Virchows Archiv A Pathol Anat (1991) 419:261–266

Virchows Archiv A
Pathological Anatomy and Histopathology

Original articles

Immunohistochemical evaluation of bone marrow lymphoid nodules in chronic myeloproliferative disorders

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Received 18 February, 1991 / Accepted 16 May, 1991

Summary. One hundred and seventy bone marrow biopsies from patients with chronic myeloproliferative disorders (CMPDs) were evaluated for the presence of lymphoid nodules (LNs) and were immunostained using a panel of monoclonal antibodies (UCHL1, 4KB5 and L26) recognizing different lymphocyte antigens. LNs were found in 35% of cases of idiopathic thrombocythaemia, 24.6% of myelofibrosis/osteomyelosclerosis, 18.2% of polycythaemia vera, 12.1% of chronic myeloid leukaemia and 19.2% of borderline cases. Varying degrees of immunohistochemical positivity for the three antibodies tested were found. LNs were always made up of variable proportions of both T- and B-lymphocytes with a prevalence of T-cells. This latter observation suggests that bone marrow LNs in CMPDs could be an expression of reactivity.

Key words: Lymphoid nodules – Chronic myeloproliferative disorders – Bone marrow – Immunohistochemistry

Introduction

Small aggregates of mature lymphocytes, or lymphoid nodules (LNs), admixed with other cell types, are frequently found in normal and pathological bone marrow (Bartl et al. 1984; Navone et al. 1985; Rywlin et al. 1974). This finding has stimulated many different hypotheses as regards their biological meaning (Bartl et al. 1984, 1985; Burkhardt et al. 1984; Frisch et al. 1984; Frisch and Bartl 1985; Faulkner-Jones et al. 1988; Horny et al. 1989; Jaeger et al. 1983; Navone and Valpreda 1983; Navone et al. 1982, 1985; Sangster et al. 1986). In chronic myeloproliferative disorders (CMPDs), LNs are reported in about 15% of cases (Frisch et al. 1984), ranging from 2.5% to 27% in the different clinical forms (Frisch et al. 1984; Hernandez-Nieto et al. 1979; Navone and Valpreda 1983; Navone et al. 1985). Their significance in the course and development of CMPDs is obscure and their immunophenotypical composition is not well known. Bone marrow immunohistochemistry using several monoclonal antibodies effective on fixed, decalcified and either paraffin- or plastic-embedded trephines plays a major role in bone marrow diagnosis (Archimbaud et al. 1987; Boqué et al. 1989; Casey and Beckstead 1990; Erber and McLachlan 1989; Gatter 1989; Hall et al. 1987; Islam et al. 1988; Schmitt-Graff et al. 1989; van der Valk et al. 1989; Vincic et al. 1989). Among these antibodies, many are able to recognize different lymphocyte subsets (Davey et al. 1988, 1990; Hall et al. 1988; Horny et al. 1990; Norton and Isaacson 1989a, b; van der Valk et al. 1989; Warnke et al. 1989) and, on the basis of several reports, a panel made up of UCHL1, 4KB5 and L26 has proved to be a sensitive, specific and useful one, able to discriminate between B- and T-lymphocytes (Chan et al. 1988; Clark et al. 1990; Hall et al. 1987; Horny et al. 1990). In order to evaluate the incidence of LNs in CMPDs and to characterize the different subsets of lymphocytes immunologically, 170 cases of CMPDs were reviewed and immunostaining with one selected T-cell marker (UCHL1) and two selected B-cell markers (4KB5 and L26) was performed.

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Materials and methods

One hundred and seventy bone marrow biopsies from patients affected by CMPDs were drawn from the files of the Institute of Pathological Anatomy at the University of Palermo. The bone marrow trephines, taken from the posterior iliac crest, had been fixed in Schaffer solution for 12 h, decalcified in EDTA for 2 h, gradually dehydrated in alcohol and embedded in paraffin. Sections 5 μm thick had been cut and stained with haematoxylin and eosin (H&E), Giemsa, periodic acid-Schiff (PAS), silver impregnation according to Gomori and Perls iron stain. The specimens, including 22 cases of polycythaemia vera (PV), 20 cases of idiopathic thrombocythaemia (IT), 33 cases of chronic myeloid leukemia (CML), 69 cases of myelofibrosis/osteomyelosclerosis (MF/OMS) and 26 cases of borderline lesions were histologically re-evaluated regarding the presence of LNs. Borderline cases corresponded to the class II (variant) of the working classification of Burkhardt et al.

