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J-Chain-Producing Immunoblastic Lymphoma in a Case of Richter's Syndrome

Immunohistochemical Evidence for a Gradual Malignant Transformation of a Single B-Cell Clone and Flow Cytophotometric Data

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Summary. A 66-year old male with Richter's syndrome died 52 month after diagnosis of chronic lymphocytic leukaemia (CLL). The clinical course was characterized by a marked IgM hypoglobulinaemia which paralleled a chronically relapsing Herpes simplex infection. Autopsy showed a large retroperitoneal and intraabdominal tumour mass and well defined supradiaphragmatic lymphomas. Histological examination revealed a composite tumour consisting of CLL B-cell type (B-CLL) and immunoblastic malignant lymphoma of B-cell type (B-IbL). The lymphocytes bear μ-chains on their surface and to a lesser extend within their cytoplasm, the obviously defective immunoblasts produce J chains exclusively. Flow cytophotometric data seem to indicate an identical diploid stem line of the two tumours. The majority of the cells are in $G_{0/1}$ phase. The CLL rarely produces mitoses, however, the IbL has a mitotic rate of 7% and a considerable proportion (33%) of cells in the phase of DNA-synthesis. This is the fourth malignant lymphoma and the second immunoblastic lymphoma to be reported that produces J chain in the absence of immunoglobulin.

Key words: B-CLL – B-immunoblastic lymphoma – Richter's syndrome – J chain

Since Lortholary et al. (1964) the term "Richter's syndrome" stands for the infrequent development of a highly malignant lymphoma in patients initially suffering from CLL. There are numerous publications dealing with the nature of the second tumour since the first description by Richter (1928), who wrote of a "reticular cell sarcoma". In the American literature the term "diffuse histiocytic lymphoma" in the sense of Rappaport (1966) has frequently been used (Armitage et al. 1978; Long and Aisenberg 1975; Trump et al. 1980; Woda and Knowles 1979). Since recent immunohistological data show that this group of lymphomas is very heterogenious (Cossman and Berard 1980; Lennert et al.

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1978; Meusers et al. 1979; Strauchen et al. 1978), consisting predominantly of blastic B-cell-, to a lesser extend of blastic T-cell lymphomas and even less commonly of truly histiocytic neoplasia, the second tumour in Richter's syndrome appears in a new light (Magrath 1981). As in the majority of the recently published cases this tumour shows B-cell characteristics (Delsol et al. 1981; Harousseau et al. 1981), the theory was put forward that the CLL and the blastic lymphoma originate from a single malignant cell clone that underwent a partial second malignant transformation. Meanwhile, the discussion on the co-incidence of two heterogenious tumours continues (Cossgriff and McCloskey 1981; Harousseau et al. 1981). Splinter et al. (1978) show two different B-cell clones, Wick et al. (1980) present evidence for a genuine histiocytic sarcoma, Choi and Keller (1981); Harousseau et al. (1981) provide convincing data for Hodgkin's disease, while in other cases interpreted as Hodgkin's disease (Cossgriff and McCloskey 1981) the tumour and its continuing cells seem to lack some important characteristics of lymphogranulomatosis. Some authors describe giant cells resembling Reed-Sternberg cells in Richter's syndrome but are convinced of the non-Hodgkin nature of the tumour as a whole (Harousseau et al. 1981; Foucar and Rydell 1980; Sebahoun et al. 1980).

We present a case of Richter's syndrome with a typical clinical picture and clinical course. The well preserved autopsy material allowed immunohistologic and cytophotometric examinations, the results which will be presented and discussed.

Case Record

At initial presentation Mr. A.M., 63 years old, showed a generalized enlargement of lymph nodes and a marked hepatosplenomegaly. The platelet count was 60,000/µl, the Hb 8.8 g%; the white cell count was 400,000/µl with 89% welldifferentiated lymphocytes, 8% blasts, 2% neutrophils, and some Gumprecht shadows. Sternal bone marrow smears were hypercellular and consisted almost exclusively of mature lymphocytes. Diagnosis at that time was: CLL.

