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Hodgkin / Reed-Sternberg cells and Hodgkin's disease in patients with B-cell chronic lymphocytic leukaemia: an immunohistological, molecular and clinical study of four cases suggesting a heterogeneous pathogenetic background

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Abstract We report the immunohistological, molecular and clinical findings in four patients affected by B-cell chronic lymphocytic leukaemia (CLL) who developed "Richter's syndrome with Hodgkin's disease (HD) features" or "CLL with Hodgkin's transformation", all characterised by the presence of typical Hodgkin / Reed-Sternberg (H/RS) cells in lymph node biopsies. In three cases the nodal involvement by CLL was demonstrated both by the presence of a predominant background of CD5/CD19/CD23+ small lymphocytes and an IgH monoclonal rearrangement revealed by PCR analysis. Conversely, in the remaining case there was neither immunohistological nor molecular evidence of lymph node involvement by CLL. In all four cases H/RS cells were Epstein-Barr virus (EBV) latent membrane protein (LMP-1) positive. These findings suggest that the presence of H/RS cells in the first three patients, who had CLL/HD nodal involvement, might be related to transformation or clonal evolution of CLL cells in H/RS cells, which is in keeping with use of the term "CLL with Hodgkin's transformation". In the fourth case a de novo HD may be postulated, representing a second malignancy presumably not clonally related to CLL. In all cases a key pathogenetic role of EBV is suggested by the expression of LMP-1 in H/RS cells. Our findings indicate that the presence of typical H/RS cells in lymph node biopsies in CLL patients may reflect a heterogeneous pathogenetic background. The different clinico-pathologic settings should be taken into consideration because of their possible implications for patients' treatment and prognosis.

Key words Hodgkin's disease · Chronic lymphocytic leukemia · Epstein-Barr virus

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Introduction

The diagnosis of Hodgkin's disease (HD) is based on the detection of Hodgkin / Reed-Sternberg (H/RS) cells with classical morphological and immunophenotypical features, scattered within an appropriate histological background. B-cell chronic lymphocytic leukaemia (CLL) is a lymphoproliferative disorder which derives from the clonal expansion of a subset of CD5+ B-lymphocytes.

An increasing number of cases of lymphoadenopathy characterised by the presence of H/RS cells, which is consistent with a diagnosis of HD, have been reported in recent years in patients with CLL: in these cases the diagnosis usually recorded is "CLL with Hodgkin's transformation" [2, 4, 9, 16, 17] or "Richter's syndrome with HD features" [1, 7, 13]. In these instances major points of interest and investigation are to elucidate the pathogenetic relationships between the two diseases [15] and to focus on the clinical implications of this peculiar condition. As far as the former point is concerned, it has recently been demonstrated by single-cell PCR assay that in some cases of Richter's syndrome with HD features H/RS cells and CLL cells belong to the same clonal population [10].

In the present study we have analysed the immunohistological and molecular findings in the lymph node biopsies taken from four B-CLL patients, which were all characterised by the presence of typical H/RS cells and thus consistent with a diagnosis of CLL with Hodgkin's transformation or Richter's syndrome with HD features. The possible pathogenetic and clinical implications of these findings are discussed.

Materials and methods

Clinical records

The four patients were all men 54–70 years old with stable A/O stage CLL, who did not need to be treated. The diagnosis of CLL was established on morphological and immunophenotypical grounds according to NCI criteria: all cases were characterised by peripheral blood lymphocytosis (12,000–21,000 lymphocytes/mm^3) and showed an interstitial and/or nodular pattern of bone marrow involvement. Subsequently, all patients developed lymphadenopathy (cervical in three cases and inguinal in one case). Following the diagnosis of HD on lymph node biopsy the patients were treated with HD-addressed therapy (ABVD in all cases, plus radiotherapy in three cases).

Histology, immunohistochemistry and molecular biology

Lymph node biopsies were processed for conventional histology (formalin-fixed, paraffin-embedded, and stained with H&E and Giemsa stains). In all cases a fragment of the lymph node biopsy was snap-frozen in NO_2 and stored at –80°C.

A large panel of antibodies was used for a complete immunophenotypical study of each case (including monoclonal antibodies recognizing CD3, CD4, CD5, CD8, CD19, CD15, CD20, CD23, CD30, BCL-2, p53, RB, and EBV LMP-1 and BZLF1). Immunohistochemical investigations were performed on both paraffin and cryostatic sections by using the APAAP method [5].

