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Haemopoiesis in human fetal and embryonic liver

Immunohistochemical determination in B5-fixed paraffin-embedded tissues

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Summary. Paraffin embedded tissue reactive monoclonal antibodies were used to study human embryonal and fetal haemopoiesis, combining optimal morphology with immunohistological determination of haemopoietic cell subtypes and their microenvironment. Seven embryonal and twelve fetal liver specimens were studied, having been fixed in B5-fixative and embedded in paraffin. The different haemopoietic lineages each showed their own immunophenotype and distribution; intercellular and microenvironmental relationships were easily determined. Erythroid cells are reactive with VIE-G4, LN1, and MT1, sometimes partly surrounding a central macrophage. Myelomonocytic cells react with LCA, MT1, MB3, LN2, and anti-lysozyme, and from 14 weeks onwards with LN3. Lymphoid cells show LCA, MT1, MT2, MB1, MB2, MB3, and LN2 reactivity. In a few cases some scarce My10$^+$ early progenitor cells were seen. An important finding is the extensive MT1-reactivity distributed over all haemopoietic lineages, and the demonstration of immature haemopoietic blast cells exclusively expressing the MT1 antigen. Further studies employing MT1 are necessary to delineate the extent of the distribution and the possible function of the antigen. Use of the MT1 mAb may contribute to the elucidation of the exact nature of the haemopoietic blast cells and their place in haemopoietic development.

Key words: Immunohistology – Monoclonal antibodies – MT1 – Progenitor cells – Haematopoiesis

Introduction

During human embryonal and fetal life the liver is the main haemopoietic organ from 6–7 weeks post conception onwards. The different haemopoietic lineages develop and differentiate in the embryonal and fetal liver from stem cells which have migrated from the yolk sac (Kelemen et al. 1979).

The morphological aspects of human haemopoiesis during embryonal and fetal life have been described extensively. Most reports are based on ultrastructural and conventional light-microscopic studies performed on routinely fixed tissues, smears or cell-suspensions (Emura et al. 1983, 1984; Enzan et al. 1978; Fukuda 1974; Gilmour 1941; Kelemen et al. 1979; Migliaccio et al. 1986). In these purely morphological studies most attention has been paid to erythropoiesis with lesser interest in myelopoiesis and lymphopoiesis.

The availability of a broad range of monoclonal antibodies (mAb) recognizing antigens present on cells of the different haemopoietic lineages has extended the possibilities for an accurate description of the presence of cells of these separate lineages and their precursors during different stages of human embryonal and fetal development. The use of these mAb is generally limited to frozen tissue sections or fresh cell suspensions (Hokland et al. 1983; Kamps and Cooper 1982; Rosenthal et al. 1983). As early human embryonal and fetal tissues often are very fragile, frozen sections of these tissues show rather poor morphology and results of immunohistology often are difficult to interpret with regard to recognized cell types and micro-environment. In studies of cell suspensions of embryonal and fetal tissues an accurate quantitative determination of cell subsets and qualitative description of cell types can be obtained as is demonstrated by the impressive work of Kelemen et al. (1979), and by several immunocytological studies on cell suspensions (Gilmour 1941; Hofman et al. 1984; Hokland et al. 1983; Kamps and Cooper 1982) but the use of cell suspensions is again unsatisfactory in terms of localisation of cell types and the nature of their micro-environment.

Recently monoclonal antibodies have been developed that react with tissue antigens resistant to fixation procedures and paraffin-embedding (Epstein et al. 1984; Poppema et al. 1987; Poppema and Hollema 1987;

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Warnke et al. 1983). These mAb, referred to as par-mAb, are a very useful diagnostic tool in cases where no frozen tissue is available or in cases where combination of cellular morphology and anatomical relationships is of importance. Mercury-containing fixatives, like B5, provide excellent morphological and immunohistological results (Poppema et al. 1987; Poppema and Hollema 1987) and are of particular advantage for use in the fragile tissues of early embryonal stages.

We studied embryonal and fetal liver haemopoiesis, employing a panel of mAb in an immunoperoxidase staining procedure on B5-fixed paraffin-embedded tissue sections. In a separate study a large panel of mAb was employed in immunohistological staining of frozen fetal liver sections (Kamps et al. 1989).

Materials and methods

Liver tissue of seven human embryos of 5 to 10 weeks, and of twelve human fetuses, varying in age from 12 to 37 weeks, was studied (informed consent was obtained, and the study was approved by the Medical Ethical Committee of the University Hospital). Age (post-conception age) was carefully determined by crown-rump length and multiple precise morphological variables in the embryonal stages, and crown-rump length, foot-length, and femur-length in fetal stages (England 1983; Moore 1982; O'Rahilly 1979). Tissues were obtained from intact embryos from medically approved abortions, and from fetal autopsies. Cause of death in the latter cases was immaturity, hyaline membrane disease, or lung hypoplasia; no further pathology was found.

