Histol Histopathol (1999) 14: 805-812

http://www.ehu.es/histol-histopathol

Histology and Histopathology

Frequent expression of haemopoietic and non-haemopoietic antigens by reactive plasma cells: an immunohistochemical study using formalin-fixed, paraffin-embedded tissue

R. Beschorner[1], H.-P. Horny [2], U.R. Petruch[3] and E. Kaiserling[2]

[1]Institute of Brain Research, University of Tübingen, Germany,

[2]Institute of Pathology, University of Tübingen, Tübingen, Germany and [3]Children's University Hospital, Tübingen, Germany

Summary. Unlike other B cells, plasma cells (PC) react with only a few antibodies against haemopoietic antigens. We investigated 36 specimens exhibiting a reactive increase in PC numbers (i.e. plasmacytosis, PC hyperplasia) with a broad panel of antibodies suitable for use on formalin-fixed, paraffin-embedded tissue, and compared the findings with those obtained in 51 cases of multiple myeloma (plasmacytoma).

Regardless of the immunostaining pattern for immunoglobulin light and heavy chains, reactive PC reacted with at least two and at most six of seventeen antibodies detecting haemopoietic antigens [Ber-H2/CD30 (91%), anti-leucocyte common antigen (LCA)/CD45 (86%), KP1/CD68 (64%), MB2 (57%), 4KB5/CD45RA (37%), DF-T1/CD43 (28%), UCHL1/CD45RO (20%), L26/CD20 (17%), MT2 (14%) and Mac387 (8%)], and with at least one and at most four of six antibodies against non-haemopoietic antigens [anti-epithelial membrane antigen (EMA) (94%), anti-vimentin (77%), anti-pan-cytokeratin/KL1 (74%), BMA120 (51%) and HMB45 (14%)].

Five antibodies stained reactive PC significantly more often than neoplastic PC: Ber-H2/CD30 ($p \leq 0.0000$), KP1/CD68 ($p \leq 0.0000$), anti-LCA/CD45 ($p \leq 0.0000$), anti-EMA ($p \leq 0.0339$) and anti-pan-cytokeratin/KL1 ($p \leq 0.0000$). The more frequent and more heterogeneous expression of antigens by reactive PC suggests that the aberrant immunoreactivity of neoplastic PC in plasmacytoma is not due to the process of malignant transformation in an early step of B-cell differentiation, but could reflect the heterogeneity of antigen expression by normal PC.

Key words: Plasma cell, Plasmacytosis, Immunohistochemistry

Offprint requests to: Prof. Dr. H.-P. Horny, Institut für Pathologie, Eberhard-Karls Universität Tübingen, Liebermeisterstrasse 8, D-72076 Tübingen, Germany

Introduction

Plasma cells (PC) are terminally differentiated B lymphocytes and are thought to express only a limited range of cellular antigens, in particular intracytoplasmic immunoglobulin (Ig), CD38 and VS38c (Jackson et al., 1988; Turley et al., 1994). There is increasing evidence that neoplastic PC in multiple myeloma (plasmacytoma) may express a great variety of haemopoietic and non-haemopoietic antigens (Durie and Grogan, 1985; Krajewski et al., 1985; Grogan et al., 1987, 1989; Möller et al., 1989; Pileri et al., 1989; Warburton et al., 1989; Wotherspoon et al., 1989; Dehou et al., 1990; Horny et al., 1992; Petruch et al., 1992; Turley et al., 1994; Cawley and Erber, 1995; Ferry et al., 1997) which led to the hypothesis that multiple myeloma possibly originates from an immature haemopoietic precursor or stem cell (Epstein et al., 1988, 1990; Grogan et al., 1989; Warburton et al., 1989; Petruch et al., 1992). However, there are relatively few reports of aberrant antigen expression by reactive PC (Delsol et al., 1984; Kurtin and Pinkus, 1985; Möller et al., 1989; Terstappen et al., 1990; Petruch et al., 1993; Krajewski et al., 1997). To investigate this issue we have performed a systematic study of reactive PC with a broad panel of antibodies suitable for use on paraffin-embedded tissue.

Materials and methods

A total of 36 tissue specimens exhibiting a marked reactive increase in PC was investigated, the diagnosis being chronic mucosal inflammation in 15 cases, malignant neoplasia in 8 cases, Castleman's disease (plasma cell type) in 4 cases and a variety of conditions involving various different sites (e.g., chronic non-specific lymphadenitis, lymph node tuberculosis, chronic non-specific dacryoadenitis) in 9 cases. Thirty-four of the specimens were obtained at operation and two at autopsy. All tissue samples were fixed in 5% buffered formalin; the bone marrow specimens were mildly