PCR analysis for the detection of the IgH rearrangement was performed on DNA extracted from paraffin-embedded tissue of lymph node biopsies and from mononuclear cells of peripheral blood, using a semi-nested PCR protocol, as previously described [6].

Results

Histology and immunohistology

The lymph node biopsies were histologically characterised in all four cases by a background mainly composed of small lymphoid cells, in which a variable number of cytomorphologically typical H/RS cells were scattered (Fig. 1A). No prominent proliferation centres were observed. Case 3 had a more polymorphous appearance characterised by the presence of scattered eosinophils and macrophages. The H/RS cells were consistently CD30+ in all cases, and also expressed the CD15 antigen (>50% of cells in one case, 10–50% of cells in one case, and <10% of cells in each of two cases). In two cases (2 and 3) H/RS cells expressed the B-cell associated antigens CD20 (case 2) and CD79a (case 3) in 10–50% and >50% of cells, respectively. T-cell-associated antigens were usually negative, with the exception of CD43, which was positive in a few H/RS cells in two cases. Epstein-Barr virus (EBV) LMP-1 was strongly expressed in H/RS cell in all four cases (Fig. 1B), whereas EBV BZLF1 protein (ZEBRA), which is expressed in EBV-infected cells in which the virus is replicating, was negative in all cases. p53 was positive in two cases and negative in the remaining two cases.

The small lymphoid cell background was composed mainly of CD5/CD19/CD23+ lymphocytes in three cases (1, 2 and 4), and of CD3/CD4/CD5+ lymphocytes in case 3.

These findings are reported in detail in Tables 1 and 2.

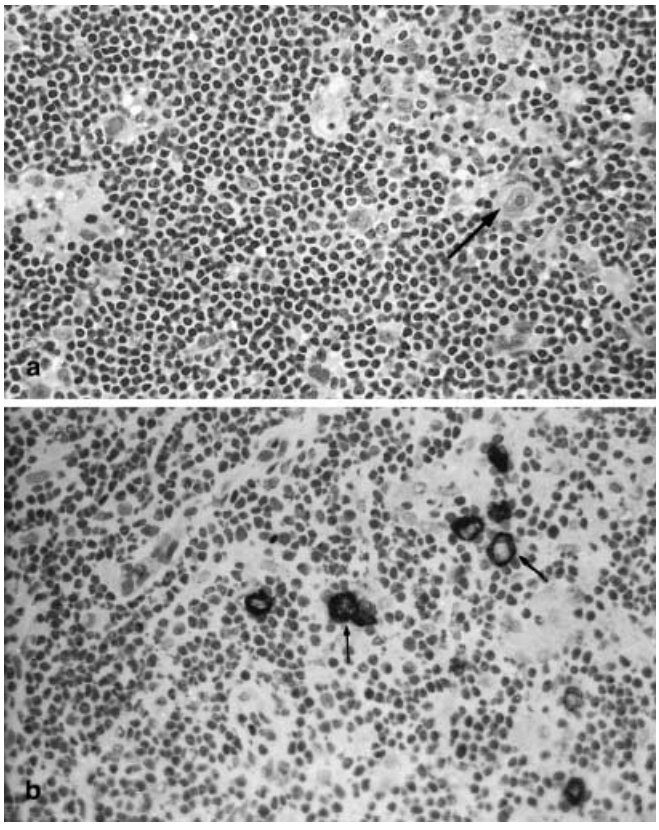

Fig. 1 a Histological features of case 1. Typical H/RS cells (*arrow*) are scattered in a background mainly composed of small lymphocytes. (H&E, ×250) **b** H/RS cells are strongly Epstein-Barr virus LMP-1 positive (*small arrows*). (APAAP, ×250)

Molecular biology

IgH PCR analysis from lymph node biopsies showed a band of monoclonal rearrangement in cases 1, 2 and 4, and a polyclonal pattern in case 3 (Fig. 2A). Furthermore, an additional smaller monoclonal band was detected in case 1 (Fig. 2A, B). In this case the peripheral blood (PB) was also studied by PCR, also showing the presence of a monoclonal band; comparative analysis of the lymph node and peripheral blood samples indicated that the larger band of lymph node and the band of PB had exactly the same size (Fig. 2B).

