Granulomatous reactions cause symptoms or clinically imitate treatment resistance in small lymphocytic lymphoma/chronic lymphocytic leukaemia more frequently than in other non-Hodgkin lymphomas

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Aims: The electronic database of the institute of pathology, Medical University of Innsbruck, was reviewed and patient histories studied to analyse systematically the coincidence of granulomatous reactions and lymphomas in a large patient collective, and to find distinct clinicopathological correlations. Five cases of small lymphocytic lymphoma/chronic lymphocytic leukaemia (CLL) associated with granulomatous reactions in lymph nodes and bone marrow were identified, all clinically associated with signs of progressive disease.

Methods: Cases were acquired by reviewing an electronic database comprising approximately 715 000 patients diagnosed between 1993 and 2003. Histochemical, immunohistochemical, and molecular techniques were used to verify diagnosis and associated infectious diseases. Clinical data were obtained from reviewing the charts.

Results: Of 2044 bone marrow and 411 lymph node non-Hodgkin lymphoma biopsy samples, CLL was most frequently associated with bone marrow (two cases) and lymph node granulomas (three cases). These granulomas were mostly composed of epithelioid cells, with or without giant cells, and in all but one case did not show necrosis. All patients with CLL had clinical symptoms primarily caused by the granulomatous disease: two suffered from acid fast bacilli infections (Mycobacterium tuberculosis and mycobacteria other than tuberculosis) and three presented with clinical manifestations of sarcoidosis (the reason a diagnostic biopsy was performed).

Conclusions: Granulomatous reactions in patients with CLL might obscure diagnosis and imitate disease progression and Richter’s transformation. Careful histological examination, exclusion of infectious agents, and a detailed clinical history are essential for correct diagnosis.

In lymph nodes from European patients, so called sarcoid-like lesions represent one of the most common granulomatous reactions, seen most frequently in the draining lymph nodes of solid tumours. In haematological disorders, sarcoid-like lesions are often seen in Hodgkin lymphomas (HL), and have also been found in approximately 4% of non-Hodgkin lymphomas (NHL). Interestingly, the development of extensive sarcoid-like lesions and sarcoidosis in patients with lymphoma and vice versa, a higher risk of developing lymphomas in patients with sarcoidosis (the “sarcoidosis–lymphoma syndrome”), have also been documented. Granulomas are rarely found in trephine bone marrow biopsies. They are found in approximately 30% of cases associated with infectious diseases, with Mycobacterium tuberculosis and Mycobacterium avium intracellulare being responsible for nearly half of these cases. Apart from infections, bone marrow sarcoid-like lesions can be associated with haematological malignancies and, in decreasing frequency, with non-haematological malignancies, sarcoidosis, and drugs. Table 1 shows an overview of the reported coincidence of lymphomas and granulomas in bone marrow and lymph nodes. To the best of our knowledge, in the past 52 years only 69 NHL cases accompanied by sarcoid-like lesions or accumulation of histiocytes in either bone marrow or in the lymph nodes have been reported. These cases have been classified according to different nomenclature systems, making a direct comparison impossible. In the above mentioned studies, small lymphocytic lymphomas did not appear to be over proportionally associated with sarcoid-like lesions, and the causes of granuloma formation have not been studied extensively.

"Patients with lymphoma are at an increased risk of developing disease and treatment related infectious complications, which may also lead to granuloma formation"

Therefore, to analyse systematically the coincidence of granulomatous reactions and NHL, on a large patient collective, and to discover distinct clinicopathological correlations, we reviewed the electronic database at the institute of pathology at the Medical University of Innsbruck, Austria, looking for patients with lymphomas and granulomatous reactions in their bone marrow and lymph nodes, and studied case histories of patients suffering from both.

Abbreviations: CLL, small lymphocytic lymphoma/chronic lymphocytic leukaemia; HL, Hodgkin lymphoma; IFN, interferon; NHL, non-Hodgkin lymphoma; PCR, polymerase chain reaction
**MATERIAL AND METHODS**

**Cases**

Cases were acquired by reviewing the electronic database of the Institute of Pathology at the Medical University of Innsbruck, Austria, comprising approximately 715 000 case files over the observation period from 1993 to 2003, looking for the coincidence of haematological neoplasms and granulomatous reactions in lymph nodes and in bone marrow biopsies. The search was performed by looking for the simultaneous occurrence of the letter combinations “LYMPHOM” or “LEUK” and “GRANULOM” or “EPITHEL” within the diagnosis paragraphs.

**Clinical data**

The clinical data of the patients with granulomatous reactions in lymph nodes and in bone marrow biopsies was obtained by reviewing the charts (table 2).

