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Fetal and neonatal development of human spleen: an immunohistological study

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SUMMARY

Localization and immunophenotype of lymphocyte subsets in fetal human spleens were studied by employing a panel of monoclonal antibodies (McAb) in an immunoperoxidase staining procedure on frozen tissue sections. Spleens varied from 15 weeks of gestational age (gestational weeks, gw) to newborn. The white pulp consisted of intermediate-sized lymphocytes; no separate compartments could be discerned. Germinal centre development was not observed. Dendritic cells stained for B2, HB5, aC3bR, anti-DRC and OKIa, but in most cases not for immunoglobulin. Although low cellular immunity is observed in neonates, T cells showed adult phenotypes in proportions comparable to the adult situation; immature OKT6(+) lymphocytes were rarely seen. Very few cells stained with anti-NK cell antibody Leu7. B cells all expressed B1, Leu14, aC3bR, T10 and OKIa, were strongly positive for BA1, and mostly stained very weakly for B2 and HB5. Almost all B cells expressed IgM and IgD simultaneously, and very few expressed IgG. IgA-positive cells were absent. At 15 gw a considerable number of IgM(+) B cells showed Leu1 staining, but this decreased during development. These cells may represent the normal counterpart of Leu1(+) IgM(+) cells observed in B-CLL and immunocytic and centrocytic malignant lymphomas. After 25 gw only very few Leu1(+) IgM(+) cells were seen. Altogether, the morphology and immunophenotype of white pulp B cells were different from the predominating adult B-cell subsets, at least until birth. These 'immature' splenic B cells may be precursors for adult splenic B-cell subsets. Considering the presumed role of the marginal zone in the immunity against TI-2 antigens, the absence of a marginal zone at birth may be a main factor in the defective immunity against these antigens in neonates.

INTRODUCTION

The development of the human lymphoid system during fetal life and the first period of life after birth has been studied extensively (Olding, 1979; Andersson *et al.*, 1981; Gathings, Kubagawa & Cooper, 1981; Hayward, 1981; Toivanen *et al.*, 1981). Particular attention has been given to reduced cellular and humoral immunity in neonates compared to adults.

More recently, the availability of a broad spectrum of specific monoclonal antibodies (McAb) has provided additional important tools to study the ontogeny of lymphoid compartments. It is now possible to define cellular lineage and stage of differentiation of the different fetal cell subsets in the various lymphoid compartments (Abo, Cooper & Balch, 1982; Kamps & Cooper, 1982; Abo *et al.*, 1983; Rosenthal *et al.*, 1983; Asma, Langlois van den Bergh & Vossen, 1984; Bofill *et al.*, 1985; Namikawa *et al.*, 1986). By employing a series of monoclonal antibodies, an immunophenotypic profile of fetal lymphoid and non-lymphoid cell subsets can be established. Together with functional *in vitro* studies of these subsets (Gathings *et al.*, 1981; Hayward, 1981; Toivanen *et al.*, 1981), this may give more insight into the functional abilities and maturation of the fetal and newborn immune system. Consequently, explanations might be found for reduced or absent immunological responses to several antigens during early life. An important part of this impaired immunity in the neonate and during the first year of life is the poor response to certain antigens, in particular polysaccharide antigens. These antigens are found on the capsule of several infectious microorganisms such as *Streptococcus pneumoniae, Neisseria meningitides* or *Haemophilus influenzae* (Gathings *et al.*, 1981). The described defective antibody responses probably contribute to the increased susceptibility of infants to, sometimes overwhelming, bacterial infections.

In adults, a comparable increased susceptibility to infections is observed in splenectomized patients (Ruben *et al.*, 1984; Amlot & Hayes, 1985). Because of this, the immune response against polysaccharide antigens has been studied in adult humans and rodents (Ruben *et al.*, 1984; Amlot & Hayes, 1985; Amlot, Grennan & Humphrey, 1985; Gray *et al.*, 1985). These antigens belong to the category of thymus-independent antigens, type 2 (TI-2). It appeared that the immune response against TI-2 antigens is mainly dependent on the presence of the

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spleen, and that particularly the microenvironment of the marginal zone might play an important role in the initiation and development of this response (Amlot *et al.*, 1985; Gray *et al.*, 1985). This marginal zone is a specific and unique lymphocyte compartment, and is not found in other lymphoid tissues (Timens & Poppema, 1985).