Tissues were fixed in B5-fixative for 3 h, subsequently rinsed in 70% alcohol for several h and embedded in paraffin. The B5-fixative was prepared as described previously (Poppema et al. 1987): 90 ml aqua dest, 6 g mercuric chloride, 2.074 g sodium acetate ($CH_3COONa.\ 3H_2O$), 10 ml 37% formaldehyde solution, pH 5.7. A series of paraffin-reactive monoclonal antibodies (Civin et al. 1984; Epstein et al. 1984; Liszka et al. 1983; Lu et al. 1987; Poppema et al. 1987; Poppema and Hollema 1987; Warnke et al. 1983) listed in Table 1, were employed in an immunoperoxidase staining procedure on 2 µm paraffin tissue sections, according to standard methods (Poppema et al. 1987). Sections were deparaffinized, dehydrated, and desublimated, and pretreated for 30 min with 0.3% methanol-H_2O_2 to block endogenous peroxidase reactivity. After a 30 min first step incubation with 100 µl of the respective mAb, sections were incubated for 15 min with 50 µl peroxidase-conjugated rabbit anti-mouse immunoglobulin antibodies. Each step was followed by a 5 min wash. Diaminobenzidin tetrahydrochloride (DAB), together with H_2O_2, was used as substrate, giving a brown reaction product. Sections were counterstained with Mayers' haemalum. Additionally, polyclonal antisera, reactive with lysozyme, keratin intermediate filaments, immunoglobulin heavy chains gamma, alpha, and mu, and kappa and lambda light chains were used (after pronase-pretreatment of the sections), and also enzymehistochemical staining for chloroacetate-esterase (CAE) according to standard methods. For conventional light-microscopy, tissue sections were haematoxylin-/eosin-, and Giemsa stained.

Although this study is generally mainly of qualitative nature, (semi-quantitative) estimations of cell numbers and ratio's were made using a counting grid mounted in the eye piece of the microscope.

Results

In all cases consistent reproducible staining results were obtained. Immunohistological staining patterns of the haemopoietic cells and their localization are summarized in Tables 2 and 3, and illustrated in Figs. 1–6. Incubation with PBS or mAb not recognizing haemopoietic antigens as a first step provided no staining at all.

Primary liver haemopoiesis was observed in all cases up to 24 weeks. The third trimester cases did also show haemopoietic activity but this was mainly of erythropoietic lineage. Also several cells of monocyte/macrophage lineage were observed.

Table 1. Paraffin-tissue reactive monoclonal antibodies

mAb	CD	Molecular weight (kD)	Reactive with:
LeuM1	15	NR	Lacto-N-fucose pentaosyl (X-hapten); on granulocytes, Reed-Sternberg cells, monocytes/macrophages (weakly); some NHL; some epithelia
My10 (HPCA-1)	34	115	Unipotent and multipotent hemopoietic progenitor cells
MT1	43	190/ 110/100	T cells, myeloid cells, monocytes, erythroid cells, not with mature B cells
LCA	45	200	Leukocyte common antigen
MT2	45R	190/200	Mature T and B cells, not reactive with germinal center cells and immature T cells
MB1	45R	100/ 110/200	B cells, 50% T cells
MB3/LN2	74	31	MHC class II related (cytoplasmic)
LN1	w75	45–85	Germinal center cells, also reactive with erythrocytes and epithelial cells
MB2	–	28	B cells (cytoplasmic), also reactive with endothelial and epithelial cells
LN3	–	28, 32	MHC class II antigen (membrane)
VIE-G4	–	41	Glycophorin-A on erythroid cells

CD = Cluster of differentiation; kD = kiloDalton; mAb = Monoclonal antibody; MHC = Major histocompatibility complex; NHL = Non-Hodgkin lymphoma.
MT-, MB-, and LN- antibodies from Biotest (Dreieich, West-Germany), LeuM1 and HPCA-1 from Becton-Dickinson (Mountain View, CA, USA), LCA from DAKO (Glostrup, Denmark), and VIE-G4 from Dr. W. Knapp, Vienna, Austria

Table 2. Reactivity of monoclonal antibodies with hemopoietic cells in human fetal liver

Cell type	LCA	MT1	MT2	MB1	MB2	MB3[1]	LN1[2]	LN3	LeuM1	My10	VIE-G4	lys[3]
Erythroid cells	–	+[4]	–	–	–	–	+	–	–	–	+	–
Myelomonocytic cells	+	+	–	±[5]	–	+	–	+[6]	+	–	–	+
Mature myeloid cells	+	+	–	–	–	+	–	+	+	–	–	+
Large macrophages	+	+	–	–	–	+	–	+[6]	–	–	–	+
Lymphocytes	+	+	+	+	+	+	–	+	–	–	–	–
Dendritic cells	[+]	+	–	–	–	+	–	+	–	–	–	+
Megakaryocytes	+	+	–	–	–	+	–	–	–	–	–	–
"Blast cells"	–	+	–	–	–	–	–	–	–	–	–	–