Table 1. Number of cases with lymphoid nodules (LNs) and correlation with age in the various chronic myeloproliferative disorders (CMPDs)

	No. of cases	No. of cases with LNs	M/F	Mean age with LNs (SD)	Mean age without LNs (SD)	*P*-value
PV	22	4	1/3	63.7 (13.4)	60.1 (13.8)	NS
IT	20	7	3/4	68.8 (13.9)	56.7 (13)	<0.05
CML	33	4	3/1	66.5 (9.8)	55.5 (13.9)	<0.05
MF/OMS	69	17	10/7	63.8 (8.7)	66.2 (10.4)	NS
Borderline cases	26	5	3/2	62.6 (5.5)	54.2 (16.8)	<0.05
Total	170	37	20/17	64.9 (9.8)	60.2 (13.8)	NS

PV, Polycythaemia vera; IT, idiopathic thrombocythaemia; CML, chronic myeloid leukaemia; MF/OMS, myelofibrosis/osteomyelosclerosis

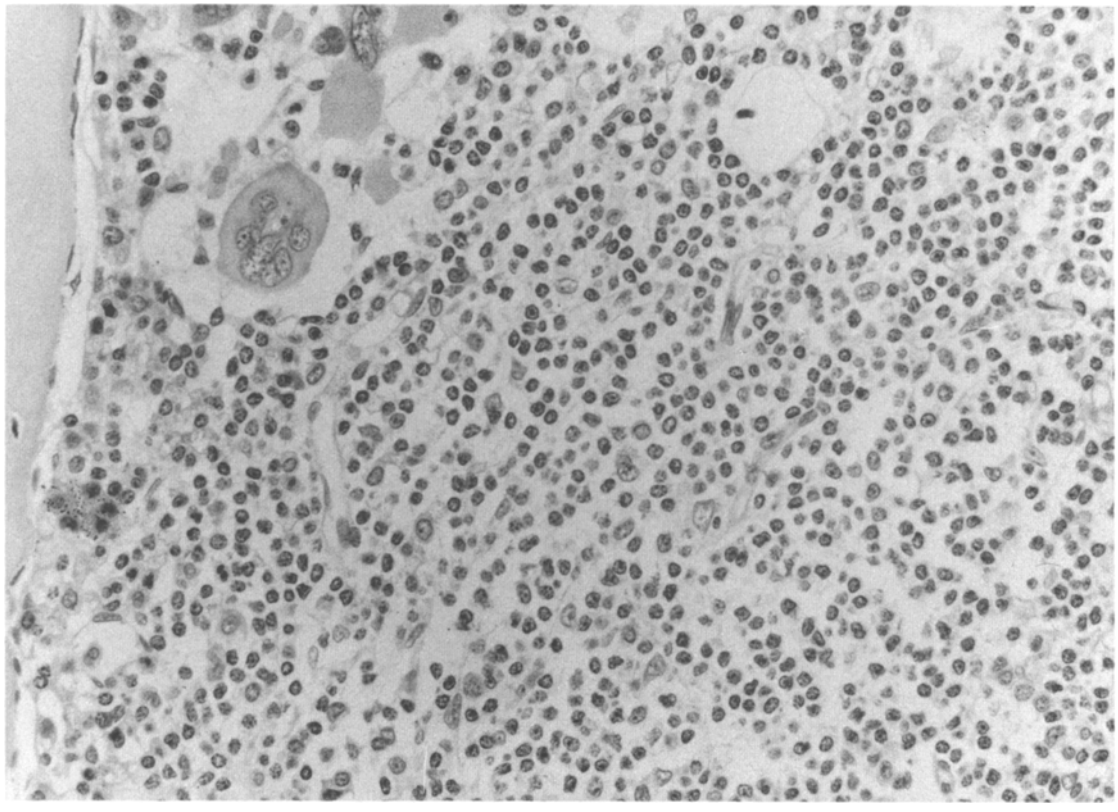

Fig. 1. A well-defined lymphoid nodule made up of lymphocytes, plasma cells, histiocytes and reticulum cells in a case of IT. H&E, ×400

(1990). LNs were classified into four types (Bartl et al. 1984): type A, nodules with germinal centres; type B, sharply demarcated nodules; type C, well-defined nodules; type D, small aggregates of lymphocytes.