Follow-up data

As far as HD is concerned, all patients achieved a response to therapy of HD (complete remission [CR] in three cases and partial remission [PR] in one case). Two patients (cases 3 and 4) are still in CR, one (case 1) is in PR on maintenance therapy, and the last one (case 2) died of pneumonia in CR after 9 months off therapy. As far as CLL is concerned, none of the four patients showed clinical evidence of disease progression after HD diagnosis.

Table 1 Immunophenotype of H/RS cells (+> 50%, +/– 10–50%, –/+ <10%, – 0)

Case no.	CD30	CD15	CD20	CD79a	CD3	CD45RO	CD43	LMP-1	p53	Rb	bcl-2
1	+/–	–/+	–	–	–	–	–	+	+/–	+	–
2	+	–/+	+/–	–	–	–	–/+	+	–	+/–	–
3	+	+/–	–	+	–	–	+/–	+	+/–	+	–/+
4	+	+	–	–	–	–	–	+	–	+/–	–

Table 2 Immunophenotype of lymphoid "background" (+> 50%, +/– 10–50%, –/+ <10%, – 0)

Case no.	T-cells			B-cells					
	CD3	CD4	CD8	CD19	CD23	CD5/CD19	bcl-2	p53	Rb
1	–/+	–/+	–/+	+	+	+	+	–	+/–
2	+/–	+/–	–/+	+	+/–	+/–	+	–	+/–
3	+	+	+/–	–/+	–	–	+/–	–	+/–
4	–/+	+/–	–/+	+	+	+	+	–	+/–

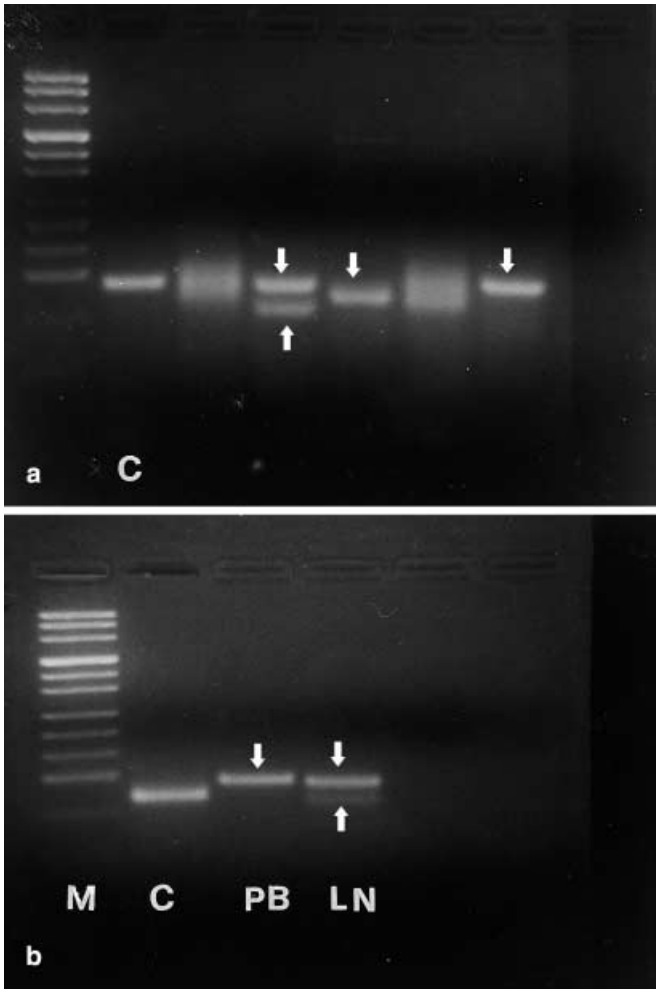

Fig. 2 a IgH PCR analysis of lymph node biopsies. Three cases (*lanes 3, 4 and 6*) show a monoclonal rearrangement band. One case (case 3, *lane 5*) shows a polyclonal pattern. An additional monoclonal band was detected in case 1 (*lane 3*). *Lane 2* polyclonal negative control. **b** Comparative IgH PCR analysis of peripheral blood (*PB*) and lymph node (*LN*) biopsy of case 1, showing two identical monoclonal rearrangement bands in the two samples. An additional monoclonal band was detected in the lymph node sample. (*C* monoclonal positive control, *M* marker)

Discussion

The occurrence of HD in CLL, so-called CLL with Hodgkin's transformation [2, 4, 9, 16, 17] or Richter's syndrome with HD features [1, 7, 13], although rare, is considered one of the commonest secondary neoplasms in patients with a previous history of B-cell CLL [14]. In a large series of 1,011 CLL patients observed in our Institution between 1984 and 1996, we observed a 0.4% rate of HD, which is comparable to the rate reported by Fayad et al. (0.5%) in a series of 1374 cases [7].