[]: weak reactivity; +: all cells positive; ±: part of the cells positive; –: all cells negative
[1] mAb LN2, recognizing the same antigen as MB3, provided similar results
[2] LN1 showed in addition clear surface staining of hepatocytes
[3] Reactivity with polyclonal rabbit anti-human lysozyme antiserum
[4] Mainly immature stages of erythroid cells were clearly stained
[5] Staining observed from 16 weeks onwards
[6] Staining observed from 14 weeks onwards

Table 3. Localization of hemopoietic cells in human fetal liver

Cell type	Age(s)	Sinusoids	Portal tracts	Liver cords
Erythroid cells	All[1]	+	–	+
Myelomonocytic cells[2]	5–12 wks	+	+	+
	>12 wks	±	+	sp
Large macrophages	5–12 wks	+	–	+
	>12 wks	+	–	–
Lymphoid cells	>14 wks	+	+	–
Dendritic cells[3]	All	+	+	–
Megakaryocytes[4]	All	sp	–	sp
"Blast cells"	All	sp	–	+

+: A substantial part of the cells is localized in this compartment
±: Few cells are observed
sp: Sporadically a cell is present
–: No cells observed
[1] "All" means that the localization as given is similar in all studied stages during the first and second trimester
[2] Including cells with mature myeloid morphology
[3] With respect to morphology and localization: putative Kupffer-cells
[4] Present in very low numbers

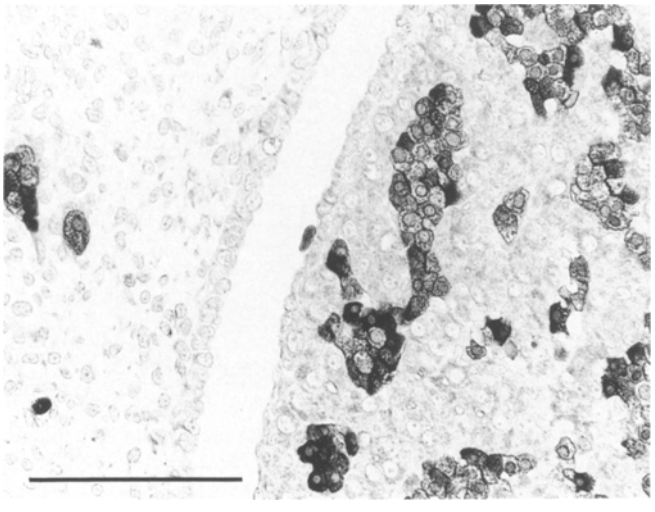

Fig. 1. VIE-G4 staining of 6 weeks human liver: all cells of erythroid lineage show membrane reactivity. (×350; *bar* = 100 μ)

The erythropoietic cell lineage was the earliest haemopoietic lineage observed in human embryonic liver and was the largest haemopoietic cell population during all stages of development. Clustered cells of erythroid origin were present from the most early stages studied (5 and 6 weeks).

Large mononuclear cells, morphologically of myelomonocytic lineage, were also present from 6 weeks with low numbers of myeloid cells with mature morphology. As described earlier (Emura et al. 1983, 1984; Enzan et al. 1978; Gilmour 1941), these cells were mainly located in the connective tissue of portal spaces (Table 3, Fig. 2a). In some cases, however, a considerable number of myelomonocytic cells was scattered throughout the liver, in the sinusoids, and in perisinusoidal liver cords (Fig. 2b). The total number of myelomonocytic cells increased with age and the various morphologically recognizable subtypes were present during all stages. From 16 weeks onwards part of the myelomonocytic cells were

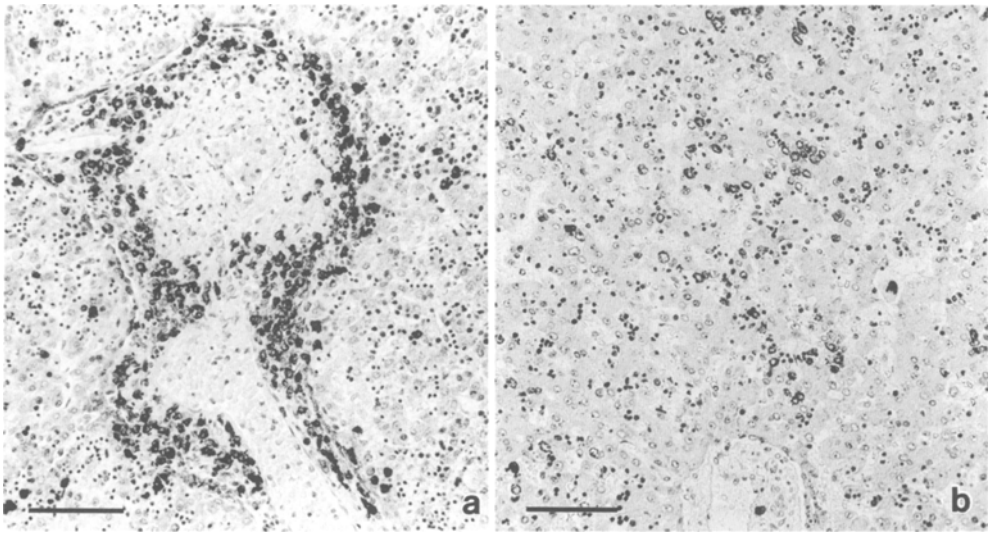

Fig. 2. Myelomonocytic cells: The main distribution pattern is shown in **A**. In 20 weeks fetal liver, mainly located in connective tissue of portal spaces, stained with LeuM1. Note several positive-staining cells in sinusoids and liver parenchyma. In some cases the distribution pattern is somewhat different as shown in **B**. In 17 weeks fetal liver, no main localization in portal spaces, but scattered cells in liver parenchyma and sinusoids (both $\times 140$; *bar* = 100 μ)