**Histological and immunohistochemical stainings**

Special stains for acid fast bacilli (Ziehl-Neelsen, Fite-Faraco) and fungi (periodic acid Schiff) were performed. Immunohistochemistry was performed in an automated immunostainer (Nexes; Ventana, Tucson, Arizona, USA), applying the streptavidin–biotin–peroxidase technique with diaminobenzidine as chromogen. The primary antibodies comprised anti-CD3 (Dako, Glostrup, Denmark; 1/50 dilution), anti-CD5 (Novocastra, Newcastle upon Tyne, UK; 1/10 dilution), anti-CD4 (Novocastra; Novacost; 1/50 dilution), anti-CD8 (Dako; 1/50 dilution), anti-CD20 (Dako; 1/700 dilution), anti-CD23 (Novocastra; 1/40 dilution), anti-Cd68 (Dako; 1/100 dilution), anti-CD79 (Dako; 1/300 dilution), and KiM1P (Dako; 1/300 dilution). Heat pretreatment in a wet autoclave at 121°C with citrate buffer (pH 6.0) for five minutes was used for antigen retrieval, except for KiM1P and CD20, for which pretreatment with pronase for eight minutes at room temperature and heat pretreatment in a microwave oven (750 W) for 10 minutes were used, respectively.

**Molecular biology**

To detect genetic material from *M tuberculosis* the total DNA from the formalin fixed, paraffin wax embedded tissue samples of the patients was extracted using a Genoscan (Qiagen, Hilden, Germany) extraction kit. For polymerase chain reaction (PCR) we used primers synthesised by an immunostainer (Nexes; Ventana, Tucson, Arizona, USA), applying the streptavidin–biotin–peroxidase technique with diaminobenzidine as chromogen. The primary antibodies comprised anti-CD3 (Dako, Glostrup, Denmark; 1/50 dilution), anti-CD4 (Novocastra, Newcastle upon Tyne, UK; 1/10 dilution), anti-CD5 (Novocastra; Novacost; 1/50 dilution), anti-CD8 (Dako; 1/50 dilution), anti-CD20 (Dako; 1/700 dilution), anti-CD23 (Novocastra; 1/40 dilution), anti-Cd68 (Dako; 1/100 dilution), anti-CD79 (Dako; 1/300 dilution), and KiM1P (Dako; 1/300 dilution). Heat pretreatment in a wet autoclave at 121°C with citrate buffer (pH 6.0) for five minutes was used for antigen retrieval, except for KiM1P and CD20, for which pretreatment with pronase for eight minutes at room temperature and heat pretreatment in a microwave oven (750 W) for 10 minutes were used, respectively.

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**Table 1** Reported bone marrow and lymph node granulomatous reactions in lymphomas

<table>
<thead>
<tr>
<th>First author</th>
<th>Location</th>
<th>Histology</th>
<th>NHL (according to different classification systems)</th>
<th>Total number of cases examined</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pease et al.</td>
<td>BM, LN</td>
<td>SG, NG</td>
<td>7 NHL, not specified; 2 multiple myelomas; 1 nodular mixed histiocytic–lymphocytic lymphoma; 2 histiocytic lymphomas</td>
<td>221 HL and NHL</td>
</tr>
<tr>
<td>O’Carroll et al.</td>
<td>BM, LN</td>
<td>SG</td>
<td>1 nodular poorly differentiated lymphoma; 2 diffuse poorly differentiated lymphomas; 4 diffuse histiocytic lymphomas; 1 diffuse mixed histiocytic–lymphocytic lymphoma; 1 unclassified splenic lymphoma</td>
<td>84 untreated NHL, CLL, and Mb; Waldenström excluded</td>
</tr>
<tr>
<td>Yu</td>
<td>BM, LN</td>
<td>SG</td>
<td>11 Lennert lymphomas of B and T cell origin</td>
<td>9 cases observed</td>
</tr>
<tr>
<td>Choe et al.</td>
<td>BM</td>
<td>SG</td>
<td>3 poorly differentiated lymphocytic lymphomas; 3 histiocytic lymphomas; 3 MM: 1 acute lymphoplastic leukemia; 1 CLL, 1 MM</td>
<td>372 NHL</td>
</tr>
<tr>
<td>Bhargava et al.</td>
<td>BM</td>
<td>SG</td>
<td>8 NHL, not specified; 1 acute lymphoplastic leukemia; 1 CLL, 1 MM</td>
<td>4988 bone marrow biopsies; 72 specimens with granulomas</td>
</tr>
<tr>
<td>Spiers et al.</td>
<td>BM</td>
<td>SG</td>
<td>11 Lennert lymphomas of B and T cell origin</td>
<td>355 NHL</td>
</tr>
<tr>
<td>Hollingsworth et al.</td>
<td>BM</td>
<td>SG</td>
<td>5 Burkitt lymphomas; 2 follicular lymphomas</td>
<td>7 cases observed</td>
</tr>
<tr>
<td>Dunphy et al.</td>
<td>BM</td>
<td>SG</td>
<td>1 immunocytoma</td>
<td>Single case observed</td>
</tr>
<tr>
<td>Kornacker et al.</td>
<td>BM</td>
<td>SG</td>
<td>1 diffuse large B cell lymphoma</td>
<td>Single case observed</td>
</tr>
<tr>
<td>Blanco et al.</td>
<td>BM</td>
<td>SG</td>
<td>3 CLL</td>
<td>3 cases observed</td>
</tr>
<tr>
<td>Haralambieva et al.</td>
<td>BM</td>
<td>SG</td>
<td>4 Burkitt lymphomas</td>
<td>67 Burkitt lymphomas</td>
</tr>
</tbody>
</table>