Table 1. Antibodies applied in the study

ANTIBODY	CD	MAIN REACTIVITY	STAINING PATTERN	SOURCE
Anti-γ*		Ig-chain γ	Cytoplasmic	Dakopatts, Hamburg, Germany
Anti-α*		Ig-chain α	Cytoplasmic	Dakopatts
Anti-μ*		Ig-chain μ	Cytoplasmic	Dakopatts
Anti-δ*		Ig-chain δ	Cytoplasmic	Dakopatts
Anti-ε*		Ig-chain ε	Cytoplasmic	Dakopatts
Anti-κ*		Ig-chain κ	Cytoplasmic	Dakopatts
Anti-λ*		Ig-chain λ	Cytoplasmic	Dakopatts
L26	20	B cells	Annular	Dakopatts
MB2		B cells	Cytoplasmic	Biotest, Dreieich, Germany
4KB5	45RA	B cells	Annular	Dakopatts
MT2		T cells	Annular	Biotest
DF-T1	43	T cells	Annular	Dakopatts
UCHL1	45RO	T cells	Annular	Dakopatts
Leu-7	57	NK cells	Annular	Becton-Dickinson, Heidelberg, Germany
Ber-H2	30	Hodgkin's cells	Cytoplasmic	Dakopatts
Anti-NE		Neutrophils	Cytoplasmic	Dakopatts
Dako-M1	15	Mature neutrophils	Annular	Dakopatts
KP1	68	Macrophages	Cytoplasmic	Dakopatts
Mac387		Macrophages, granulocytes	Cytoplasmic	Dakopatts
PG-M1	68	Macrophages	Cytoplasmic	Dakopatts
Anti-LCA	45	Leucocytes	Annular	Dakopatts
Anti-GA		Erythroid cells	Annular	Dakopatts
Anti-GPIIIa *	61	Megakaryocytes	Cytoplasmic	Dakopatts
Anti-vWF		Endothelial cells	Cytoplasmic	Dakopatts
BMA120		Endothelial cells	Cytoplasmic	Behringwerke, Marburg, Germany
Anti-EMA		Epithelial cells	Cytoplasmic/annular	Dakopatts
KL1		Epithelial cells	Cytoplasmic	Dianova, Hamburg, Germany
Anti-CEA		Carcino-embryonic antigen	Cytoplasmic	Camon, Wiesbaden, Germany
HMB45		Melanocytes	Cytoplasmic	Enzo, Farmingdale, New York, USA
Anti-vimentin		Mesenchymal cells	Cytoplasmic	Dakopatts

* Rabbit polyclonal antibody; all others mouse monoclonal. Anti-NE = anti-neutrophil elastase; Anti-LCA = anti-leucocyte common antigen; Anti-GA = anti-glycophorin A; Anti-GPIIIa = anti-glycoprotein IIIa; Anti-vWF = anti-von Willebrand factor; Anti-EMA = anti-epithelial membrane antigen

decalcified in edetic acid, as described elsewhere (Horny and Kaiserling, 1988). Tissue blocks were embedded in paraffin and cut at 4 μm. Sections were stained with haematoxylin and eosin, Giemsa, the periodic acid-Schiff reaction and the naphthol AS-D chloroacetate esterase (NASDCE) reaction (Leder, 1964). Immunostaining was performed by the avidin-biotin-peroxidase complex method of Hsu et al. (1981) with the antibodies listed in Table 1. The specificity of immunostaining with antibodies detecting cytoplasmic antigens was investigated by changing the dilution of the antibodies. Only relatively consistent staining of PC at all dilutions was recorded as specific. Staining of PC at routinely used dilutions that disappeared or was markedly weaker at higher dilutions was recorded as non-specific. Immunostaining with antibodies against the different Ig light chains and heavy chains was compared and the ratios (κ:α and γ:α, respectively) analysed using the Wilcoxon test. The number of PC stained by all other antibodies (including those against the Ig heavy chains μ, δ and ε) was evaluated semiquantitatively on a four-grade scale (Table 2) and analysed by Fisher's exact test. Possible differences in antigen expression between reactive and neoplastic PC and among PC in the various different diagnostic groups (chronic mucosal inflammation, malignant neoplasms and Castleman's disease) were also investigated by Fisher's exact test.

Results

The male/female ratio of the 36 patients was 1:0.6. The median age was 54 years (range: 2 months to 80 years). The PC exhibited typical morphology by light microscopy in all cases, with basophilic cytoplasm, an eccentric nucleus with the characteristic cart-wheel heterochromatin pattern and a perinuclear halo (Fig. 1A). Immunostaining for the Ig heavy chains γ and α was found to be polytypic (γ:α ratio: 0.3-3.0) in twenty-four cases. In twelve cases, increased numbers of Igγ-positive PC were found, with a γ:α ratio of up to 8.0. Immunostaining for Ig light chains was polytypic in all cases (κ:λ ratio: 0.7-3.0), which was regarded as definitive proof that the PC proliferation was reactive (Fig. 1B). Anti-μ stained up to 50% of PC in nearly 80% of the cases, whereas anti-δ reacted with only a few PC in about half of the cases. No immunoreactivity of PC with anti-ε was detected in any of the specimens. No statistical differences in the expression of Ig light chains and heavy chains among reactive PC in the different diagnostic groups were found.