Major points of interest and investigation in these cases are the clonal relationship between CLL and H/RS cells, the possible pathogenetic role of oncogenes, "tumour suppressor genes" (such as p53) and viruses (such as EBV), and finally the clinical implications of this diagnosis. As far as the first point is concerned, Ohno et al., using single-cell PCR analysis and DNA sequencing, have recently demonstrated a clonal relationship between CLL cells and H/RS cells in three out of four cases of Richter's syndrome with HD features [10]. Furthermore, a possible pathogenetic role of EBV has been suggested in a significant number of cases [9, 11, 13]. However, the clinical implications of this parcular condition for patients' treatment and prognosis are still not clear, partly because relatively few cases have been reported, although it has been suggested that HD in CLL patients is more probably characterised by poor response to therapy and short survival [7].

In three out of the four cases described in the present study the immunohistological findings of the lymph node biopsy showed nodal involvement by CLL, characterised by the presence of CD5/CD19/CD23+ small lymphocytes associated with scattered H/RS cells with typical morphological and phenotypical features (CD30+; CD15 +/–). In the fourth case, in contrast, the immunohistological investigation did not provide evidence of nodal CLL involvement, the small lymphoid cell background being mainly composed of CD3/CD4/CD5+ lymphocytes, as is generally observed in de novo classical HD. The immunohistological findings were confirmed by IgH PCR analysis,

which showed the presence of a band of monoclonal rearrangement in the former three cases and a polyclonal pattern in the fourth case, the latter being consistent with lacking CLL nodal involvement. Furthermore, the comparative analysis of the PCR findings in the lymph node and in the PB samples of case 1 showed two monoclonal bands within the lymph node, the larger one being of the same size of the the monoclonal band detected in the PB, suggesting the presence of a same clonal population. The meaning of the smaller monoclonal band detected in the lymph node is currently a matter of speculation, although the possibility that it might be due to a different IgH monoclonal rearrangement in H/RS cells should be considered.

These findings suggest that H/RS cells in lymph node biopsies in CLL patients may be observed within two different clinico-pathologic settings. The first – and more frequent – one is characterised by immunohistological and molecular evidence of CLL involvement of the same lymph node, and is presumably related to a transformation (clonal evolution) of CLL cells in H/RS cells, which is consistent with use of the term "CLL with Hodgkin's transformation". The second – and probably less frequent – may basically be considered similar to de novo classical HD, representing a second primary malignancy that is presumably not clonally related to CLL. This distinction is similar to the one reported by Ohno et al., who have considered two different types of Richter's syndrome with HD features [10]. An additional point of discussion is whether these two distinct pathogenetic settings might also have different clinical and prognostic implications: in the present study all cases were treated and showed a response to HD-addressed chemotherapy (ABVD) with or without subsequent radiotherapy, but it will be necessary to analyse a larger series of cases to clarify the prognostic impact and the response to HD therapy in CLL patients.

Furthermore, our results suggest that the term "CLL with Hodgkin's transformation" is more appropriate than "Richter's syndrome with HD features": this impression is supported by the indolent and stable course of CLL associated with responsive HD in all our four patients, whereas the clinical syndrome referred to as "Richter's syndrome" indicates the transformation or the association of a progressive CLL with "high-grade" non-Hodgkin's lymphoma, which usually shows a poor response to therapy and has a poor prognosis [12].

Finally, in this study we have shown that EBV LMP-1 was expressed by H/RS cells in all four cases, whereas EBV BZLF1 protein (ZEBRA) was not expressed in any of these cases, indicating that EBV is neither replicating in infected H/RS cells nor switching from latency to the replication phase [3, 8]. These findings confirm some previous observations suggesting a key role of EBV [9, 11, 13], whose genome is integrated in the absence of active viral replication, in the pathogenesis of these cases. The high percentage of EBV-positive cases (4/4=100% in this study), similar to the proportion of HIV+ patients with HD, might be related to the underlying immunodepression of CLL patients.