Immunostaining was performed by the avidin-biotin complex method using the monoclonal antibodies 4KB5 (CD45RA), UCHL1 (CD45RO) and L26 (Dakopatts Copenhagen, Denmark). Endogenous peroxidase activity was blocked by incubation for 15 min with 3% hydrogen peroxide methanol. Incubation with primary antibody was performed overnight at 4° C and normal mouse serum was used instead of primary antibodies as negative control. Estimation of UCHL1, 4KB5 and L26-positive lymphocytes was made by the following scale (Schmitt-Graff et al. 1989): −, no staining of LN cells; (+), occasional staining of very rare LN cells; +, staining of less than 20% of LN cells; ++, staining of between 20% and 50% of LN cells; +++, staining of more than 50% of LN cells.

Results

The distribution of LNs in the several forms of CMPD together with the mean age of the groups with and without LNs are shown in Table 1. Table 2 reproduces in detail the quantitative distribution of the different types. Among the 170 cases reviewed, 37 revealed the presence of at least one lymphoid nodule. "A"-type nodules were never encountered; in CML cases only very small clusters of lymphocytes of the "D" type were detected, while the other disorders showed a more balanced distribution of the nodules resulting in a slight prevalence (46%) of the "C" type (Fig. 1). The aggregates were generally peripheral or paratrabecular and, close to the foci of active haematopoiesis and particularly of megakaryo-

Table 2. Qualitative and quantitative distribution of lymphoid nodules in CMPDs

	No. of cases with LNs	Type A	Type B	Type C	Type D
PV	4	–	2 (50%)	2 (50%)	–
IT	7	–	2 (28.5%)	3 (43%)	2 (28.5%)
CML	4	–	–	–	4 (100%)
MF/OMS	17	–	6 (35.3%)	9 (53%)	2 (11.7%)
Borderline	5	–	–	3 (60%)	2 (40%)
Total	37	–	10 (27%)	17 (46%)	10 (27%)

Table 3. Immunohistochemical reactivity of the monoclonal antibodies tested with LNs in the different CMPDs

	No. of cases with LNs	UCHL1	4KB5	L26
PV	4	++	++	+
IT	7	+++	++	+
CML	4	+++	++	–
MF/OMS	17	+++	++	++
Borderline	5	++	++	++
Total	37			

–, No staining of LN cells; (+), occasional staining of very rare LN cells; +, staining of less than 20% of LN cells; ++, staining of between 20% and 50% of LN cells; +++, staining of more than 50% of LN cells

poiesis, scattered lymphocytes were often detected. The results of immunostaining are schematically reproduced in Table 3. Although staining restricted to lymphoid cells was achievable only in a few cases because of a variable degree of reaction with the myeloid lineage with two of the antibodies used (UCHL1 and 4KB5), lymphocytes expressed a clear ring-shaped membrane positivity, appearing therefore clearly distinguishable from the diffusely stained myeloid cells. Moreover, their nodular aggregation was strikingly enhanced and in some instances immunohistochemical reactions revealed the presence of little lymphoid clusters not previously detected in the H&E or Giemsa staining.

In PV, UCHL1 stained at least 40% of the lymphocytes, whereas the rest of them revealed a clear positivity for 4KB5. LNs in IT were made up of T-lymphocytes as expressed by the strong ring-shaped staining to UCHL1 (80% of nodule cells), whereas small amounts of 4KB5+ cells were scattered close to the clusters of megakaryocytes. Only a very low percentage (<20%) of lymphocytes was L26+. The behaviour of CML cases differed significantly from that of the other disorders, showing the lowest percentage of LNs (12.1%), consisting of very small and equivocal clusters hardly visible in routine staining. LNs were present only in cases showing a considerable number of megakaryocytes. Most of the aggregated lymphocytes were UCHL1+ (70%), whereas rare scattered 4KB5+ (30%) cells were also detected. No L26+ cells were seen. The group of MF/OMS showed rather a high incidence of nodules occurring in 24.6% of cases. About 50% of the lymphocytes were UCHL1+: they were predominantly distributed in the centre of the nodules while the peripheral cells showed a clear ring-shaped positivity for 4KB5 and, to a lesser extent, for L26 (Fig. 2a–d). No significant immunohistochemical differences were seen between the cases of MF and OMS. In borderline cases of CMPDs, LNs were predominantly of C type, showing a rather different composition with 80% of UCHL1+ cells in some instances and 20% in others (Fig. 3a, b). Smaller aggregates were composed mainly of B-cells both L26+ and 4KB5+.