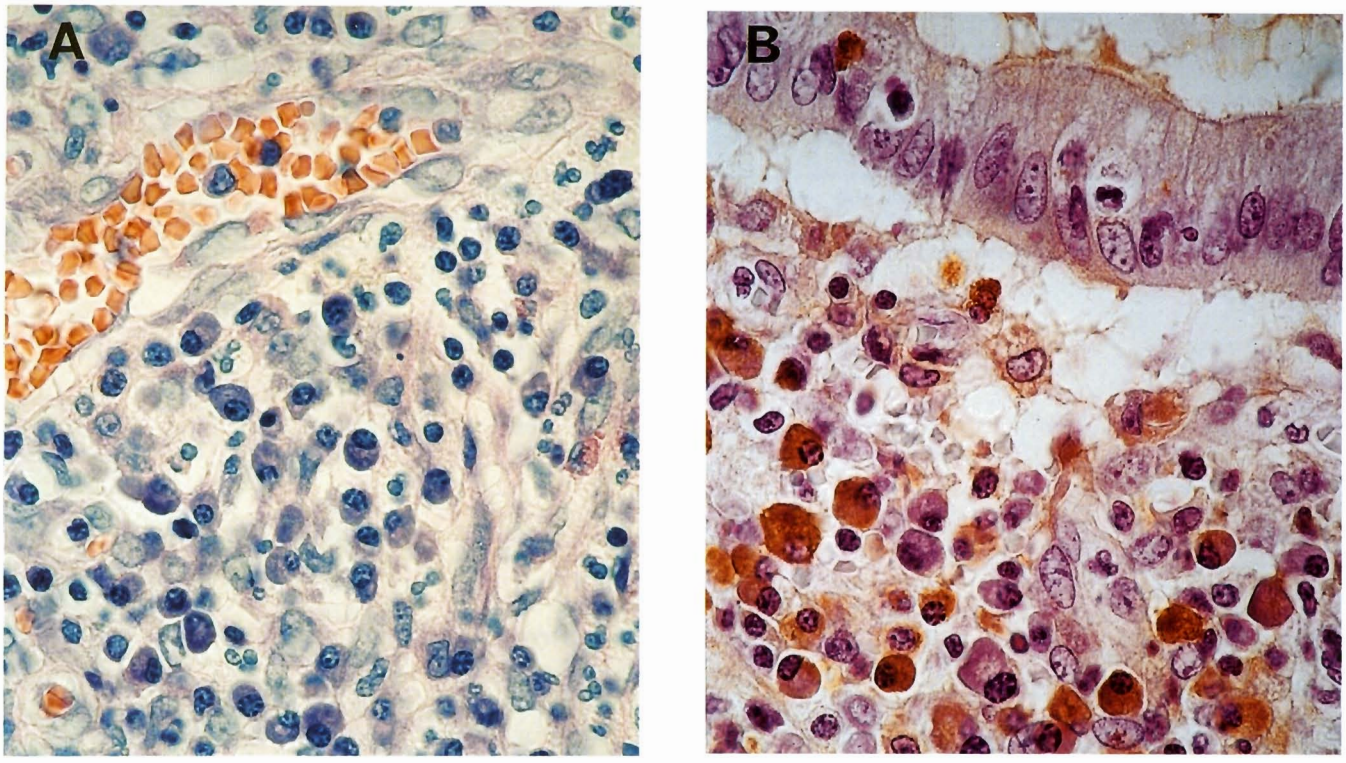


Fig. 1. Acute appendicitis. **A.** Plasma cells (PC) in the submucosal tissue with typical cytomorphology. Giemsa, x 567. **B.** Immunostaining for immunoglobulin light chains confirmed the PC proliferation to be polytypic. Approximately 50% of the PC in the submucosal tissue are kappa-positive. ABC, x 567

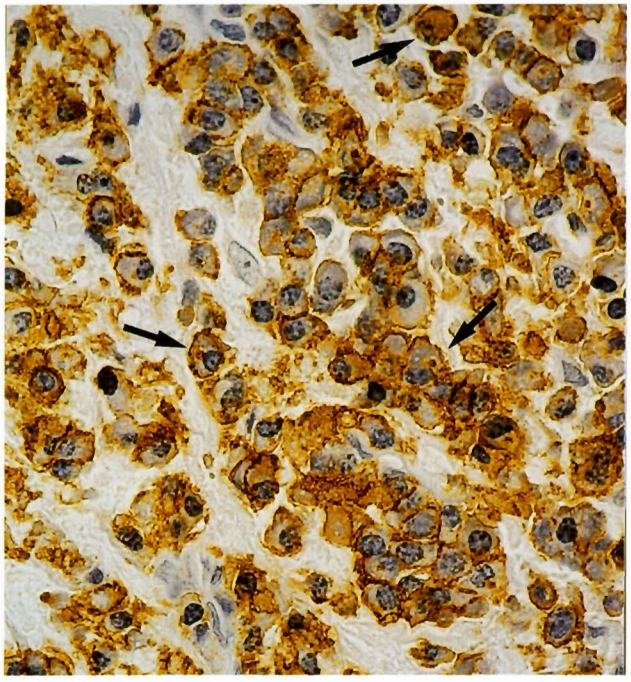


Fig. 2. Odontogenic apical granuloma. Nearly all the plasma cells (PC) exhibit annular staining for leucocyte common antigen. A few PC show non-specific, cytoplasmic or perinuclear staining (arrows). ABC, x 567