MB1-reactive; this subset could morphologically not be related to a particular cell subtype or stage of differentiation. LeuM1 stained myeloid cells with mature morphology and mononuclear cells. The latter cells may represent cells of monocyte/macrophage lineage and/or early myeloid cells; this could not be determined with certainty. Additional histochemical stains were used to obtain further information on the presence of different myelomonocytic subsets. In the Giemsa-stain the presence of eosinophils was recognized by red-staining granules. Mature granulocytes with lobulated nuclei as well as immature myeloid mononuclear cells with cytoplasmic granules were seen. Staining for CAE showed a positive reaction in granulocytes and in somewhat larger mononuclear cells.

At 5 and 6 weeks large macrophages and primitive mesenchymal cells with "dendritic" morphology (most probably Kupffer-cells), were present, both cell types in number increasing with age. Some erythroid cells were observed surrounding macrophages (Fig. 3): so-called "Bessis-islands" (Enzan et al. 1978; Kelemen et al. 1979). However, only a small number of all cells of erythroid lineage was seen in very close proximity of macrophages and the total number of erythroid cells mostly exceeded fifty- to hundredfold the number of macrophages.

Lymphoid cells were obvious from 14 weeks onwards: at first only scattered intermediate-sized lymphoid cells were seen and at 16 weeks and later predominantly small lymphoid cells were found (Tables 2 and 3) showing surface staining for μ heavy chain immunoglobulin (Ig) and kappa or lambda light chains. In contrast, the intermediate-sized cells showed cytoplasmic mu-staining; kappa or lambda staining was not observed in such cells. Faint, diffuse staining for gamma- and alpha-heavy chains and kappa and lambda light chains was also observed in larger cells with monocyte/macrophage morphology with locations as described in Table 3 for these cell types. This staining probably has to be considered as passive absorption of Ig by these cells.

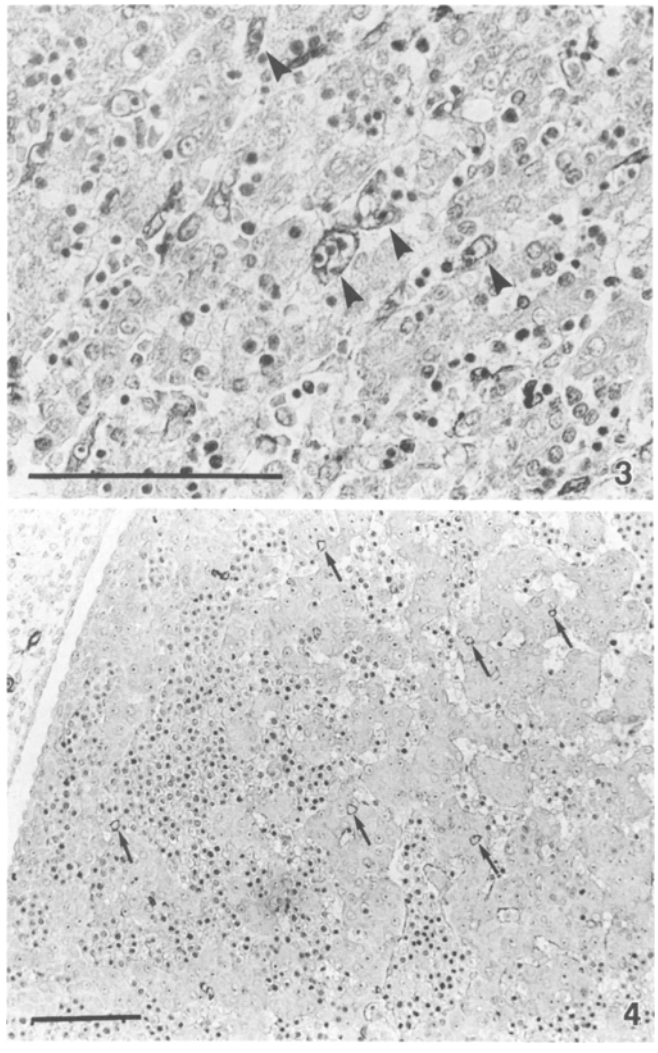

Fig. 3. LN2 staining of 20 weeks fetal liver: positive staining macrophages and Kupffer cells; some macrophages (*arrows*) enclose erythroid cells ($\times 350$; *bar* = 100 μ)