Discussion

The presence of either nodular or scattered diffuse lymphocytes in bone marrow biopsies of patients with CMPDs is an event which, even if often remarked, has not been explained in detail (Burkhardt et al. 1984; Frisch et al. 1984; Frisch and Bartl 1985; Hernandez-Nieto et al. 1979; Horny et al. 1989; Jaeger et al. 1983; Navone and Valpreda 1983; Navone et al. 1985). A review of LN incidence in CMPDs in different series is reported in Table 4.

In our study, high incidences were detected in cases of IT, MF/OMS and PV (35%, 24.6% and 18.2% respectively), while the CML group showed the lowest prevalence (12.1%). In the borderline group the results were unreliable owing to its lack of homogeneity (Burkhardt et al. 1986, 1990). The correlation between the presence of LNs and age in the several CMPDs led to different results. The mean age of patients with LNs was significantly higher in IT, CML and borderline cases, in agreement with other reports (Thiele et al. 1989), probably due to the decrease in immunoregulatory function of the elderly, which causes a delayed antibody response (Suzuki et al. 1984). The PV and the MF/OMS groups behaved quite differently, showing no significant age differences between the patients with and without LNs. The forms often associated with fibrosis (IT, PV, MF/OMS) have high incidences of LNs (Frisch et al. 1984; Jaeger et al. 1983; Navone and Valpreda 1983; Navone et al. 1985) whereas in CML and particularly in its histological subtypes showing a unilinear granulocytic proliferation devoid of megakaryocytes and rarely transforming into myelofibrosis (Georgii et al. 1990; Thiele et al. 1988) LNs are hardly ever found. Moreover, the high incidences of LNs in MF/OMS and PV groups irrespective of age suggest that in these cases, especially those of MF, their presence could be related to a relationship between stromal microenvironment and immunological processes. The latter could be either of autoimmune origin (Navone et al. 1985) or related to an activation of the complement system (Gordon et al. 1981). An interaction of immune complexes with plate-