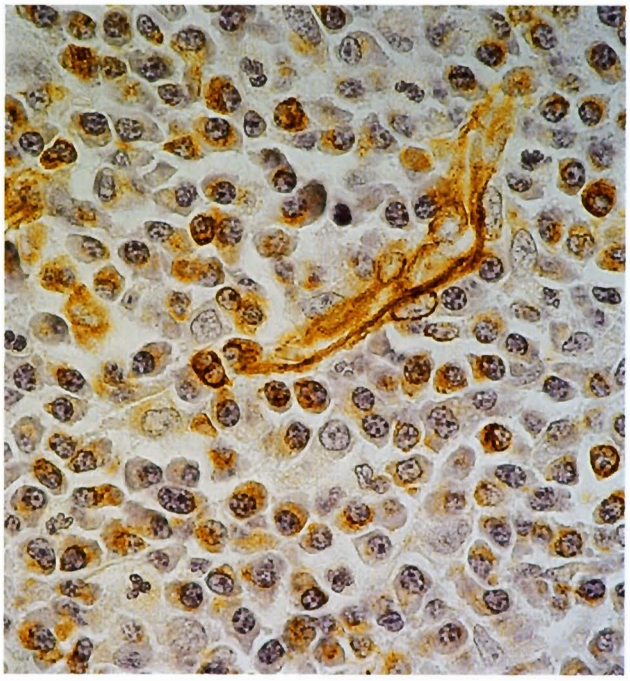


Fig. 3. Chronic non-specific lymphadenitis. There is immunostaining of plasma cells of varying intensity for vimentin. ABC, x 567

Table 2. Immunoreactivity of normal/reactive and neoplastic plasma cells (PC).

ANTIBODY	REACTIVITY (% of cases)						p[b]	NON-SPECIFIC REACTIVITY (% of cases)	
	Reactive PC*					Neoplastic PC[a]		Reactive PC	Neoplastic PC[a]
	No/uncertain reactivity	(+)	+	++	≥ 1% PC reactive	≥ 1% PC reactive			
B-cell associated									
L26 (CD 20)	83	14	3	0	17	26	-1.0000	0	0
MB2	100	0	0	0	0	0	-0.8889	57	75
4KB5 (CD 45RA)	63	37	0	0	37	18	0.6821	0	0
T-cell associated									
MT2	86	14	0	0	14	2	0.8440	0	0
DF-T1 (CD 43)	72	22	6	0	28	59	-0.1570	0	0
UCHL1 (CD 45RO)	80	17	3	0	20	47	-0.2495	0	0
Other lymphoid cell markers									
Leu-7 (CD 57)	100	0	0	0	0	8	-0.9796	0	0
Ber-H2 (CD 30)	9	23	34	34	91	10	≤ 0.0000	0	0
Myelomonocytic antigens									
Anti-neutrophil elastase	100	0	0	0	0	4	1.0000	0	0
KP1 (CD 68)	36	42	14	8	64	2	≤ 0.0000	0	0
PG-M1 (CD 68)	100	0	0	0	0	not done	--------	0	0
Mac387	92	8	0	0	8	0	0.8331		
Dako-M1 (CD 15)	100	0	0	0	0	2	-1.0000	0	0
Leucocyte common antigen									
Anti-leucocyte common antigen (CD 45)	14	19	25	42	86	40	≤ 0.0000	0	0
Erythroid and megakaryocytic antigens									
Anti-glycophorin A	100	0	0	0	0	0	1.0000	0	0
Anti-glycoprotein IIIa (CD 61)	100	0	0	0	0	2	-1.0000	0	0
Anti-von Willebrand factor	100	0	0	0	0	0	1.0000	0	0
Non-haemopoietic antigens									
BMA120	100	0	0	0	0	0	1.0000	51	53
Anti-epithelial membrane antigen	6	22	28	44	94	65	0.0339	0	0
KL1	100	0	0	0	0	0	≤ 0.0000	74	8
Anti-carcino-embryonic antigen	100	0	0	0	0	6	-0.9994	0	0
HMB45	100	0	0	0	0	0	0.9992	14	6
Anti-vimentin	23	14	43	20	77	44	0.1373	0	0
NASDCE	0	0	0	0	0	12	-0.5888	0	0

*: proportion of plasma cells (PC) reactive: (+), <10%; +, 10-50%; ++, >50%; [a]: findings of Petruch et al. (1997) in 51 cases of multiple myeloma (plasmacytoma); [b]: probability of error (negative if neoplastic PC were stained more frequently than normal/reactive PC)

All the antibodies detecting B-cell antigens stained PC, but in varying numbers of cases: MB2, 57%; 4KB5, 37%; L26, 17%. MB2 reacted with the majority of the PC in five cases, but 4KB5 and L26 never did so. In 22% of the cases, the PC reacted with none of the B-cell markers. All of the T-cell-associated antibodies stained PC (DF-T1, 28%; UCHL1, 20%; MT2 14%), but there was no case in which the majority of PC was stained. The PC were unreactive with all of the antibodies against T cells in about 60% of cases. Non-reactivity of PC with all the T-cell and B-cell antibodies applied was observed in only 6% of cases. The antibody Ber-H2 stained PC in most cases (91%), the majority being stained in 34%. CD57-positive PC were not detected in any case. The antibodies detecting myelomonocytic antigens stained varying numbers of reactive PC. KP1-positive PC were demonstrated in 64% of the cases, the majority of the PC being stained in 8%. Mac387 stained only a few PC in 8% of cases, and no PC reacted with Dako-M1, anti-neutrophil elastase or PG-M1. The PC in most cases (86%) were reactive for the leucocyte common antigen (LCA), the majority being stained in about half of these. In addition, PC in some cases showed non-specific, weak, cytoplasmic or perinuclear staining for LCA (Fig. 2). No immunoreactivity of PC with any of the antibodies detecting erythroid or megakaryocytic antigens (glycophorin A, glycoprotein IIIa and von Willebrand factor) was found.