Fig. 4. Distribution of My10 (CD34) positive cells (*arrows*) in 6 weeks embryonic liver ($\times 140$; *bar* = 100 μ)

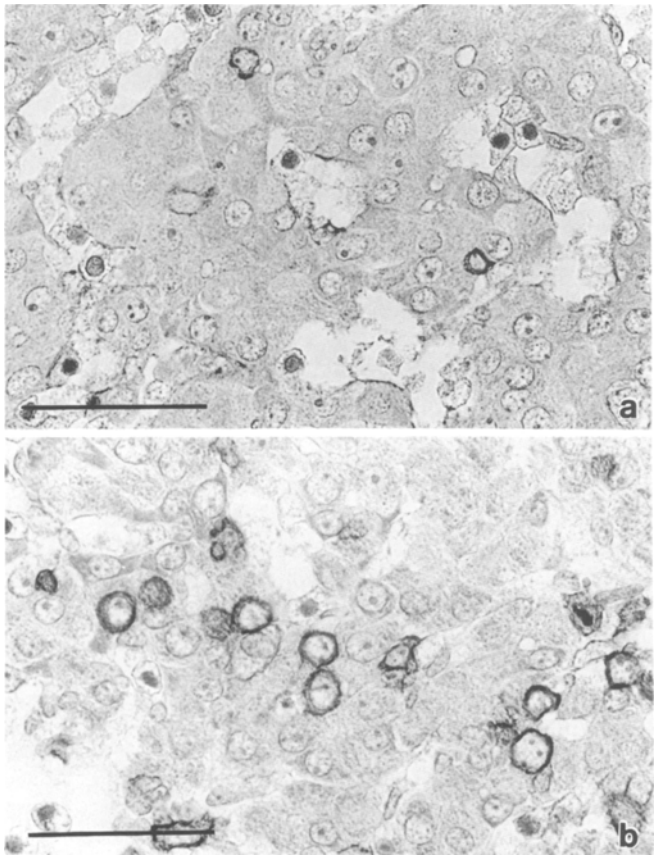

Fig. 5. 6 weeks embryonic liver: **A** (*upper*) My10-positive progenitor cells in liver parenchyma (larger magnification of Fig. 4); note My10 staining of sinusoidal walls. **B** (*lower*) MT1-positive blast cells (both $\times 560$; *bar* = 50 μ)

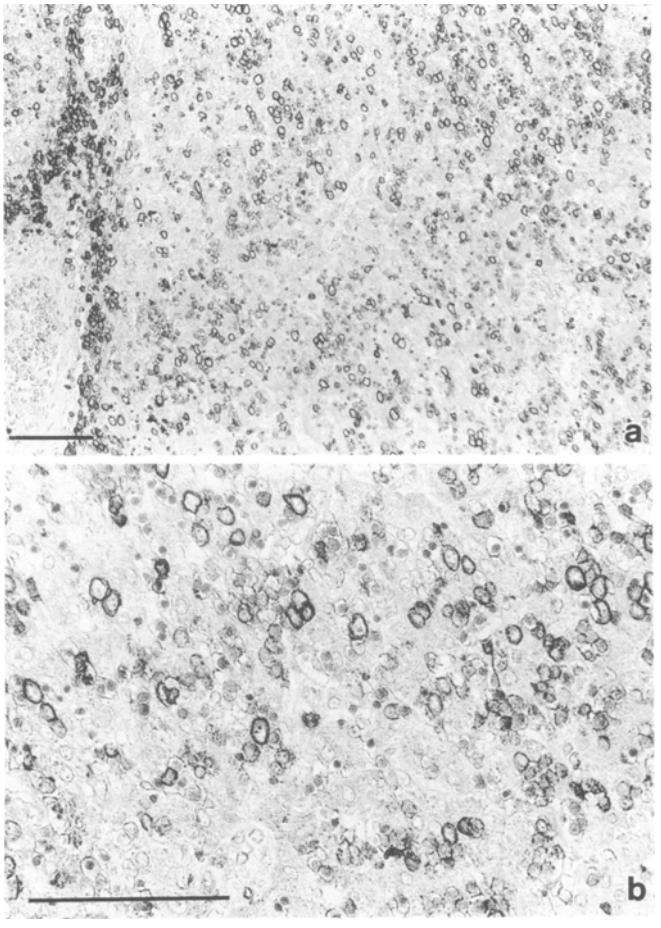

Fig. 6. **A** MT1-positive cells in the second trimester (20 weeks) in human fetal liver ($\times 140$; *bar* = 100 μ). **B** larger magnification ($\times 350$; *bar* = 100 μ)

The presence of My10+ cells was limited to some of the non-autopsy cases in which the tissue was rapidly obtained and processed. It appeared therefore that, although My10 reactivity of early progenitor cells can be demonstrated in paraffin-embedded tissues, the antigen recognized on progenitor cells can probably only withstand fixation and paraffin embedding when obtained under optimal conditions. My10 (CD34) reactivity was observed in intermediate-sized cells ("large lymphocyte-size") characterized by a small cytoplasmic rim and a nucleus with dispersed chromatin and occasional small nucleoli in a minority of cases (not age-related). These cells were very scarce (less than 1 per 1000 haemopoietic cells) and mainly localized in liver parenchyma and the perisinusoidal spaces (Figs. 4, 5a). In all cases My10 showed additional reactivity of walls of small vessels and liver sinusoids.