After initial improvement with chemotherapy using prednisone and chlorambucil, symptoms and blood findings stayed constant for 24 months until a necrotizing Herpes simplex nasolabialis and a facial puritus occurred. In addition, an antibody deficiency syndrome with a marked decrease of IgM fraction to 0.4 mg/ml was diagnosed.

Partial remission was induced by applying polyagent chemotherapy with cyclophosphamide, oncovine and prednisone (COP-scheme) giving rise to a regression of lymph node, spleen and liver enlargement. Papulous non-itching eruptions continued to exist on face, forearms and hands, as well as ulcers on the oral mucosa and lower lip. At that time there was a leukocytosis of 20,500/µl with a relative lymphocytosis of 83%; haemoglobin and platelet count were within normal limits.

Two years after the first onset of the papulous skin lesions, leukaemic skin infiltrates were demonstrated histologically. Cutaneous irradiation was performed. Three years after the initial diagnosis severe Herpes simplex relapse with weight loss occured, while the peripheral blood variables remained unchanged. During the following 11 months the patient was hospitalized for most of the time due to frequently relapsing Herpes labialis which tended to generalize, and responded only temporarily to immunoglobulin injections. A therapeutic trial with trophosfamide was unsuccessful, lymph nodes, liver and spleen enlarged, while the white cell count fell from 112,000/µl to almost normal values. Three days prior to death he underwent laparatomy due to increasing signs of intestial obstruction. Large bulky tumour masses were resected, because the established diagnosis of CLL the tissue obtained was discorded.

The autopsy of a cachectic male (165 cm; 45 kg) revealed enlarged, rubbery and well-defined lymph nodes in the cervical, axillary and mediastinal region. Below the diaphragm coherent, infiltrat-

ing bulky tumour masses originating from the retroperitoneal region were observed. The surgical wound at the abdominal wall was overgrown by tumour. The urinary bladder wall, rectum, uretha and funiculi spermatici were also infiltrated. The bone marrow showed a blastomatous expansion of reddish grey colour, the skull vault showed coin-like osteolytic metastasis.

The estimated tumour mass of the well defined supradiaphragmatic lymph nodes was about 500 g, that of the infradiaphragmatic tumour about 3,000 g.

Herpes labialis and skin infiltrations were no longer present; there was no other significant disease.

Methods

Histological Techniques. 3 to 4 μm thick sections from various formalin-fixed and paraffin-embedded tissue samples were stained with hematoxilin eosin, Giemsa, PAS, and Gomöri's silver stain. Immunohistological examination was done by applying the peroxidase-anti-peroxidase technique (Sternberger et al. 1970; Taylor and Burns 1974; Mepham et al. 1975). The following commercial antisera were used: rabbit antihuman μ-, α-, γ-, ε-, λ-chain and anti-human lysozyme (Dako, Denmark); anti-human J-chain (Nordic Immunology, Netherlands), all in a dilution of 1:100; a 1:40 diluted swine anti-rabbit immunoglobulins and a 1:100 diluted PAP-complex produced in rabbit (both Dako, Denmark) were used as a linking and labeling agent, respectively. Peroxidase reaction was visualized with 3,3-diaminobenzidine (Fluka, Switzerland) as substrate agent according to the method of Graham and Karnovsy (1966). This procedure was followed by counterstaining with Meyer's hemalaun, dehydration and mounting with Eukitt.

Intrinsic controls using normal rabbit serum instead of first antibody were always negative. A rabbit anti-human albumin antibody (Dako, Denmark) was also applied and gave no intracytoplasmic positive results in lymphoid cells ruling out transmembranous serum diffusion that can provoke artifacts in immunostaining.