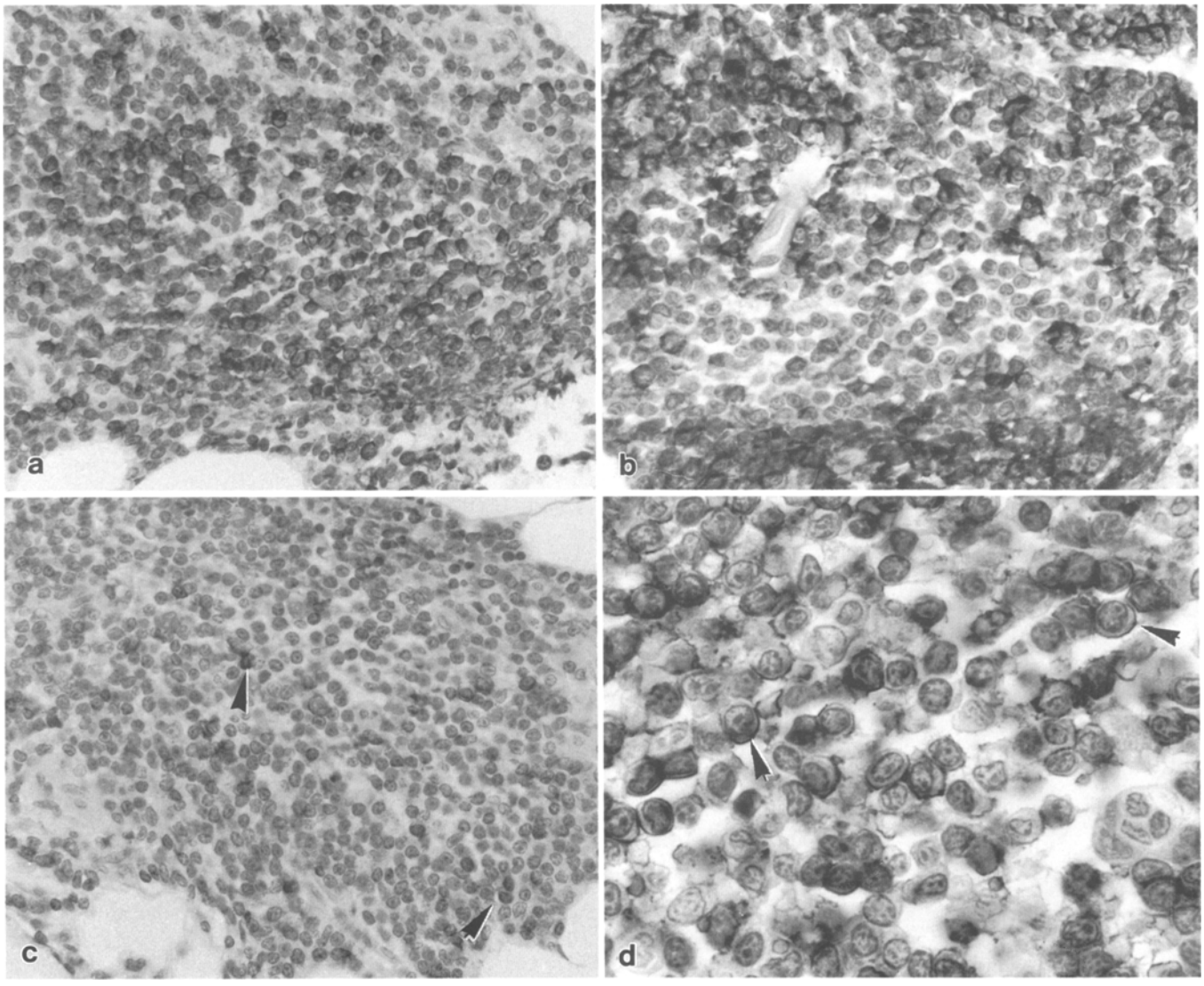

Fig. 2a–d. A lymphoid nodule in a case of myelofibrosis shows different patterns of reactivity with the three monoclonal antibodies tested. In detail, most of UCHL1+ cells are located in the centre of the nodule (**a**), whereas 4KB5 (**b**) and a few L26 (**c**) cells are mainly peripheral (*arrows*). At higher magnification (**d**), lymphocytes positive to UCHL1 express a clear membrane positivity (*arrows*). ABC method with haematoxylin counterstain; **a, b** ×400; **c** ×500; **d** ×1000

Table 4. Incidence of LNs in CMPDs in different series

Reference	Normal	PV	IT	LMC	MF/OMS	Borderline
Hernandez-Nieto et al. (1979)				3%	33.3%	
Frisch et al. (1984)		27%	20%	4%	23% (MF) 11% (OMS)	
Bartl et al. (1985)	2%	24.6%	18%	4%	20% (MF) 10% (OMS)	
Navone et al. (1985)	15%	20.2%		6.4%	15.7%	
Thiele et al. (1989)					12%	
Present series		18.2%	35%	12.1%	24.6%	19.2%

lets and consequent mesenchymal activation is also possible (Caligaris-Cappio et al. 1981; Rondeau et al. 1983).

In our study, the size, morphology and position of LNs were apparently not specifically related to the several different forms of CMPDs, while the immunohistochemical results differed in various diseases expressing different percentages of positivity of T- and B-markers.

In IT and CML, T-lymphocytes represented the prevailing cellular subset of LNs, whereas a lower percentage of cells was 4KB5+ and only a few lymphocytes