Apart from the haemopoietic cell lineages described, undifferentiated blast cells were seen. These had a relatively small amount of cytoplasm and a nearly round nucleus with light chromatin and a small rim of more condensed chromatin along the nuclear membrane. Nucleoli were usually prominent but inconspicuous in some smaller cells. Immunohistochemical staining of these scattered blast cells in the liver parenchyma showed exclusive reactivity with MT1 in serial sections (Table 2; Figs. 5b, 6). These cells were not reactive with polyclonal antisera against the intermediate filament cytokeratin, which stains parenchymatous liver cell cytoplasm, or with LN1, which shows membrane staining of liver cells. Although MT1 stains the majority of haemopoietic cells, the MT1-positive blast cells were easily recognized in serial sections by morphology (size) and specific localization. In particular in serial sections MT1+ cells showed clearly different localization and morphology when compared with My10 reactive cells. The blast cells described were present from 5 weeks onwards in slightly decreasing percentage in all first and second trimester cases. The number ranged from $\pm 1\%$ of all haemopoietic cells at 5 weeks to $\pm 5\%$ at the beginning of the second trimester to far less than 1% (<1 per mille) at the end of the second trimester. No blast cells were observed in any of the third trimester fetal livers. MT1-positive blast cells were not observed in adult livers with extramedullary haemopoiesis.

Discussion

Our study has used techniques which make it possible to determine the distribution of immunohistochemically

stained cells as well as their micro-anatomic localization. Although we have used B5-fixative, the mAb employed (Table 1) can also be used on tissues fixed in other fixatives, like buffered formalin (Warnke et al. 1983; Epstein et al. 1984; Poppema et al. 1987; own observations). Nevertheless, prolonged fixation (>24 h) produces a considerable decrease in immunohistochemical reactivity.

Both MT1 and LCA mAb (Poppema et al. 1987; Poppema and Hollema 1987; Warnke et al. 1983) show reactivity in more than one haemopoietic lineage. Our results demonstrate that LCA recognizes cells in all differentiation stages of lymphoid and myelomonocytic origin, megakaryocytes, macrophages and Kupffer-cells. The MT1-antigen shows an even broader distribution (Figs. 5, 6); all haemopoietic lineages, including immature blast cells, are stained with MT1 mAb, with exclusion of mature B-cells. Mature erythroid cells react faintly.

Other antigens expressed in the different haemopoietic lineages are surface and cytoplasmic (Poppema et al. 1987; Quaranta et al. 1984) MHC class II related antigens. In our study these are recognized by MB3, LN2, and LN3 in all haemopoietic cells except for the morphologically recognizable erythroid cells and the described MT1+ undifferentiated blast cells. These results are in accordance with known cellular expression of MHC class II antigens (Bodger et al. 1983; Natali et al. 1987; Sieff et al. 1982).

The presence and localization of all morphologically identifiable erythroid cells is easily determined by reactivity with VIE-G4 and LN1 and more weakly with MT1. Cells of erythroid lineage are present in circumscribed clusters in the hepatic parenchyma, the place of early erythropoietic development (Emura et al. 1984; Fukuda 1974; Gilmour 1941; Kelemen et al. 1979; Migliaccio et al. 1986), and along and inside the sinusoids where further maturation takes place (Emura et al. 1984; Fukuda 1974; Gilmour 1941; Kelemen et al. 1979; Migliaccio et al. 1986). Part of the erythroid development is reported to occur in so-called "Bessis-islands": erythroid cells enclosed by or surrounding a central macrophage (Kelemen et al. 1979). Such islands could easily be identified with the techniques employed in this study. Although these central macrophages are thought to play an essential role in maturation of the erythroid cells (Emura et al. 1984; Kelemen et al. 1979), the total number of macrophages compared to the number of erythroid cells is small and in all stages very few of the described erythroblastic islands were present. An explanation for this discrepancy may be that these macrophages play a role during a limited stage of erythropoietic development or that specialized macrophages produce soluble factors that may be essential for erythropoiesis. The staining results with VIE-G4, which reacts with glycophorin A (GpA) on all morphologically recognizable erythroid cells (Liszka et al. 1983), indicate that the VIE-G4-antigen not only is stable to cryopreservation and freeze-thawing (Liszka et al. 1983), but also to fixation and paraffin-embedment (Fig. 1), as also observed by others (Magyarlaki and Kelenyi 1988). VIE-G4, together with LN1 and MT1 is therefore particularly well-suited for the use in (immuno-)histological studies of erythropoiesis and may also be of interest in diagnostic histopathology of malignancies of erythroid origin, especially in cases where only fixed (paraffin-embedded) tissue is available.