BM, bone marrow; CLL, small lymphocytic lymphoma/chronic lymphocytic leukaemia; HL, Hodgkin lymphoma; LN, lymph node; Mb, myeloblastoma; MM, multiple myeloma; NG, necrotising granulomas; NHL, non-Hodgkin lymphoma; SG, sarcoid granulomas.

**Table 2** Patients’ clinical data

<table>
<thead>
<tr>
<th>Case</th>
<th>Sex</th>
<th>NHL</th>
<th>Clinical manifestations at diagnosis of granuloma</th>
<th>IFN</th>
<th>Age</th>
<th>Age</th>
<th>Follow up (months)</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M</td>
<td>CLL</td>
<td>Cough, suspicious pulmonary lesions with lymphadenopathy</td>
<td>No</td>
<td>72</td>
<td>72</td>
<td>120</td>
<td>Alive</td>
</tr>
<tr>
<td>2</td>
<td>F</td>
<td>CLL</td>
<td>Indolent axillary, cervical, and bihilar lymphadenopathy</td>
<td>No</td>
<td>74</td>
<td>74</td>
<td>18</td>
<td>Alive</td>
</tr>
<tr>
<td>3</td>
<td>F</td>
<td>CLL</td>
<td>Bilhar lymphadenopathy resistant to cytotoxic treatment</td>
<td>Yes</td>
<td>54</td>
<td>65</td>
<td>133</td>
<td>Alive</td>
</tr>
<tr>
<td>4</td>
<td>F</td>
<td>CLL</td>
<td>Rapidly growing generalised lymphadenopathy, fever</td>
<td>No</td>
<td>63</td>
<td>71</td>
<td>93</td>
<td>Dead</td>
</tr>
<tr>
<td>5</td>
<td>M</td>
<td>CLL</td>
<td>Rapidly growing mediastinal lymphadenopathy, fever</td>
<td>No</td>
<td>41</td>
<td>55</td>
<td>169</td>
<td>Alive</td>
</tr>
</tbody>
</table>

*Age at diagnosis of NHL; †age at diagnosis of granulomas.
CLL, small lymphocytic lymphoma/chronic lymphocytic leukaemia; IFN, interferon; NHL, non-Hodgkin lymphoma; TBC, tuberculosis.
automated DNA synthesiser (GenXpress, Maria Woerth, Austria) against the *M tuberculosis* complex specific target IS6110 repetitive element, as described by Lachnik and co-authors, and a commercially available ready to use Taq man buffer containing Taq polymerase, reaction buffer, and dNTPs (Applied Biosystems, Rotkreuz, Switzerland). PCR was performed for 50 cycles; the amplification products were visualised using a 121 bp specific fluorescent labelled probe that recognises the IS6110 repetitive element PCR copies in an ABI prism 7000 real time PCR cycler (Applied Biosystems).