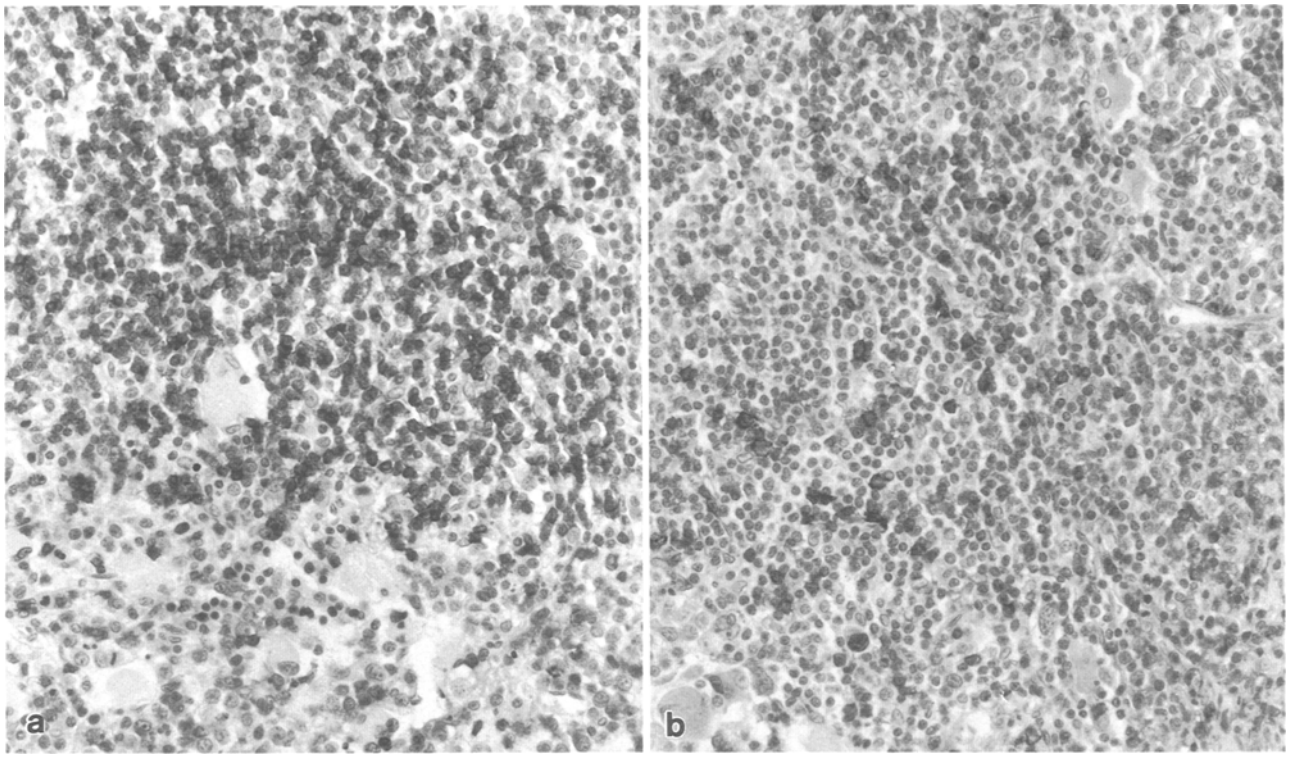

Fig. 3a, b. Two borderline cases show different degrees of reactivity for the same monoclonal antibody; whereas in the first (**a**) UCHL1 positive lymphocytes represent 80% of nodule cells, in the other (**b**) they account only for 30%. ABC method with haematoxylin counterstain. ×250

reacted with L26. The behaviour of PV and MF/OMS was different: in these groups the percentage of UCHL1+ lymphocytes was lower whereas the majority of LN cells was made up of B-lymphocytes, mainly 4KB5+. This variation of LN cellular composition could suggest a different meaning for the nodules in the several CMPDs. Those forms in which LN incidence increases with age show an overwhelming majority of T-cells; as they are generally considered mature and expression of reactivity (Clark et al. 1986; Horny et al. 1989), it follows that the same reactive inference could be attributed to the presence of LNs. In diseases such as PV and MF where LN incidences are not related to age, B-lymphocytes represent the main cellular subset supporting theories suggesting the importance of immunological mechanisms in the development of fibrosis (Caligaris-Cappio et al. 1981; Gordon et al. 1981; Rondeau et al. 1983).

A different response of LN cells to the two B-markers tested was observed: most B-cells reacted with 4KB5 whereas only a few of them were L26+. 4KB5 (CD45RA) generally stains mantle lymphocytes more intensely than germinal centre cells (Chan et al. 1988; Davey et al. 1990), whereas L26, probably recognizing an intracellular epitope of the B-cell CD20 antigen (Mason et al. 1990), is a highly specific B-cell marker with germinal centre cells most intensely stained (Chan et al. 1988; Ishii et al. 1984). Further studies could clarify whether this latter heterogeneous response has pathogenetic significance.

References

Archimbaud E, Islam A, Preisler HD (1987) Immunoperoxidase detection of myeloid antigens in glycolmethacrylate-embedded human bone marrow. J Histochem Cytochem 35:595–599

Bartl R, Frisch B, Burkhardt R, Jaeger K, Pappenberger R, Hoffmann-Fezer G (1984) Lymphoproliferation in the bone marrow: identification and evolution, classification and staging. J Clin Pathol 37:233–254

Bartl R, Frisch B, Burkhardt R (1985) Bone marrow biopsies revisited. A new dimension for haematologic malignancies. Karger, Basel

Boqué C, Pujol-Moix N, Linde MA, Murcia C, Guanyabens C, Soler J (1989) Use of monoclonal anti-actin as a megakaryocyte marker in paraffin wax-embedded bone marrow biopsy specimens. J Clin Pathol 42:982–984

