressed by the non-neoplastic T-cells (c-Maf).

PTGC have been consistently associated with NLPHL. Our results further support the hypothesis that PTGC and NLPHL may both be manifestations of an abnormal follicle centre reaction. c-Maf staining was most pronounced in the light zone of GC of the reactive lymph nodes (tables 2 and 3). In transformed follicles of PTGC cases there was an increased frequency of c-Maf+ T-cells together with loss of zonal distribution of c-Maf reactivity. Frequent c-Maf+ T-cell rosettes around L&H cells was a common characteristic of all NLPHL cases, and c-Maf+ rosette formation were also detected between broken-up borders of GC and the mantle zone in transformed follicles of PTGC, albeit at low frequencies. PTGC and NLPHL nodules contained very few GATA3+ and T-bet+ T-lymphocytes, which is consistent with an enlarged mantle zone like B-cell rich area.

In conclusion, we have shown that CHL associated lymphocytes are mostly T-cell expressing T-cells, whereas NLPHL cases have a high frequency of c-Maf+/CD57+ T-cells as rosettes or single cells. The T-cell TF expression pattern of the HL associated T-cells is consistent with the T-cell TF pattern observed in the distinct lymphoid compartments in which HL subtypes are diagnosed. This implies that background T-cells of HL are not randomly distributed T-cells, but an expansion of the native T-cells or an influx of T-cells with the same profile as the native T-cells in the corresponding lymphoid compartments.

**Take-home messages**

- Background T-cells of Hodgkin’s lymphoma (HL) are not randomly distributed T-cells: the reactive T-cells in classical Hodgkin’s lymphoma (CHL) are predominantly immunoreactive for T-bet and in NLPHL for c-Maf.
- T-cell transcription factor profiles of HL indicate that the two types of HL are two different diseases within two distinctive lymphoid compartments.
- Raised numbers of c-Maf+/CD57+ T-cell rosettes are characteristic for NLPHL.

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