Because of the special role of the spleen in immunity, we decided to study the immunophenotypes of splenic lymphoid cells and the development of the different lymphoid compartments during gestation and in neonates. The study was performed by employing a panel of monoclonal antibodies in an immunoperoxidase staining procedure on frozen tissue sections.

MATERIALS AND METHODS

Spleens of 30 fetuses, varying from 15 weeks of gestational age (gestational weeks, gw) to newborn, were studied. The spleens were obtained from autopsies. Cause of death in most cases was immaturity, respiratory insufficiency, and congenital heart defect, with no further abnormalities found. Few cases revealed other, or additional diagnoses: spina bifida (18 gw), Potter's syndrome (32 gw), cytochrome-*c* oxidase deficiency (40 gw), and anencephaly (40 gw). Additionally, some spleens were studied from infants aged 1, 1·5 and 4 years, respectively. These were all removed because of trauma.

Gestational age was confirmed by foot and femur length measurements (Wigglesworth, 1984), and correlated to the date of the last menstrual period. Tissue blocks of each spleen were snap-frozen in liquid freon and stored at −70° until use.

A panel of well-known monoclonal antibodies (Sutherland *et al.*, 1981; Foon, Schroff & Gale, 1982; Gerdes *et al.*, 1982; Naiem *et al.*, 1983; Weiss, Tedder & Fearon, 1984; Schwarting, Stein & Wang, 1985), listed in Table 1, was used to define the immunophenotype of the cell subsets in the studied spleens. McAb 1G8 was developed in our laboratory (L. Visser *et al.*, manuscript in preparation). The reactivity of this antibody is identical to Leu14 (CD 22), but 1G8 is an antibody of IgM class, whereas Leu 14 is of IgG class. The antibodies were used in dilutions considered to be optimal, as determined in previous experiments.

A two-step immunoperoxidase procedure was performed on 5-μm thick, acetone-fixed, frozen tissue sections of spleen, as described previously (Timens & Poppema, 1985). Rabbit anti-mouse antibody conjugated to horseradish peroxidase (DAKO, Glostrup, Denmark) was used as the second step, and 3-amino-9-ethylcarbazole (AEC), together with H_2O_2, as the enzyme-histochemical reagent, giving a reddish-brown reaction product.

For conventional histology, tissue blocks were fixed in formalin or B5 and subsequently embedded in paraffin or plastic. Sections were stained with haematoxylin and eosin, and the morphology of the different cell types and their microscopic environment was studied.

Leu1/IgM double labelling

After incubation with Leu1 for 30 min, sections were incubated with a mixture of fluorescein-conjugated goat anti-mouse IgG (Cappel Lab., Cochranville, PA), detecting Leu1 (which is of the IgG class), and rhodamin-conjugated rabbit anti-human IgM (polyclonal, Nordic, Tilburg, The Netherlands) labelling the IgM(+) cells.

Table 1. Reactivity of the monoclonal antibodies

McAb	CD	MW of antigen	Reactive with:
B1	CD20	30,000	B cells
B2	CD21	140,000	B cells, dendritic cells (C3d receptor)
HB5	CD21	140,000	B cells, dendritic cells (C3d receptor)
Leu14	CD22	135,000	B cells
1G8	CD22	135,000	B cells
BA1	CD24	45,000	B cells, granulocytes
OKIa	—	29,000, 34,000	HLA-DR antigen
aC3bR	—	205,000	C3b receptor: B cells, dendritic cells
aDRC1	—	NR	Dendritic cells; some B cells (very weakly)
OKT6	CD1	49,000, 12,000	Thymocytes
Leu5	CD2	40,000–50,000	E-rosette receptor on T cells
OKT3	CD3	19,000	T cells, thymocytes
Leu3	CD4	55,000	T-helper/inducer cells
66IIG5	CD7	41,000	T cells
OKT8	CD8	32,000–43,000	T-cytotoxic/suppressor cells
OKT9	—	180,000—90,000	Transferrin receptor
OKT10	—	46,000, 12,000	Thymocytes; activated T and B lymphocytes; bone marrow B cells; immature myeloid cells
Leu7	—	NR	Large granular lymphocytes; NK/K cells
CALLA	CD10	100,000	Common acute lymphocytic leukaemia antigen