Burkhardt R, Bartl R, Jaeger K, Frisch B, Kettner G, Mahl G, Sund M (1984) Chronic myeloproliferative disorders (CMPD). Pathol Res Pract 179:131–186

Burkhardt R, Bartl R, Jaeger K, Frisch B, Kettner G, Mahl G, Sund M (1986) Working classification of chronic myeloproliferative disorders based on histological, haematological and clinical findings. J Clin Pathol 39:237–253

Burkhardt R, Jaeger K, Kettner G, Helmer G (1990) Chronic myeloproliferative disorders: prognostic importance of new working classification. J Clin Pathol 43:357–364

Caligaris-Cappio F, Vigliani R, Novarino A, Camussi G, Campana D, Gavosto F (1981) Idiopathic myelofibrosis: a possible role for immune-complexes in the pathogenesis of bone marrow fibrosis. Br J Haematol 49:17–21

Casey TT, Beckstead JH (1990) Plastic versus paraffin embedded for histopathology and immunocytochemistry (letter). Am J Surg Pathol 14:500

Chan JKC, Ng CS, Hui PK (1988) A simple guide to the terminolo-

gy and application of leukocyte monoclonal antibodies. Histopathology 12:461–480
Clark P, Normansell DE, Innes DJ, Hess CE (1986) Lymphocyte subsets in normal bone marrow. Blood 67:1600–1606
Clark JR, Williams ME, Swerdlow SH (1990) Detection of B- and T-cells in paraffin-embedded tissue sections. Diagnostic utility of commercially obtained 4KB5 and UCHL-1. Am J Clin Pathol 93:58–69
Davey FR, Olson S, Kurec AS, Eastman-Abaya R, Gottlieb AJ, Mason DY (1988) The immunophenotyping of extramedullary myeloid cell tumors in paraffin-embedded tissue sections. Am J Surg Pathol 12:699–707
Davey FR, Tarek Elghetany M, Kurec AS (1990) Immunophenotyping of haematologic neoplasms in paraffin-embedded tissue sections. Am J Clin Pathol 93 [Suppl 1]:S17–S26
Erber WN, McLachlan J (1989) Use of APAAP technique on paraffin wax embedded bone marrow trephines. J Clin Pathol 42:1201–1205
Faulkner-Jones BE, Howie AJ, Boughton BJ, Franklin JM (1988) Lymphoid aggregates in bone marrow: study of eventual outcome. J Clin Pathol 41:768–775
Frisch B, Bartl R (1985) Histology of myelofibrosis and osteomyelosclerosis. In: Lewis SM (ed) Myelofibrosis. Pathophysiology and clinical management. Dekker, New York, pp 51–86
Frisch B, Bartl R, Burkhardt R, Jaeger K, Mahl G, Kettner G (1984) Classification of myeloproliferative disorders by bone marrow histology. Bibl Haematol 50:57–80
Gatter KC (1989) Diagnostic immunocytochemistry: achievements and challenges. J Pathol 159:183–190
Georgii A, Vykoupil KF, Buhr T, Choritz H, Doehler U, Kaloutsi V, Werner M (1990) Chronic myeloproliferative disorders in bone marrow biopsies. Pathol Res Pract 186:3–27
Gordon BR, Coleman M, Kohen P, Day NK (1981) Immunologic abnormalities in myelofibrosis with activation of the complement system. Blood 58:904–910
Hall PA, Lindeman R, Butler MG, Amess JAL, D'Ardenne AJ (1987) Demonstration of lymphoid antigens in decalcified bone marrow trephines. J Clin Pathol 40:870–873
Hall PA, D'Ardenne AJ, Stansfeld AG (1988) Paraffin section immunohistochemistry. I. Non-Hodgkin's lymphoma. Histopathology 13:149–160
Hernandez-Nieto L, Morey Sureda M, Granena A, Montserrat Costa E, Feliu E, Rozman C (1979) Les nodules lymphoides de la moelle dans la splénomégalie myéloide: comparaison avec la leucémie myéloide chronique. Nouv Rev Fr Haematol 21:251–256
Horny HP, Engst U, Waltz RS, Kaiserling E (1989) "In situ" immunophenotyping of lymphocytes in human bone marrow: an immunohistochemical study. Br J Haematol 71:313–321
Horny HP, Campbell M, Steinke B, Kaiserling E (1990) Acute myeloid leukemia: immunohistologic findings in paraffin-embedded bone marrow biopsy specimens. Hum Pathol 21:648–655