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References

1. Brecher M, Banks PM (1990) Hodgkin's disease variant of Richter's syndrome: report of eight cases. Am J Clin Pathol 93:333–339
2. Butts C, Drouin J, Taylor R, Mcleish W (1995) Hodgkin's disease in CLL. Am J Hematol 48:134–135
3. Carey M, Kolman J, Katz DA, Gradoville L, Barberis L, Miller G (1992) Transcriptional sinergy by the Epstein-Barr virus transactivator ZEBRA. J Virol 66:4803–4813
4. Choi H, Keller RH (1981) Coexistence of chronic lymphocytic leukemia and Hodgkin's disease. Cancer 48:48–57
5. Cordell JL, Falini B, Erber WN, Ghosh AK, Abdulaziz Z, MacDonald S, Pulford KA, Stein H, Mason DY (1984) Immunoenzymatic labelling of monoclonal antibodies using immune complexes of alkaline phosphatase and monoclonal anti-alkaline phosphatase (APAAP complexes). J Histochem Cytochem 32:219–229
6. Diss TC, Peng HZ, Wotherspoon AC, Isaacson PG, Pan L (1993) Detection of monoclonality in low-grade B-cell lymphomas using the polymerase chain reaction is dependent on primer selection and lymphoma type. J Pathol 169:291–295
7. Fayad L, Robertson LE, O'Brien S, Manning JT, Wright S, Hagemeister F, Cabanillas F, Keating MJ (1996) Hodgkin's disease variant of Richter's syndrome: experience at a single institution. Leuk Lymphoma 23:333–337
8. Grogan E, Jenson H, Countryman J, Heston L, Gradoville L, Miller G (1987) Transfection of a rearranged viral DNA fragment, Wzhet, stably converts latent Epstein-Barr viral infection to productive infection in lymphoid cells. Proc Natl Acad Sci USA 84:1332–1336
9. Momose H, Jaffe ES, Shin SS, Chen YY, Weiss LM (1992) Chronic lymphocytic leukemia/small lymphocytic lymphoma with Reed-Sternberg-like cells and possible transformation to Hodgkin's disease: mediation by Epstein-Barr virus. Am J Surg Pathol 16:859–872
10. Ohno T, Smir BN, Weisenburger DD, Gascoyne RD, Hinrichs SD, Chan WC (1998) Origin of the Hodgkin/Reed-Sternberg cells in chronic lymphocytic leukemia with "Hodgkin's" transformation. Blood 91:1757–1761
11. Petrella T, Yaziji N, Collin F, Rifle G, Morlevat F, Arnould L, Fargeot P, Depret O (1997) Implication of the Epstein-Barr virus in the progression of chronic lymphocytic leukemia/small lymphocytic lymphoma to Hodgkin-like lymphomas. Anticancer Res 17:3907–3913
12. Robertson LE, Pugh W, O'Brien S, Kantorjan H, Hirsh-Ginsberg C, Cork A, Mc Laughlin P, Cabanillas F, Keating MJ (1993) Richter's syndrome: a report on 39 patients. J Clin Oncol 11:1985–1989
13. Rubin D, Hudnall SD, Aisenberg A, Jacobson JO, Harris NL (1994) Richter's transformation of chronic lymphocytic leukemia with Hodgkin's-like cells is associated with Epstein-Barr virus infection. Mod Pathol 7:91–98
14. Travis LB, Curtis RE, Hankey BF, Franmeni JF (1992) Second cancers in patients with chronic lymphocytic leukemia. J Natl Cancer Inst 84:1422–1427
15. Tsang WYW, Chan JKC, Ng CS (1993) The nature of Reed-Sternberg-like cells in chronic lymphocytic leukemia. Am J Clin Pathol 99:317–323
16. Weisenberg E, Anastasi J, Adeyanju M, Variakojis D, Vardiman JW (1995) Hodgkin's disease associated with chronic lymphocytic leukemia: eight additional cases, including two of the nodular lymphocyte predominant type. Am J Clin Pathol 103:479–484
17. Williams J, Schned A, Cotelingam JD, Jaffe ES (1991) Chronic lymphocytic leukemia with coexistent Hodgkin's disease: implication for the origin of the Reed-Sternberg cells. Am J Surg Pathol 15:33–42