Myelomonocytic cells are mostly found located in the mesenchymal tissue of the portal triads similar as described by others (Enzan et al. 1987; Fukuda 1974; Gilmour 1941; Kelemen et al. 1979). It appears that sometimes considerable numbers of immunohistologically identified myelomonocytic cells are present in a more diffuse distribution in the liver parenchyma and in sinusoids. LeuM1 mAb and anti-lysozyme show reactivity restricted to cells of myelomonocytic lineage and are therefore particularly useful for the detection of scarce myelomonocytic cells in the early embryonal stages of haemopoietic development. LeuM1 stains less cells than anti-lysozyme. In considering the known reactivity of LeuM1 on adult tissues (Table 1), we may speculate that the mononuclear LeuM1+ cells represent early cells in the myeloid rather than cells of monocyte/macrophage lineage. With respect to the staining specificities of LN3 and LN2/MB3 our results suggest that during development in myelomonocytic cells, expression of cytoplasmic MHC class II-related antigens (Poppema et al. 1987; Quaranta et al. 1984), as recognized by LN2 and MB3, precedes surface expression of MHC class II antigens as recognized by LN3.

Lymphoid cells are present in small numbers in all stages. These cells are partly scattered throughout the sinuses but surprisingly, from 16 weeks onwards a considerable number of lymphoid cells is also observed in the perivascular connective tissue in portal tracts and around central veins. This is similar to the localization of myelopoiesis. With respect to the mAb reactive with cells of lymphoid lineage and their staining pattern in the different haemopoietic lineages it can be concluded that MT2 and MB2 are exclusive markers of the lymphoid lineage (Poppema et al. 1987; Poppema and Hollema 1987). Most lymphoid cells are B-cells with a rather mature immunophenotype. The lymphoid cells of intermediate size, i.e. clearly larger than mature small lymphocytes and lacking expression of kappa and/or lambda light chain immunoglobulin, meet the definition of pre-B cells (Kamps and Cooper 1982).

A surprising finding in our study is the observation of immature blast cells showing exclusive reactivity with MT1. Separate studies with a large panel of mAb on frozen fetal liver tissue sections (Kamps et al. 1989), including tissue of the cases in the present study, showed cells with similar size and localization reactive with MT1 and a mAb directed against the intermediate filament vimentin present in cells of mesenchymal origin (Dellagi et al. 1983). These cells did not react with other mAb's including OKIa recognizing HLA-DR and CD34 recognizing the earliest known antigen on haemopoietic cells

(Civin et al. 1984; Lu et al. 1987). The MT1+ blast cells are observed in all stages during the first and second trimester where primary fetal haemopoietic activity is observed, but not in adult liver extramedullary haemopoiesis (unpublished observations). In addition, such blast cells were not present in haemopoiesis in fetal and adult spleen (Timens et al. 1988; and unpublished observations) and were also not described in adult bone marrow (van der Valk et al. 1989). Similar undifferentiated blast cells, different from small undifferentiated presumptive haemopoietic stem cells, were morphologically recognized by others in second trimester fetal liver. Although their nature could not be determined accurately these cells were considered to be early progenitor cells of one or more haemopoietic cell lineages (Emura et al. 1983, 1984; Fukuda 1974; Gilmour 1941; Kelemen et al. 1979; Migliaccio et al. 1986; Peschle et al. 1984). Some authors suppose on morphological grounds that these blast cells belong to the erythroid lineage (haemocytoblasts or transitional cells) (Fukuda 1974; Gilmour 1941; Kelemen et al. 1979). From both our findings and those of others, the presence of MT1-positive blast cells seems to be a unique feature of primary fetal liver haemopoiesis.

In conclusion, this study demonstrates that paraffin-reactive monoclonal antibodies provide a useful tool in the study of embryonal and fetal haemopoiesis. An important finding is the broad distribution of the antigen recognized by the MT1 mAb over all haemopoietic cell lineages and its presence in early stages of development. Further studies are necessary to determine the extent of the distribution of the antigen, including isolation of the haemopoietic blast cells described, in order to elucidate the nature of this cell and to determine its place in the development of the different haemopoietic cell lineages.

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References

Bodger MP, Izaguirre CA, Blacklock HA, Hoffbrand AV (1983) Surface antigenic determinants on human pluripotent and unipotent hematopoietic progenitor cells. Blood 61(5):1006–1010

Civin CI, Strauss LC, Brovall C, Fackler MJ, Schwartz JF, Shaper JH (1984) Antigenic analysis of hematopoiesis: III. A hematopoietic progenitor cell surface antigen defined by a monoclonal antibody raised against KG-1a cells. J Immunol 133(1):157–165

Dellagi K, Vainchenker W, Vinci G, Paulin D, Brouet JC (1983) Alteration of vimentin intermediate filament expression during differentiation of human hemopoietic cells. EMBO J 2(9):1509–1514

Emura I, Sekiya M, Ohnishi Y (1983) Four types of presumptive hemopoietic stem cells in the human fetal liver. Arch Histol Jap 46(5):645–662

Emura I, Sekiya M, Ohnishi Y (1984) Ultrastructural identification of the hemopoietic inductive microenvironment in the human embryonic liver. Arch Histol Jap 47(1):95–112