B1 and B2 were a gift from Dr L. M. Nadler (Dana Farber Cancer Institute, Harvard Medical School, Boston, MA), HB5 was from Dr T. F. Tedder (Dana Farber Cancer Institute), and anti-immunoglobulin antibodies were from Dr J. Haaijman (RIV, Rijswijk, The Netherlands). Leu antibodies were purchased from Becton-Dickinson (Mountain View, CA), OK antibodies were from Ortho Diagnostic Systems (Raritan, NJ), BA1 was from Hybritech (San Diego, CA), and CALLA, anti-DRC1 and anti-C3bR were from DAKO (Glostrup, Denmark).

Leu1/1G8 labelling

Sections were incubated with a mixture of Leu1 and 1G8 (CD 22) for 30 min. Subsequently, the sections were incubated with a mixture of fluorescein-conjugated goat anti-mouse IgG, detecting Leu1, and rhodamin-conjugated goat anti-mouse IgM (Cappel), detecting 1G8, being of IgM subclass.

RESULTS

White pulp development

In 15-week gestational age fetal spleens, few small clusters of intermediate-sized lymphocytes were observed (Fig. 1a). During gestation up to birth, these primitive white pulp cell clusters increased with respect to the amount of cells, and gradually they became more organized, forming a follicle-like shape (Fig. 1b). However, all lymphocytes were of equal, intermediate size, and no distinct lymphocyte compartments could be discerned in all subsequent gestational stages. Development of a germinal centre reaction was not observed in any of the studied fetal or neonate spleens.

Presence of B cells during splenic development

At 15 gw small perivascular accumulations of B cells were observed, which became more prominent during fetal development, until at the time of birth a sort of primitive follicle was formed. In all developmental stages the cells stained for B1, Leu14, aC3bR, T10, OKIa, and relatively strongly for BA1. Staining for T10 was strong in the first and second trimester and very weak after 26 gw. In most cases B2 (anti-C3dR) showed very weak staining of B cells, and in some (early) cases B2 reactivity of B cells seemed absent. HB5, a monoclonal antibody, also recognizing the C3d receptor, showed a staining pattern similar to B2. Almost all B cells were sIgM(+) as well as sIgD(+) (Fig. 2). No cells stained for IgA, whereas an IgG stained cell was occasionally observed. Kappa and lambda showed complementary staining of virtually all B cells in a ratio of 2–3 to 1.

Staining for CALLA was not present in the primitive white pulp. Although CALLA staining in the red pulp was difficult to evaluate, in most cases few weakly CALLA-positive lymphoid cells were observed. In the third trimester generally no CALLA staining was seen.

At 15 gw up to 20% of the splenic B cells stained for Leu1, as confirmed by double staining for Leu1/IgM and Leu1/1G8. With increasing gestational age, a gradual decrease in the relative amount of B cells, staining for Leu1 was observed, until after about 25 gw only very few Leu1 (+) B cells were seen, comparable to the adult situation.

The red pulp showed very few scattered B cells, with immunophenotypes similar to the described white pulp B cells. Except for the described Leu1 and T10 staining, all splenic B cells exhibited a constant immunophenotype during all observed stages of fetal development.

Presence of T cells in fetal and neonate spleen

At early gestational age T cells were scattered throughout the splenic tissue. At 17–18 gw very small groups of T cells were found surrounding small arterioles, and scattered individual T cells were also present. A progressive accumulation of T cells was observed with increasing gestational age (Fig. 3). In the early stages these T-cell areas were not very well organized and there was no sharp demarcation between T-cell and B-cell areas.