Cytophotometry. A fresh non-fixed tissue sample from bone marrow, cervical lymph node and from the abdominal wall tumour was measured for the DNA-content of the nuclei by means of flow cytophotometry. For this purpose single cell suspensions were prepared by mincing the material mechanically in an acid pepsin solution. After DNA-specific fluorchrome marking with ethidium bromide and 4'6-diamino-2-phenylindole (Zante et al. 1976; Haag 1980) 10^5 cells were measured in the impulse cytophotometer ICP 22 (Phywe, Göttingen, Germany). The evaluation of the resulting distribution of the relative DNA-content by means of a special computer program (Haag 1980) yielded the percentage of cells in $G_{0/1}$-, S-and G_2+M phase. The resulting diagrams furnished information concerning the ploidy of the cell genome.

Results

Histology

Cervical, axillary and mediastinal lymph nodes show a complete effacement of their structure and a diffuse infiltration pattern by small or slightly enlarged lymphocytes with minute nucleoli and a narrow rim of cytoplasm. So-called proliferation centres (Lennert et al. 1978) are rare, there is no pseudo-follicular pattern. The capsule is infiltrated by plasmocytoid cells and scanty immunoblasts. Except for the capsular infiltration the picture is in keeping with the diagnose of B-CLL. The large abdominal tumour is formed in the largest part by large monomorphic immunoblasts and to a lesser extend by lymphocytes. The immunoblasts have a small paranuclear halo. Bipolar mitoses are frequent, atypical ones however, are not present. PAS-positive cellular inclusions are very rare. Giant cell formation within the immunoblastic population is rare (see Figs. 1 and 4, inset).

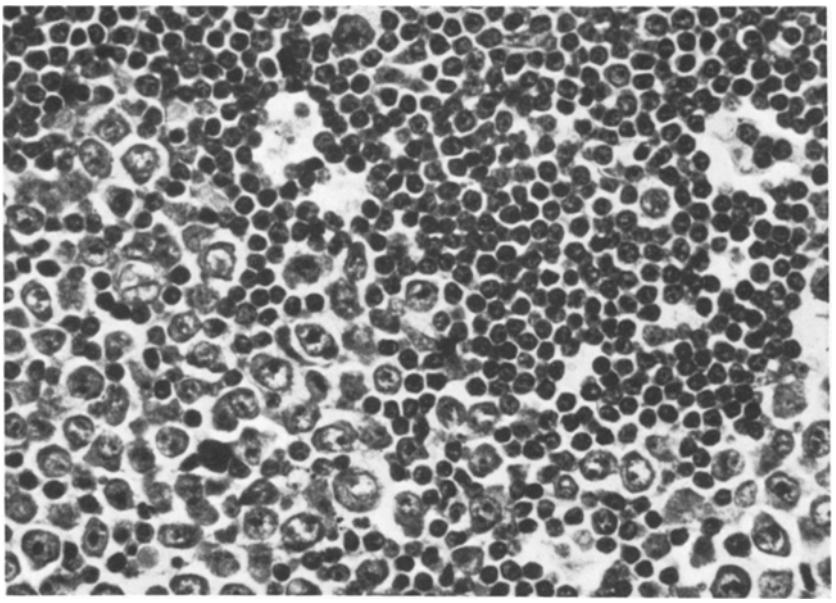

Fig. 1. Lymph node area with mixed infiltration. Lymphocytic population partially displaced by immunoblasts. Giemsa; ×350

Within the residual lymphocytic areas there are round, rather well defined foci with prominent capillaries and venules and a mixed cellular population of lymphocytes, various reticulum cells, eosinophilic granulocytes, macrophages incorporating nuclear debris, and some large multilobated giant cells similar to Reed Sternberg cells (see Fig. 2b). Immunoblasts are absent in these "pseudo-granulomatous" foci.