Of the non-haemopoietic antigens, staining for epithelial membrane antigen (EMA) was seen in large numbers of PC in nearly all of the cases. KL1 (anti-pan-cytokeratin) stained PC in 74% of the cases. PC were not immunoreactive for carcino-embryonic antigen (CEA) in any of the cases. BMA120 stained PC in about half of

the cases, the majority being stained in 14%. PC in 77% of the cases were immunoreactive for vimentin, the majority being reactive in 20% (Fig. 3). The antibody HMB45, which preferentially detects activated and neoplastic melanocytes, stained a few PC in 14% of the cases.

When the specificity of staining by antibodies detecting cytoplasmic antigens was tested, staining of PC by MB2, BMA120, KL1 and HMB45 was found to be non-specific. Further investigations by Petruch et al. (1992) have shown that these antibodies also produce non-specific staining in neoplastic PC (Table 2).

No statistical differences in antigen expression amongst the reactive PC in chronic mucosal inflammation, malignant neoplasms and Castleman's disease were found (Fig. 4).

The findings of the study were then compared with those of Petruch et al. (1992) in 51 cases of neoplastic proliferation of PC (multiple myeloma, plasmacytoma). Normal PC were found to react more frequently than neoplastic PC for many of the haemopoietic and non-haemopoietic antigens investigated. Reactive PC were stained significantly more often by the antibodies Ber-H2/CD30 ($p \leq 0.0000$), KP1/CD68 ($p \leq 0.0000$), anti-LCA/CD45 ($p \leq 0.0000$), anti-EMA ($p=0.0339$) and KL1 ($p \leq 0.0000$). None of the antibodies reacted significantly more often with neoplastic PC than reactive PC (Table 2).

Discussion

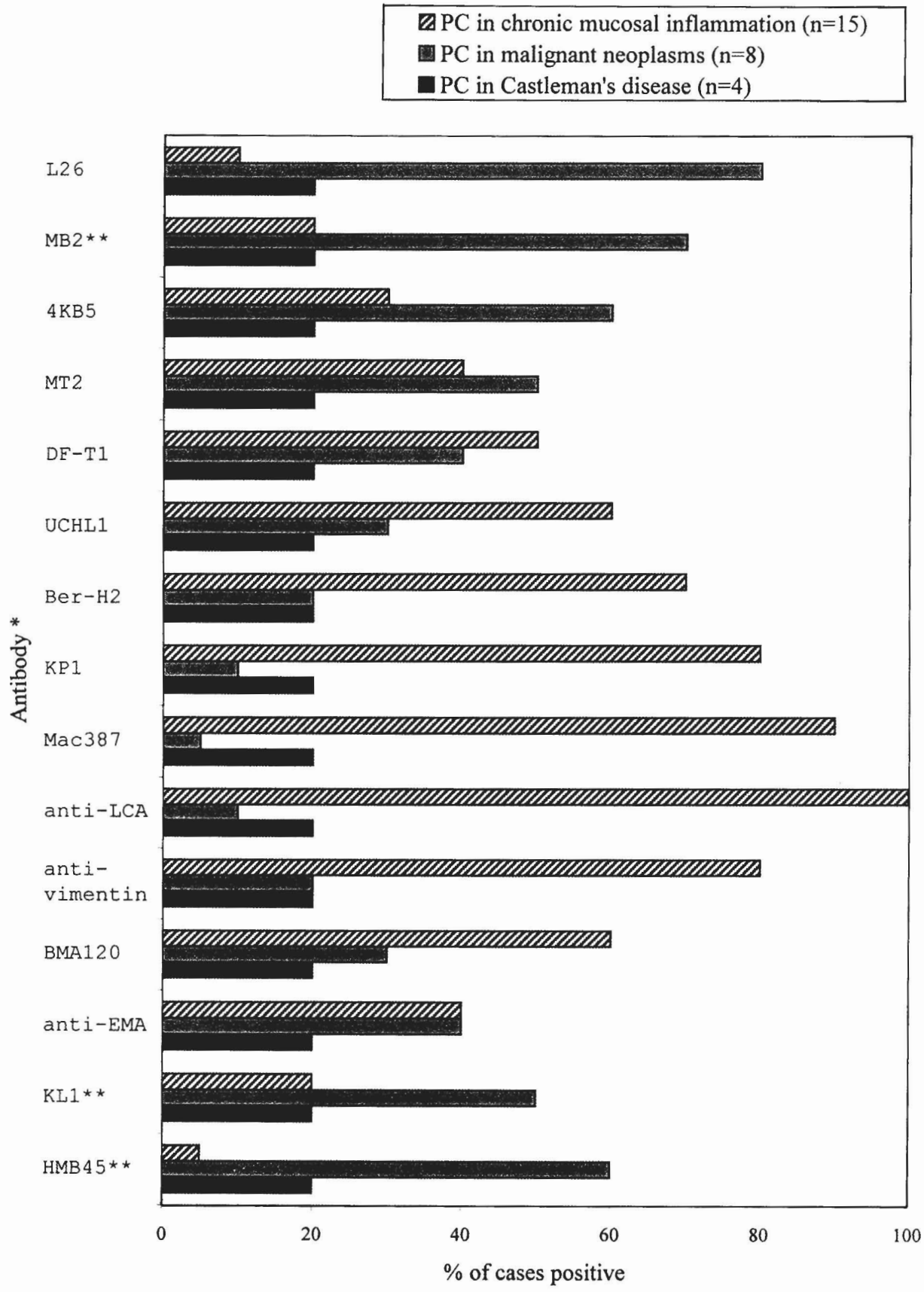
Our findings show that reactive PC express various haemopoietic and non-haemopoietic antigens. We found Ig light chain staining more reliable than heavy chain staining for confirmation of the reactive nature of the plasma cell proliferation. Although in some cases immunostaining for Ig heavy chains appeared to be monotypic, with a γ:α ratio of up to 8:1, the polytypic nature of the PC proliferation was proved definitively in all cases by Ig light chain staining (κ:λ ratio: 0.7-3.0, (Eckert et al., 1986; Peterson et al., 1986; Buss et al., 1988) (Fig. 1). We found that normal (reactive) PC react frequently both with antibodies associated with B-cell and T-cell differentiation, and with certain antibodies detecting non-haemopoietic antigens. The antigen for which PC were immmunoreactive in the greatest number of cases (94%) was the non-haemopoietic cell marker EMA. Ber-H2, which detects the Ki1 antigen (CD30), was the most frequently reactive haemopoietic cell-associated antibody, reacting with PC in 91% of the cases.