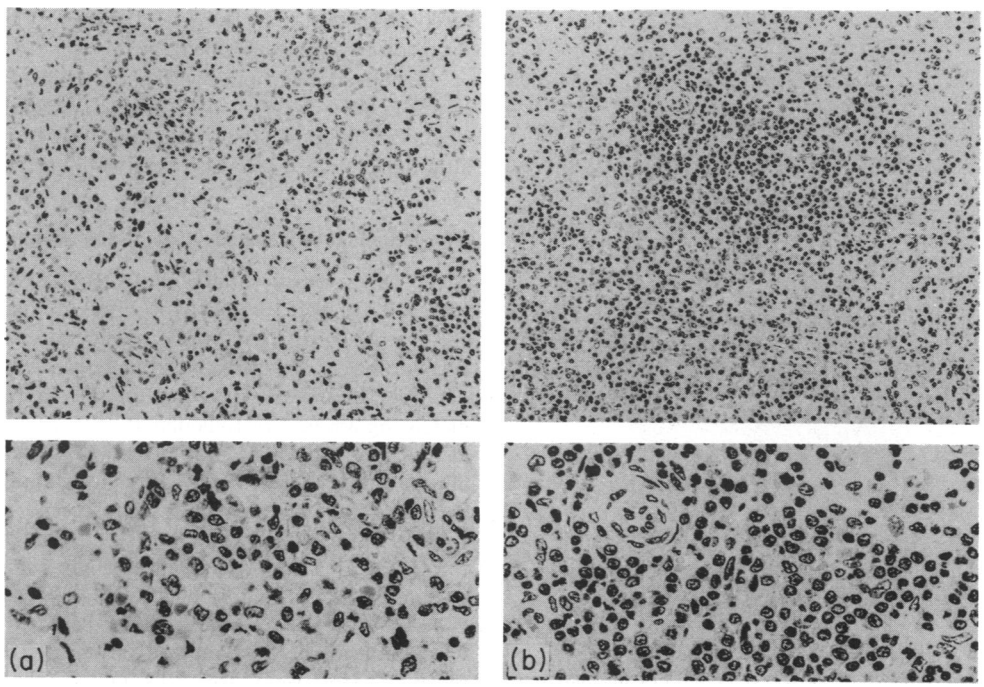

Figure 1. Haematoxylin and eosin stained sections of spleen: magnifications ×112 (above) and ×280 (below). (a) 17 gw: scattered intermediate-sized lymphocytes, sometimes in small clusters: (b) 40 gw: primitive white pulp area.