RESULTS

Between 1993 and 2003 approximately 715 000 cases were evaluated at our institute, of which 10 472 were bone marrow biopsies; 2044 bone marrow biopsy specimens were infiltrated by NHL and only three by HL; 981 samples were diagnosed as chronic lymphocytic leukaemia/small lymphocytic lymphoma (CLL). In the same period, 411 primary nodal NHLs were diagnosed. Independent of the primary diagnosis, within the observation period, sarcoid-like lesions were diagnosed in 56 lymph nodes, 37 of which were associated with solid neoplasms, three with HL (two classic HL and one nodular paragranuloma), and one with synchronous CLL (patient 2). We identified two more patients with CLL from all of the patients diagnosed with NHL with synchronous (patient 1) or metachronous (patient 3) sarcoid-like lesions in their bone marrow biopsies. Two additional patients with CLL from our database had lymph node granulomas in the setting of infection with *M tuberculosis* (patient 4) and mycobacteria other than *M tuberculosis* (patient 5). All patients with HL and sarcoid-like lesions presented with B symptoms, but had no additional peculiarities in their clinical history. Unexpectedly, all patients with CLL and granulomatous reactions had a distinct clinical history (table 2). Three patients presented with manifestations that typically occur in sarcoidosis (bilateral lymphadenopathy, arthralgia, increased angiotensin converting enzyme concentrations; patients 1, 2, and 3). In addition, in patients 1 (fig 1A) and 2 the diagnostic biopsy was performed because of the progressive lymphadenopathy suspicious of sarcoidosis, and coincidental (synchronous) “dormant” CLL with sarcoid-like lesions was diagnosed histologically. The detection of concomitant CLL in the lymph node of patient 2 was difficult (fig 1C, D); lymphocytic infiltration beyond the lymph node capsule, the disappearance of hilar structures as a result of expansion, and the monotonous lymphocytic morphology were important morphological keys to consider ancillary immunohistochemical studies for diagnosing CLL. In patient 3, CLL was known and Richter’s syndrome was clinically suspected because the progressive bilateral lymphadenopathy was resistant to the fludarabine/interferon α (IFNα) treatment regimen (fig 1B). Therefore, a newly staging bone marrow biopsy was performed, which showed a sarcoid-like lesion and only minimal residual CLL. CLL was also known in patients 4 and 5. They developed rapidly progressive lymph node swellings.
with fever and increased lactate dehydrogenase, clinically suspicious of Richter’s syndrome; patient 5 had recently undergone cyclophosphamide/oncovine/prednisone chemotherapy. The patients were diagnosed as infected with \textit{M tuberculosis} and mycobacteria other than tuberculosis (\textit{M avium intracellulare}), respectively (fig 1E, F), involving the mediastinal lymph nodes and, in patient 5, the small intestine. Both patients were given specific treatment, but patient 4 unfortunately died of septicemia.

**DISCUSSION**

Although the presence of granulomatous reactions in patients with NHL is known, they have been rarely observed in CLL.\(^{11-14} \) In our study group, all five patients with CLL became symptomatic as a result of a granulomatous process within the setting of their previously or newly (patients 1 and 2) diagnosed NHL. Three patients had sarcoid-like lesions, and sarcoidosis, and two suffered from mycobacterial infections.

Sarcoid-like lesions represent non-specific histological patterns that may be associated with viral infections, tuberculosis, sarcoidosis, or lymphoproliferative disorders, so that in addition to a careful histological examination, special stains and PCR analyses should be performed to exclude the presence of an infectious agent.\(^{15} \) Although a distinction is made between sarcoid-like lesions and sarcoidosis, some evidence points to the possibility that sarcoidosis represents a generalised equivalent of sarcoid-like lesions.\(^+\) Several authors noted the presence of both sarcoidosis and NHL in the same patient.\(^{16} \) Clinically, sarcoidosis is known to induce sarcoidosis/sarcoid-like lesions that may be caused by phospholipids from necrotic material or chronic antigen stimulation.\(^{17} \) In sarcoid-like lesions, putative (for example, tumour derived) antigens are thought to cause a hypersensitivity reaction mediated by T cells that stimulate monocytes to form an epithelioid granuloma.\(^{18} \) Continuous IFN\(_\alpha\) administration in viral infection can activate T cells and macrophages.\(^{19} \) An accumulation of CD4\(^+\) T cells has been seen in IFN\(_\alpha\) associated skin eruptions.\(^{20} \)

Indeed, in our study, one female patient with CLL developed fludarabine/IFN\(_\alpha\) resistant biliary lymphadenopathy, with an increased angiotensin converting enzyme concentration (62 U/litre) and raised local CD4/CD8 ratio in the bone marrow sarcoid-like lesions, and therefore was diagnosed as suffering from sarcoidosis. Interestingly, all these symptoms, including the biliary lymphadenopathy, resolved after withdrawing IFN\(_\alpha\) treatment. Kornacker et al noted sarcoidosis in two patients with NHL without IFN\(_\alpha\) treatment and made the intriguing suggestions of (1) a probable spread of an infectious agent during immunosuppression leading to granuloma formation, or (2) chemotherapy induced elimination of immunosuppressive cells that inhibit immune activation resulting in sarcoid-like lesion formation.\(^1\)