Immunostaining demostrates γ, μ, κ, λ, chains in some single plasmocytoid cells and mature plasmacells. μ chain is present in a large proportion of the lymphocytic population forming a net and narrow rim of positivity around the nucleus which corresponds to an intracytoplasmic binding site; an overall light positivity for μ chain of many other lymphocytes is probably due to remnants of a surface binding (see Fig. 3) μ is negative in and on all blastic tumour cells, the letters containing no light or heavy chains whatsoever.

Interestingly enough, about 80% of the immunoblasts are highly positive for J-chains in their cytoplasm (see Fig. 4). (This unusual phenomenon was reproducible and noticed for the first time by Prof. H. Stein from the German Lymph Node Registry in Kiel).

The pseudo-granulomatous foci are highly positive for lysozyme the Reed-Sternberg-like cells included (see Fig. 2a). The bone marrow showes a confluent lymphocytic population and little, dispersed immunoblastic proliferation. Some of the pseudo-granulomatous foci are seen in the perivascular spaces. Haemato-poiesis is almost completly displaced.

Spleen and liver show an infiltration pattern typical of B-CLL, restricted to the white pulp and the periportal tracts, respectively. In the kidneys there are perivascular lymphocytic and subcapsular immunoblastic infiltrates.

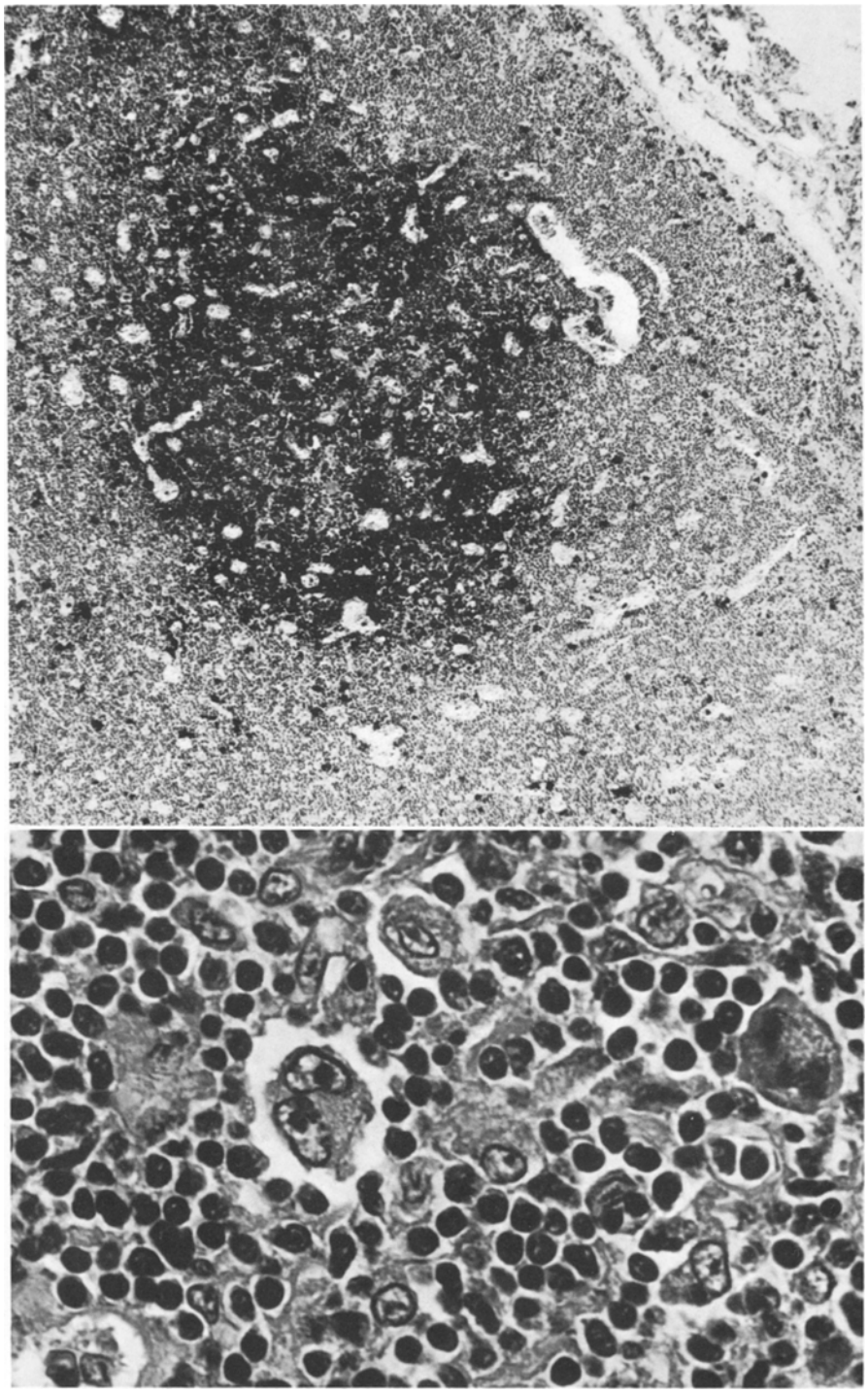

Fig. 2a, b. Pseudo-granulomatous focus in the B-CLL area. **a** Immunostaining for lysozyme. Diaminobenzidine (DAB), haemalaun; × 74. **b** Its cellular constituents: lymphocytes, histiocytes, macrophages, vascular proliferations, some eosinophils and very few Reed-Sternberg-like cells. Giemsa; × 740

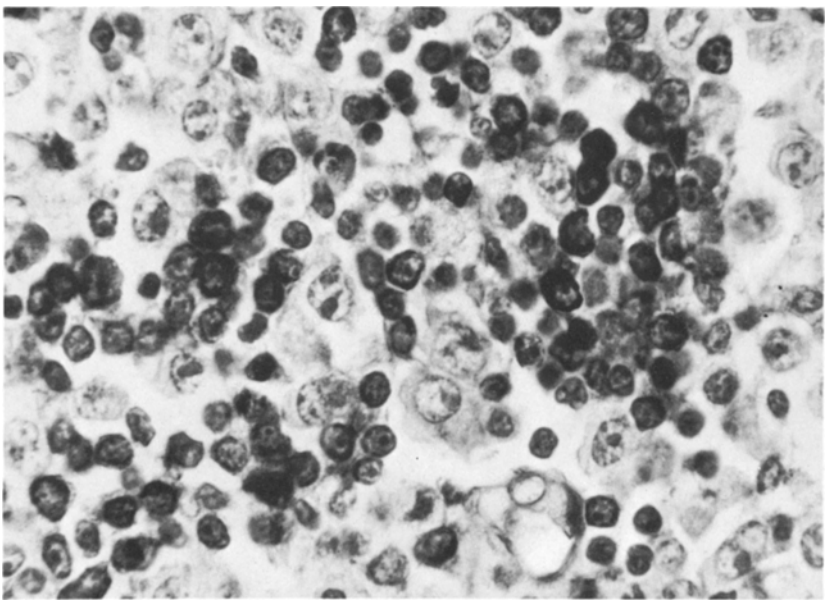

Fig. 3. Immunohistological demonstration of μ chain in and on lymphocytes of CLL. Note the non-stained immunoblasts. DAB, hemalaun; ×830