Reactive PC in all the cases were stained by at least two of the antibodies detecting haemopoietic antigens and in 23 cases (64%) by four or more. Our findings are somewhat at variance with those of other authors, who found expression of CD30 (Ber-H2) in at most 30% of reactive PC (Norton and Isaacson, 1987; Hall et al., 1988; Möller et al., 1989; Schwarting et al., 1989). Other investigations into the immunoreactivity of PC with the B-cell-associated antibodies used in this study also produced differing results: Poppema et al. (1987) and Dehou et al. (1990) reported PC to be non-reactive with MB2, and Möller and Mielke (1989) found strong staining with 4KB5 but none with L26. Other studies using flow cytometry and different antibodies to detect CD20 reported either a lack of reactivity of PC (Harada et al., 1993) or consistent reactivity of up to 79% of PC (Terstappen et al., 1990). No reactivity of PC with the T-cell-associated antibodies UCHL1 and MT2 was observed by Poppema et al. (1987) and Clark et al. (1990). Using the same antibody to detect LCA as in this study, Kurtin and Pinkus (1985) reported only occasional PC in paraffin-embedded tissue to be reactive for CD45 (LCA), but Terstappen et al. (1990) found regular immunostaining in up to 94% of the PC in cell suspensions of bone marrow aspirates using flow cytometry with Hle-1 fluorescein isothiocyanate, which detects LCA. Like Kurtin and Pinkus (1985) we observed weak cytoplasmic or perinuclear staining of PC in a few cases with anti-LCA, which we found to be non-specific (Fig. 2).

Immunostaining of a very small number of PC by KP1 was reported by Bolz et al. (1991). To our knowledge, there are no reports of immunoreactivity of PC with PG-M1, Mac387, Leu-7, anti-neutrophil-elastase, Dako-M1, anti-glycophorin A, anti-glycoprotein IIIa or anti-von Willebrand factor.

PC in all the cases investigated reacted with at least one of the antibodies against non-haemopoietic antigens; in 26 cases (72%) they reacted with three or four. It has been shown that PC may express EMA (Delsol et al., 1984; Pinkus and Kurtin, 1985; Hall et al., 1988). Nevertheless, we were surprised by the proportion of cases (94%) with reactivity for EMA. Reactive PC have been reported to be negative for cytokeratin (KL1), CEA and vimentin (Delsol et al., 1984; Figarella-Branger et al., 1990). To our knowledge, no other studies have investigated immunoreactivity of PC with BMA120 or HMB45. We found more frequent staining of PC in paraffin sections with Ber-H2, DF-T1, UCHL1, MT2, anti-LCA (Fig. 2), KL1, anti-vimentin (Fig. 3) and anti-CEA (Delsol et al., 1984; Kurtin and Pinkus, 1985; Norton and Isaacson, 1987; Poppema et al., 1987; Schwarting et al., 1989; Clark et al., 1990; Figarella-Branger et al., 1990), than other workers have done, KL1 (like MB2, BMA120 and HMB45) producing non-specific staining.

Neoplastic PC have also been shown to express various haemopoietic and non-haemopoietic antigens (Durie and Grogan, 1985; Grogan et al., 1987, 1989; Epstein et al., 1990; Terstappen et al., 1990; Van Camp et al., 1990; Leo et al., 1992; Petruch et al., 1992; Harada et al., 1993; Pellat-Deceunynck et al., 1994; Ferry et al., 1997; Lin and Weiss, 1997), which has been explained by the postulated origin of plasmacytoma from an immature haemopoietic precursor or stem cell (Epstein et al., 1988, 1990; Grogan et al., 1989; Warburton et al., 1989; Petruch et al., 1992). As we



* antibodies that stained PC in at least 10% of cases in one or more group

** antibodies with non-specific staining of PC

Fig. 4. Immunoreactivity of normal plasma cells (PC).

observed an unexpectedly frequent expression of various haemopoietic and non-haemopoietic antigens by reactive PC as well, it is possible that the antigen expression observed in plasmacytoma is not due to derivation from an immature haemopoietic stem cell, but rather might reflect heterogeneity of antigen expression by normal PC (Table 2).

Acknowledgements. The authors wish to thank Ms. U. Späth and Ms. H. Ableiter for excellent technical assistance, PD Dr. rer. nat. D. Schenzle for help with the statistical analysis, Dr. M. Ruck for help in preparation of the manuscript and DAKO Diagnostica GmbH, Hamburg, Germany, for their generous contribution towards the cost of reproduction of the colour photographs.