England MA (1983) A colour atlas of life before birth: Normal fetal development, Wolfe, London

Enzan H, Takahashi H, Kawakami M, Yamashita S, Ohkita T (1978) Light and electronmicroscopic observations on hepatic hematopoiesis of human fetuses: I. Granulocytopoiesis in the hepatic mesenchymal tissue. Acta Path Jap 28(3):411–426

Epstein AL, Marder RJ, Winter JN, Fox RI (1984) Two new monoclonal antibodies (LN1, LN2) reactive in B5 formalin-fixed, paraffin-embedded tissues with follicular center and mantle zone human B lymphocytes and derived tumors. J Immunol 133(2):1028–1036

Fukuda T (1974) Fetal hemopoiesis: II. Electron-microscopic studies on human hepatic hemopoiesis. Virchows Arch [B] 16:249–270

Gilmour JR (1941) Normal hemopoiesis in intra-uterine and fetal life. J Pathol Bacteriol 52:25–55

Hofman FM, Danilovs J, Husmann L, Taylor CR (1984) Ontogeny of B cell markers in the human fetal liver. J Immunol 133(3):1197–1201

Hokland P, Rosenthal P, Griffin JD, Nadler LM, Daley JF, Hokland M, Schlossman SF, Ritz J (1983) Purification and characterization of fetal hematopoietic cells that express the common acute lymphoblastic leukemia antigen (CALLA). J Exp Med 157:114–129

Kamps WA, Cooper MD (1982) Microenvironmental studies of pre-B and B cell development in human and mouse fetuses. J Immunol 129(2):526–531

Kamps WA, Timens W, de Boer TGJ, Spanjer HH, Poppema S (1989) In situ study of hemopoiesis in human fetal liver. Scand J Immunol 30:399–408

Kelemen E, Calvo W, Fliedner TM (1979) Atlas of human hemopoietic development. Springer, Berlin Heidelberg New York

Liszka K, Majdic O, Bettelheim P, Knapp W (1983) Glycophorin A expression in malignant hematopoiesis. Am J Hematol 15:219–226

Lu L, Walker D, Broxmeyer HE, Hoffman R, Hu W, Walker E (1987) Characterization of adult marrow hematopoietic progenitors highly enriched by two color cell sorting with My10 and major histocompatibility class II monoclonal antibodies. J Immunol 139:1823–1829

Magyarlaki T, Kelenyi G (1988) Use of monoclonal antibodies in formol-paraffin sections. Virchows Arch [A] 412:487–492

Migliaccio G, Migliaccio AR, Petti S, Mavilio F, Russo G, Lazarro D, Testa U, Marinucci M, Peschle C (1986) Human embryonic hemopoiesis: Kinetics of progenitors and precursors underlying the yolk sac to liver transition. J Clin Invest 78:51–60

Moore KL (1982) The Developing Human. Saunders, Philadelphia

Natali PG, Segatto O, Ferrone S, Tosi R, Corte G (1987) Differential tissue distribution and ontogeny of DC-1 and HLA-DR antigens. Immunogenetics 19:109–116

O'Rahilly R (1979) Early human development and the chief sources of information on staged human embryos. Europ J Obstet Gynec Reprod Biol 9/4:273–280

Peschle C, Migliaccio G, Lazarro D, Petti S, Mancini G, Care A, Russo G, Mastroberardino G, Migliaccio AR, Testa U (1984) Hemopoietic development in human embryos. Blood Cells 10:427–441

Poppema S, Hollema H (1987) Monoclonal antibodies in lymphoma diagnosis and classification. In: Ruiter DJ, Fleuren GJ, Warnaar SO (eds) Application of monoclonal antibodies in tumor pathology. Martinus Nijhoff, Dordrecht, The Netherlands, pp 117–130

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

Quaranta V, Majdic O, Stingl G, Liszka K, Honigsman H, Knapp W (1984) A human Ia cytoplasmic determinant located on multiple forms of invariant chain (gamma, gamma 2, gamma 3). J Immunol 132(4):1900–1905

Rosenthal P, Rimm IJ, Umiel T, Griffin JD, Osathanondh R, Schlossman SF, Nadler LM (1983) Ontogeny of human hematopoietic cells: analysis utilizing monoclonal antibodies. J Immunol 131:232–237

Sieff C, Bicknell D, Caine G, Robinson J, Lam G, Greaves MF (1982) Changes in cell surface antigen expression during hemopoietic differentiation. Blood 60(3):703–713

Timens W, Rozeboom-Uiterwijk T, Poppema S (1988) Immunoarchitecture of human fetal lymphoid tissues. Virchows Arch [A] 413:563–571

Van der Valk P, 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

Warnke RA, Gatter KC, Phil D, Falini B, Hildreth P, Woolston RE, Pulford K, Cordell JL, Cohen B, De Wolf-Peeters C, Mason DY (1983) Diagnosis of human lymphoma with monoclonal antileukocyte antibodies. N Engl J Med 309:1275–1281