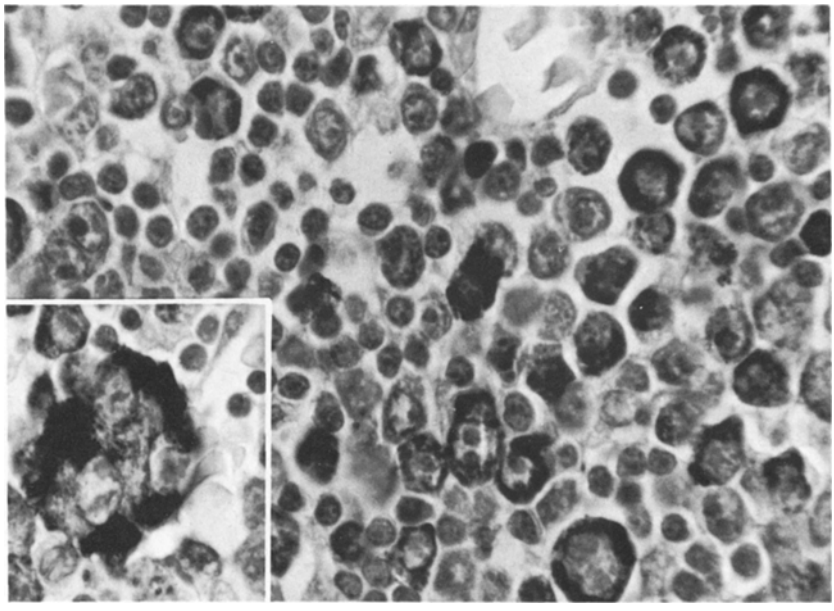

Fig. 4. Immunohistochemical demonstration of J chains in the cytoplasm of immunoblasts. *Inset*: multinuclear J chain producing giant cell. DAB, haemalaun; ×830

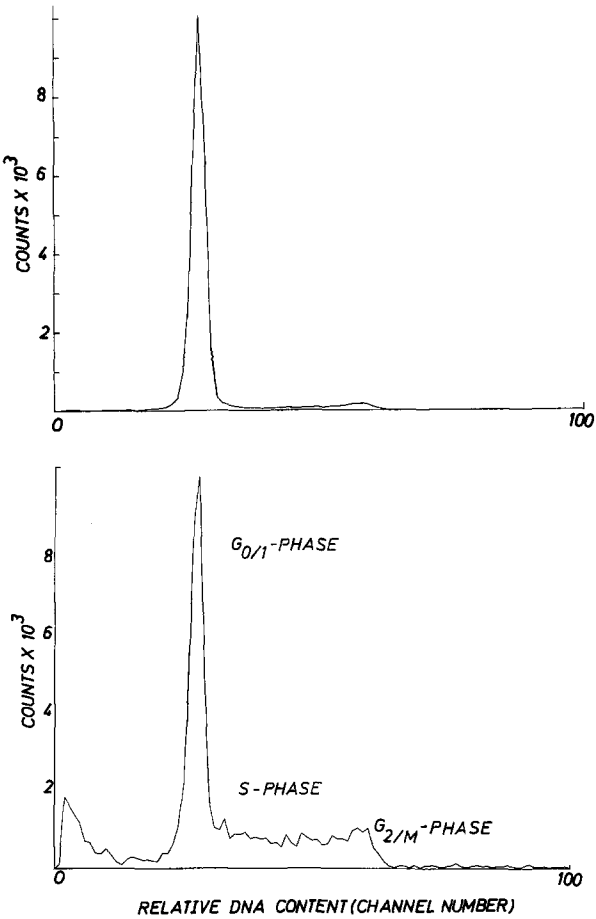

Fig. 5a, b. DNA histopgrams obtained by automated flow cytophotometry.
a Cervical lymph node. Diploid distribution pattern; low S phase fraction.
b Abdominal wall tumour. Diploid distribution pattern; increased number of cells in S phase and G_2+M phase (left side from $G_{0/1}$ peak corresponds to nuclear debris of necrotizing tumour cells)

The osteolytic metastasis consists predominantly of immunoblasts; lymphoplasmocytoid cells are situated in the marginal zone. Osteolysis is caused by a dense layer of osteoclasts forming typical lacunar excavations in the osteoid.

Flow Cytophotometry

As Fig. 5a and b show, the cells of the cervical lymph node and of the bone marrow have a diploid DNA-distribution pattern. Neither a stem line shifting nor a polyploidy can be noted. As the curves are partially identical the CLL cells and the vastly predominating immunoblasts of the abdominal wall tumour (Fig. 5b) have the same DNA-content.