References

Bolz S., Ruck P., Schaumburg-Lever G., Horny H.-P., Radzun H.-J. and Kaiserling E. (1991). Immunelektronenmikroskopische Befunde an Zellen des Makrophagensystems mit den monoklonalen Antikörpern KP1 (CD68) und KiM1P. Verh. Dtsch. Ges. Path. 75, 318.

Buss D.H., Prichard R.W. and Cooper M.R. (1988). Plasma cell dyscrasias. Hematol. Oncol. Clin. North Am. 24, 603-615.

Cawley B. and Erber W. (1995). Reactivity of the workshop B-cell blind panel with normal and malignant plasma cells. In: Leucocyte typing. Schlossman S.F., Boumsell L., Gilks W., Harlan J.M., Kishimoto T., Morimoto C., Ritz J., Shaw S., Silverstein R., Springer T., Tedder T.F. and Todd R.F. (eds). Oxford University Press. Oxford-New York-Tokyo. pp 663-664.

Clark J.R., Williams M.E. and Swerdlow S.H. (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.

Dehou M.-F., Schots R., Lacor P., Arras N., Verhavert P., Klöppel G. and Van Camp B. (1990). Diagnostic and prognostic value of MB2 monoclonal antibody in paraffin-embedded bone marrow sections of patients with multiple myeloma and monoclonal gammopathy of undetermined significance. Am. J. Clin. Pathol. 94, 287-291.

Delsol G., Gatter K.C., Stein H., Erber W.N., Pulford K.A.F. and Zinne K. (1984). Human lymphoid cells express epithelial membrane antigen. Implication for diagnosis of human neoplasms. Lancet 8412, 1124-1128.

Durie B.G.M. and Grogan M. (1985). CALLA-positive myeloma: An aggressive subtype with poor survival. Blood 66, 229-232.

Eckert F., Schmid L., Kradolfer D. and Schmid U. (1986). Bone-marrow plasmacytosis - an immunohistological study. Blut 53, 11-19.

Epstein J., Barlogie B., Katzmann J. and Alexanian R. (1988). Phenotypic heterogeneity in aneuploid multiple myeloma indicates pre-B cell involvement. Blood 71, 861-865.

Epstein J., Xiao H. and He X. (1990). Markers of multiple hematopoetic-cell lineages in multiple myeloma. N. Engl. J. Med. 322, 664-668.

Ferry J.A., Young R.H. and Scully R.E. (1997). Testicular and epididymal plasmacytoma: a report of 7 cases, including three that were the initial manifestation of plasma cell myeloma. Am. J. Surg. Pathol. 21, 590-598.

Figarella-Branger D., Gambarelli D., Perez-Castillo M., Garbe L. and Grisoli F. (1990). Primary intracerebral plasma cell granuloma: a light, immunocytochemical, and ultrastructural study of one case. Neurosurgery 27, 142-147.

Grogan T.M., Durie B.G.M., Lomen C., Spier C., Wirt D.P., Nagle R., Wilson G.S., Richter L., Vela E., Maxey V., McDaniel K. and Rangel C. (1987). Delineation of a novel pre-B cell component in plasma cell myeloma: Immunochemical, immunophenotypic, genotypic, cytologic, cell-culture, and kinetic features. Blood 70, 932-942.

Grogan T.M., Durie B.G.M., Spier C.M., Richter L. and Vela E. (1989). Myelomonocytic antigen positive multiple myeloma. Blood 73, 763-769.

Hall P.A., D'Ardenne A.J. and Stansfeld A.G. (1988). Paraffin section immunohistochemistry. I. Non-Hodgkin's lymphoma. Histopathology 13, 149-160.

Harada H., Kawano M.M., Huang N., Harada Y., Iwato K., Tanabe O., Tanaka H., Sakai A., Asaoku H. and Kuramotu A. (1993). Phenotypic difference of normal plasma cells from mature myeloma cells. Blood 81, 2658-2663.

Horny H.-P. and Kaiserling E. (1988). Lymphoid cells and tissue mast cells of bone marrow lesions in systemic mastocytosis: A histological and immunohistological study. Br. J. Haematol. 69, 449-455.

Horny H.-P., Saal J. and Kaiserling E. (1992). Primary splenic presentation of plasma cell dyscrasia: report of two cases. Hematol. Pathol. 6, 155-160.

Hsu S., Raine L. and Fanger H. (1981). Use of avidin-biotin-peroxidase complex (ABC) in immunoperoxidase techniques: A comparison between ABC and unlabeled antibody (PAP) procedures. J. Histochem. Cytochem. 29, 577-580.

Jackson N., Ling N.R., Ball J., Bromidge E., Nathan P.D. and Franklin I.M. (1988). An analysis of myeloma plasma cell phenotype using antibodies defined at the IIIrd international workshop on human leucocyte differentiation antigens. Clin. Exp. Immunol. 72, 351-356.

Krajewski S., Zapata J.M., Krajewska M., VanArsdale T., Shabaik A., Gascoyne R.D. and Reed J.C. (1997). Immunohistochemical analysis of in vivo patterns of TRAF-3 expression, a member of the TNF receptor-associated factor family. J. Immunol. 159, 5841-52.