Ishii Y, Takami T, Yuasa H, Takei T, Kikuchi K (1984) Two distinct antigen systems in human B lymphocytes: identification of cell surface and intracellular antigens using monoclonal antibodies. Clin Exp Immunol 58:183–192
Islam A, Archimbaud A, Henderson ES, Han T (1988) Glycol methacrylate (GMA) embedding for light microscopy. II. Immunohistochemical analysis of semithin sections of undecalcified marrow cores. J Clin Pathol 41:892–896
Jaeger K, Burkhardt R, Bartl R, Frisch B, Mahl G (1983) Lymphoid infiltrates in chronic myeloproliferative disorders (MPD). Verh Dtsch Ges Pathol 67:239–242
Mason DY, Comans-Bitter WM, Cordell JL, Verhoeven MAJ, Dongen JJM van (1990) Antibody L26 recognizes an intracellular epitope on the B-cell associated CD20 antigen. Am J Pathol 136:1215–1222
Navone R, Valpreda M (1983) Presenza di noduli linfatici nel midollo osseo in soggetti affetti da policitemia vera e da poliglobulie secondarie. Pathologica 75:235–240
Navone R, Vigliani R, Valpreda M (1982) Studio dei noduli linfatici del midollo osseo in una casistica autoptica. Pathologica 74:231–240
Navone R, Valpreda M, Pich A (1985) Lymphoid nodules and nodular lymphoid hyperplasia in bone marrow biopsies. Acta Haematol (Basel) 74:19–22
Norton AJ, Isaacson PG (1989a) Lymphoma phenotyping in formalin-fixed and paraffin wax-embedded tissues. I. Range of antibodies and staining patterns. Histopathology 14:437–446
Norton AJ, Isaacson PG (1989b) Lymphoma phenotyping in formalin-fixed and paraffin wax-embedded tissues. II. Profiles of reactivity in the various tumour types. Histopathology 14:557–579
Rondeau E, Solal-Celigny P, Dhermy D, Vroclans M, Brousse N, Bernard JF, Boivin P (1983) Immune disorders in agnogenic myeloid metaplasia: relations to myelofibrosis. Br J Haematol 53:467–475
Rywlin AM, Ortega RS, Dominguez CJ (1974) Lymphoid nodules of bone marrow: normal and abnormal. Blood 43:389–400
Sangster G, Crocker J, Nar P, Leyland MJ (1986) Benign and malignant (B-cell) focal lymphoid aggregates in bone marrow trephines shown by means of an immunogold-silver technique. J Clin Pathol 39:453–457
Schmitt-Graff A, Skalli O, Gabbiani G (1989) alpha-Smooth muscle actin is expressed in a subset of bone marrow stromal cells in normal and pathological conditions. Virchows Arch [B] 57:291–302
Suzuki K, Hirokawa K, Hatakeyama S (1984) Age-related change of distribution of immunoglobulin containing cells in human bone marrow. Virchows Arch [A] 404:243–251
Thiele J, Simon K-G, Fischer R, Zankovich R (1988) Follow-up studies with sequential bone marrow biopsies in chronic myeloid leukaemia and so-called primary (idiopathic) osteo-myelofibrosis. Evolution of histopathological lesions and clinical course in 40 patients. Pathol Res Pract 183:434–445
Thiele J, Steinberg T, Zankovich R, Fischer R (1989) Primary myelofibrosis-osteomyelosclerosis (agnogenic myeloid metaplasia): correlation of clinical findings with bone marrow histopathology and prognosis. Anticancer Res 9:429–436
Valk P van der, Mullink H, Huijgens PC, Tadema TM, Vos W, Meijer CJLM (1989) Immunohistochemistry in bone marrow diagnosis. Value of a panel of monoclonal antibodies on routinely processed bone marrow biopsies. Am J Surg Pathol 13:97–106
Vincic L, Weston S, Riddel RH (1989) Bone core biopsies. Plastic or paraffin? Am J Surg Pathol 13:329–334
Warnke RA, Pulford KAF, Pallesen G, Ralfkiaer E, Brown DC, Gatter KC, Mason DY (1989) Diagnosis of myelomonocytic and macrophage neoplasms in routinely processed tissue biopsies with monoclonal antibody KP1. Am J Pathol 135:1089–1095