The bone marrow containing mainly CLL cells shows a nearly identical distribution of cell cycle phases compared with the cervical node: more than 90% of the cells are in $G_{0/1}$ phase, 5%, respectively, in phase G_2+M. The cells of the immunoblastic lymphoma however, have only a ca. 60% proportion in $G_{0/1}$phase and a strikingly high percentage of proliferating cells: 33% of cells are in S phase, 7% in G_2+M phase.

The proportion of S phases within the cervical lymph node is within normal limits, while in the bone marrow it is significantly below the normal range of 12 to 18% – a fact which is probably due to the extremely reduced haematopoiesis. The high proportion of phases S and G_2+M is an expression of the highly malignant nature of the tumour.

Discussion

Against the background of the quite numerous reports of Richter's syndrome (Armitage et al. 1978; Choi and Keller 1981; Cossgriff and McCloskey 1981; Delsol et al. 1981; Fialom et al. 1979; Fitzgerald et al. 1980; Foucar and Rydell 1980; Goldstein and Baden 1977; Harousseau et al. 1981; Hoerni et al. 1980; Long and Aisenberg 1975; Nowell et al. 1981; Sebahoun et al. 1980; Splinter et al. 1978; Trump et al. 1980; Wick et al. 1980), this case seems to be clinically typical in many respects: namely the advanced age, sex, the relatively benign course of the CLL with good response to therapy, a secondary immunodeficiency and a myelopathy by displacement; then a sudden deterioration with rapid and untreatable tumour growth (see Armitage et al. 1981) with an impressive pre-terminal decrease of white blood counts. Recurrent Herpes labialis has not yet been described but is easily explained by the chronic IgM-deficiency. As in other cases, it was the autopsy that led to the definitive diagnosis. A limited but infiltrative and destructive tumour mass (in this case infradiaphragmatic) is typical of B-Ibl (Levine et al. 1981) and of Richter's syndrome (Armitage et al. 1981); Osteolytic lesions in the skull have already been observed by Trump et al. (1980). A clear proof of the velocity of tumour propagation is the blastic infiltration of the laparotomy wound dating from an operation just three days prior to death.

As in other cases of Richter's syndrome IgM-dysglobulinaemia was evident: the cases of Schindler et al. (1978) show an IgM-deficiency; Long and Aisenberg (1975) saw one IgM-deficiency and one monoclonal IgM paraprotein; IgM paraproteins have been observed by Choi and Keller (1981) and Trump et al. (1980) in the letters case displaying cold agglutinating properties. As in Foucar's and Rydell's cases 2 and 3 (1980), a subpopulation of lymphocytes had a net positive cytoplasmic ring of IgM which might reflect a retograde dedifferentiation of the CLL towards a pro-lymphocyte (Kjeldsberger and Marty 1981) corresponding to the pre-B-cell of the bone marrow or to the B_o-cell according to Salomon and Seligman (1974). (The typical B-cell however is surface positive, cytoplasmic negative and is an immature cell not reactive to antigen (B_1-cell) (Stein 1981; Aisenberg 1981; Long and Aisenberg 1975)). Alternatively there might have been an abortive commitment and differentiation towards a memory cell in which "differentiation arrest leads to immunoblastic-histiocytic lymphoma" (Magrath 1981). Evidence for such a bidirectional process are presented by Dick and Macas (1978) who describe in some cases of CLL (stage III in their classification) a "bidirectional transformation" when small immature lymphocytes together with "pleomorphic blasts" can be observed.