Kurtin P.J. and Pinkus G.S. (1985). Leukocyte common antigen - a diagnostic discriminant between hematopoietic and nonhematopoietic neoplasms in paraffin sections using monoclonal antibodies: Correlation with immunologic studies and ultrastructural localisation. Hum. Pathol. 16, 353-365.

Leder L.-D. (1964). Über die selektive fermentcytochemische Darstellung von neutrophilen myeloischen Zellen und Gewebsmastzellen im Paraffinschnitt. Klin. Wschr. 42, 553.

Leo R., Boeker M., Peest D., Hein R., Bartl R., Gessner J.E., Selbach J., Wacker G. and Deicher H. (1992). Multiparameter analyses of normal and malignant human plasma cells: CD38++, CD56+, CD54+, clg+ is the common phenotype of myeloma cells. Ann. Hematol. 64, 132-139.

Lin B.T. and Weiss L.M. (1997). Primary plasmacytoma of lymph nodes. Hum. Pathol. 28, 1083-1090.

Möller P., Matthaeie-Maurer U. and Moldenhauer G. (1989). CD30 (Ki1) antigen expression in a subset of gastric mucosal plasma cells and in a primary gastric plasmacytoma. Am. J. Clin. Pathol. 91, 18-23.

Möller P. and Mielke B. (1989). Extensive analysis of tissue distribution of antigens defined by new clustered and unclustered B-cell antibodies. In: Leucocyte typing IV; White cell differentiation antigens. Knapp W., Dörken B., Gilks W.R., Rieber E.P., Schmidt R.E., Stein H. and von dem Borne A.E.G.Kr. (eds). Oxford University Press. Oxford-New York. pp 175-177.

Norton A.J. and Isaacson P.G. (1987). Detailed phenotypic analysis of

B-cell lymphoma using a panel of antibodies reactive in routinely fixed wax-embedded tissue. Am. J. Pathol. 128, 225-240.

Pellat-Deceunynck C., Bataille R., Robillard N., Harousseau J.-L., Rapp M.-J., Juge-Morineau N., Wijdenes J. and Amiot M. (1994). Expression of CD28 and CD40 in human myeloma cells: A comparative study with normal plasma cells. Blood 84, 2597-2603.

Peterson L.C., Brown B.A., Crosson J.T. and Mladenovic J. (1986). Application of the immunoperoxidase technic to bone marrow trephine biopsies in the classification of patients with monoclonal gammopathies. Am. J. Clin. Pathol. 85, 688-693.

Petruch U.R., Horny H.-P. and Kaiserling E. (1992). Frequent expression of haemopoietic and non-haemopoietic antigens by neoplastic plasma cells: an immunohistochemical study using formalin-fixed, paraffin-embedded tissue. Histopathology 20, 35-40.

Petruch U.R., Horny H.-P., Beschorner R., Weber R., Tiemann M., Menke D.M., Banks P. and Kaiserling E. (1993). Zum Immunphänotyp reaktiver und neoplastischer Plasmazellen. Eine systematische immunhistochemische Studie an Plasmocytosen, multiplen Myelomen und extramedullären Plasmocytomen. Verh. Dtsch. Ges. Path. 77, 327.

Pileri S., Poggi S., Baglioni P., Montanari M., Sabattini E., Galeni E., Tazzari P.L., Gobbi M., Cavo M., Falini B., Stein H. and Tura S. (1989). Histology and immunohistology of bone marrow biopsy in multiple myeloma. Eur. J. Haematol. 43, 52-59.

Pinkus G.S. and Kurtin P.J. (1985). Epithelial membrane antigen - a diagnostic discriminant in surgical pathology: Immunohistochemical profile in epithelial, mesenchymal, and hematopoietic neoplasms using paraffin sections and monoclonal antibodies. Hum. Pathol. 16, 929-940.

Poppema S., Hollema H., Visser L. and Vos H. (1987). Monoclonal antibodies (MT1, MT2, MB1, MB2, MB3) reactive with leukocyte subsets in paraffin-embedded tissue sections. Am. J. Pathol. 127, 418-429.

Schwarting R., Gerdes J., Dürkop H., Falini B., Pileri S. and Stein H. (1989). Ber-H2: a new anti-Ki-1(CD30) monoclonal antibody directed at a formol-resistant epitope. Blood 74, 1678-1689.

Terstappen L.W.M.M., Johnsen S., Segers-Nolten I.M.J. and Loken M.R. (1990). Identification and characterization of plasma cells in normal human bone marrow by high-resolution flow cytometry. Blood 76, 1739-1747.

Turley H., Jones M., Erber W., Mayne K., de Waele M. and Gatter K. (1994). VS38: a new monoclonal antibody for detecting plasma cell differentiation in routine sections. J. Clin. Pathol. 47, 418-422.

Van Camp B., Durie B.G.M., Spier C., De Waele M., Van Riet I., Vela E., Frutiger Y., Richter L. and Grogan T. (1990). Plasma cells in multiple myeloma express a natural killer cell-associated antigen: CD56 (NHK-1; Leu-19). Blood 76, 377-382.

Warburton P., Joshua D.E., Gibson J. and Brown D. (1989). CD10-(CALLA)-positive lymphocytes in myeloma: evidence that they are a malignant precursor population and are of germinal centre origin. Leuk. Lymphoma 1, 11-20.

Wotherspoon A.C., Norton A.J. and Isaacson P.G. (1989). Immunoreactive cytokeratins in plasmacytomas. Histopathology 14, 141-150.

Accepted January 18, 1999