To date, sarcoid-like lesions have only rarely been seen in the lymph nodes and bone marrow of patients with CLL. Choe et al investigated 372 cases of bone marrow granulomas and found no cases of NHL, whereas Bharage and Farhi reported one case of CLL in 21 patients with haematological diseases associated with sarcoid-like lesions.\(^{21} \) Marruchella et al reported the case of a 59 year old woman with CLL treated with chlorambucil and fludarabine, who subsequently developed sarcoidosis with diffuse lung involvement and biliary lymphadenopathy.\(^{22} \) In our study, most of the patients with haematological neoplasms and concomitant granulomatous (especially sarcoid-like) lesions had CLL, perhaps in part because of the frequent occurrence and long clinical history of CLL, similar to that seen in testicular germ cell tumours.\(^{23} \) However, studies on T cell function in CLL have revealed a wide range of abnormalities, including absolute CD8\(^+\) lymphocytosis and abnormalities in ligand expression. The elimination of specific CD8\(^+\) immunosuppressive cells and CD4\(^+\) T helper type 1 and 2 cell subsets through chemotherapy might also contribute to sarcoid-like lesion formation.\(^1\) Furthermore, treatment associated T cell dysfunction and subset disturbances in CLL, as specific loss of CD4\(^+\) helper cells—induced by alkylating agents, purine analogues, especially fludarabine, and steroids—\(^{23} \) lead to disturbed immune surveillance for infections, especially those caused by mycobacteria, \textit{Listeria monocytogenes}, pneumocystis, \textit{Candida} spp, \textit{Aspergillus} spp, cytomegalovirus, varicella zoster virus, and herpes simplex virus, all of which may trigger granuloma formation.\(^{24} \)

The treatment

**Table 3** Histological, histochemical, and immunohistochemical findings

<table>
<thead>
<tr>
<th>Case</th>
<th>Granuloma location</th>
<th>Histology</th>
<th>CD4/CD8*</th>
<th>Zielh-Neelsen</th>
<th>PAS</th>
<th>Fite-Faraco</th>
<th>TBC-PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 BM</td>
<td>Microgranuloma, no giant cells; CLL</td>
<td>1/1</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>2 LN</td>
<td>Circumscribed granulomas, epithelioid and Langhans giant cells; CLL</td>
<td>5/1</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>3 BM</td>
<td>Circumscribed granulomas, epithelioid cells, no giant cells; CLL</td>
<td>10/1</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>4 LN</td>
<td>Necrotising granulomas, epithelioid and Langhans giant cells; CLL</td>
<td>2/1</td>
<td>Positive</td>
<td>Negative</td>
<td>Positive</td>
<td>Positive</td>
<td></td>
</tr>
<tr>
<td>5 LN</td>
<td>Large granulomas, foamy macrophages; CLL</td>
<td>No T cells</td>
<td>Positive</td>
<td>Positive</td>
<td>Positive</td>
<td>Negative</td>
<td></td>
</tr>
</tbody>
</table>

*Within the granulomas.

BM, bone marrow; CLL, small lymphocytic lymphoma/chronic lymphocytic leukaemia; LN, lymph node; PAS, periodic acid shiff; PCR, polymerase chain reaction; TBC, tuberculosis.
associated T cell dysfunctions in CLL result mainly from the fact that the therapeutic agents used inhibit not only lymphocyte RNA and DNA synthesis and repair, but also cytokine induced STAT1 activation, and interfere with the interleukin 2 cascade, which is essential for cell mediated immunity function. Indeed, we found severe mycobacterial infections in two patients with CLL, one of whom unfortunately died of septicemia. Clinically, both patients presented with a rapidly progressive generalised lymphadenopathy and lactate dehydrogenase increase, but low leucocyte counts, so that Richter’s transformation was clinically suspected, although the first histological examination detected mycobacteriosis.

In summary, symptoms caused by granulomatous reactions can occasionally lead to the detection of an otherwise dormant CLL or, in cases with known CLL, clinically mimic disease progression or treatment resistance. Treatment with IFNα, but also infections, especially mycobacterioses, and CLL related or treated induced changes in lymphocyte communication may cause granulomatous reactions. Careful histological examination applying special staining procedures, combined with modern methods for molecular detection of infectious agents, considering also details of the clinical history, should lead to the correct diagnosis.

ACKNOWLEDGEMENTS

We thank S. Josef-St, A. Struebl, and M. Koseva for excellent assistance.

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REFERENCES