We are impressed by the loss of immunological markers in our case. It corresponds with findings of Hokland and Ellegaard (1981) reporting surface marker changes of B-CLL in the course of time. Whether these alterations reflect a loss of functional capacities as Wolos and Davey (1981) can show in their mixed lymphocyte culture experiments, is open to question. Nevertheless, in our case the malignant lymphocytes were light-chain negative; the immunoblasts produced only a small fraction of the IgM-molecule i.e. J-chain, the junction chain of the pentamer and even this molecule was lacking in about 20% of the cells. So we believe that in our case a transformation, with a parallel dedifferentiation of the cell has occured. To our knowledge this is the fourth exclusively J-chain producing tumour reported. The first description of this phenomenon was by Mason and Stein (1981).

In our case some Reed-Sternberg-like giant cells can be demonstrated. They are situated within pseudogranulomatous foci and are lysozyme positive. Lennert et al. (1978) saw equivalent cells in cases of CLL (see also: Long and Aisenberg 1975; Foucar and Rydell 1980). In Richter's syndrome this kind of cell has been noted by several authors (Schindler et al. 1978; Goldstein and Baden 1977; Sebahoun et al. 1980). These foci are probably more prominent in other cases (Long and Aisenberg 1975; Foucar and Rydell 1980) and may have been dominant, leading to the diagnosis of Hodgkin's disease. Like Goldstein and Baden (1977) we also found them in the bone marrow. Colby et al. (1981) report a case that was misdiagnosed as lymphocyte predominant Hodgkin's disease because of the numerous Hodgkin-like cells. It was, in fact a CLL. Dick and Maca (1978) believe this cell to be a "pleomorphic blast", a degenerating lymphocyte. Harousseau et al. (1981) distinguish in their 25 cases of Richter's syndrome between Reed-Sternberg-like cells and true Reed-Sternberg cells and present 2 cases of true Hodgkin's disease, one of the nodular sclerosing and one of the epitheloid cell type.

The flow cytophotometric data show that the CLL-population has a very low proliferation activity. This is in keeping with the results of Lang et al. (1980) who consider the main factor of tumour growth in low grade malignant lymphomas to be reduced cell elimination and, conversely, an increased proliferation rate of the cells in high grade malignant lymphomas. The immunoblastic lymphoma, with its high proportion of S- and G_2+M phases, reflects this phenomenon. According to Braylan et al. (1980), lymphomas with an S phase share of more than 5% are to be classified as highly malignant. An other interesting finding was the euploidy of this composite lymphoma. This is inconsistent with results of Fitzgerald et al. (1980) concerning the ploidy in Richter's syndrome. M. Richter, who measured the mitotic angles of the dividing cells in the supervening tumour (1928) did not report on a hyperdiploid mitosis. A pseudohyperdiploidy caused by translocations or deletions, which Morita et al. (1981) find in mitogenstimulated CLL-cells in about 50%, cannot be be ruled out by our method. Nowell et al. (1981) observed in a case of Richter's syndrome pseudo-diploid lymphocytes with a $3q^+$- and $14q^+$-translocation that appeared in originally diploid CLL-cells; in the final stage this tumour developed a hypertriploidy and became a "diffuse histocytic" malignant lymphoma.

The fact that 60% of the immunoblasts are in $G_{0/1}$phase supports the interpretation of recent results of in vitro studies by Dardick et al. (1981) using a T-lymphocyte/concanavalin A-model, suggesting that the full range of morphological alterations observed in lymphocyte transformation can occur in the G_1phase. This refutes Taylor's theory (1974) that lymphocyte transformation is correlated to the cell cycle.

While the B-cell nature of the second malignancy in Richter's syndrome seems to become well established, there are reports of true histiocytic neoplasia supervening CLL (Wick et al. 1980). Nevertheless the term "histiocytic lymphoma" for the characterization of the second tumour should be dropped for the reasons mentioned above.

Perhaps there is a pathological principle hiding behind the composit lymphomas CLL-IbL, CLL-immunocytoma, CLL-plasmocytoma, immunocytoma-IbL, that could be described as a "gradual malignization of a single B-cell clone". To support this theory, additional cytogenetic, cytophotometric, immunological and immunohistological data will have to be collected.

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