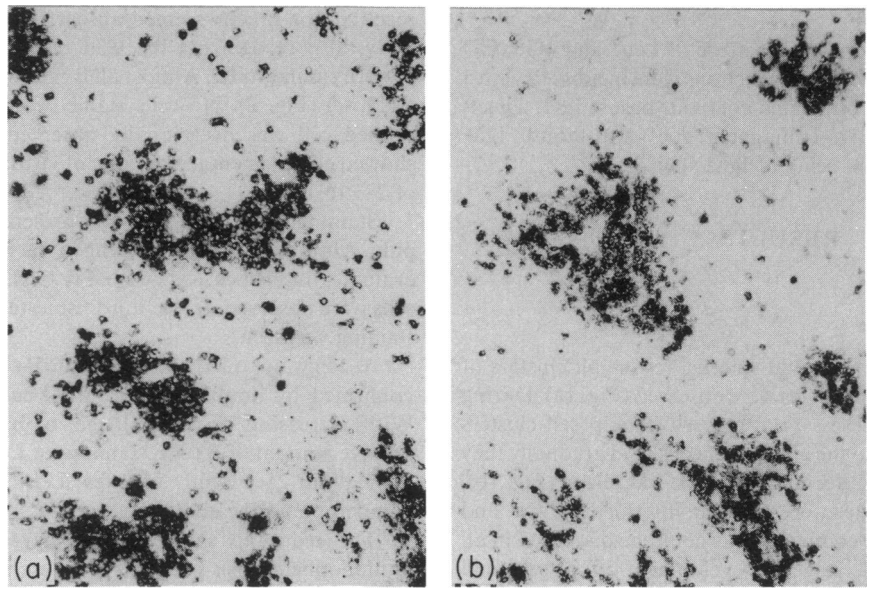

Figure 2. Immunoperoxidase staining of 26 gw fetal spleen for (a) IgM, and (b) IgD (magnification ×98); almost all B cells are stained for IgM and IgD.

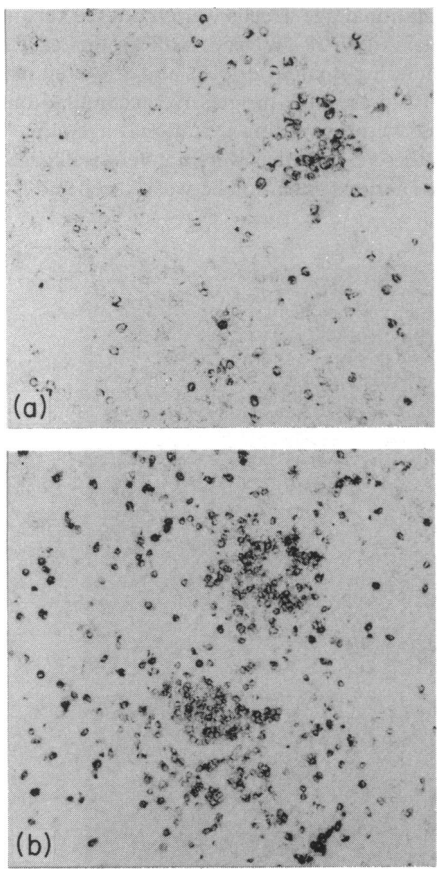

Figure 3. Spleen section stained with 66IIG5 (CD7): (a) 18 gw: scattered cells, sometimes in small clusters; (b) 26 gw: somewhat larger T-cell clusters (magnification ×112).

At the end of the second trimester a well-organized area of T cells was found, situated around arterioles. This fetal periarterial lymphocyte sheath (PALS) was adjoining the primitive B-cell follicles. In these B-cell follicles very few scattered T cells were found.

The T cells showed immunophenotypes similar to T cells of the PALS in adult spleen (Timens & Poppema, 1985). All T cells stained for Leu1, Leu5, OKT3, T10 and 66-II-G5, and additionally for Leu3 or T8 in a ratio of 2:1. OKT6(+) cells were rarely observed. Apart from the staining of T cells, Leu1 showed B-cell reactivity as described above.

Leu7 in adults reactive with natural killer cells and germinal centre T cells (Abo *et al.*, 1982, 1983; Poppema, Visser & De Ley, 1983; Pizzolo *et al.*, 1984) generally showed no staining at all, although a stained cell was occasionally seen.

Non-lymphoid constituents of fetal and neonate spleen

At 17–18 gw very few individual aDRC(+) follicular dendritic cells (FDC) were present, localized in the described B-cell clusters. A dendritic meshwork, formed by FDC in the primitive B-cell follicles, was observed from 22 gw. During gestation the dendritic meshwork increased in size in proportion to the increasing amount of follicular B cells. FDC stained with B2, aC3bR, anti-DRC, and, although weakly, with OKIa. Generally no detectable immunoglobulin staining of FDC was present, although occasionally some spleens (mostly in the third trimester) showed FDC staining for IgM. Interdigitating cells of the T-cell area (IDC) stained strongly for OKIa, and were present in the earliest formed clusters of T cells at about 18 gw.

OKT9, which recognizes the transferrin receptor, reacted with a considerable amount of cells, scattered throughout the red pulp. Most were large cells, most probably myeloid cells, and some very large cells, presumably macrophages, were also stained. Also, clusters of large cells in the red pulp showed

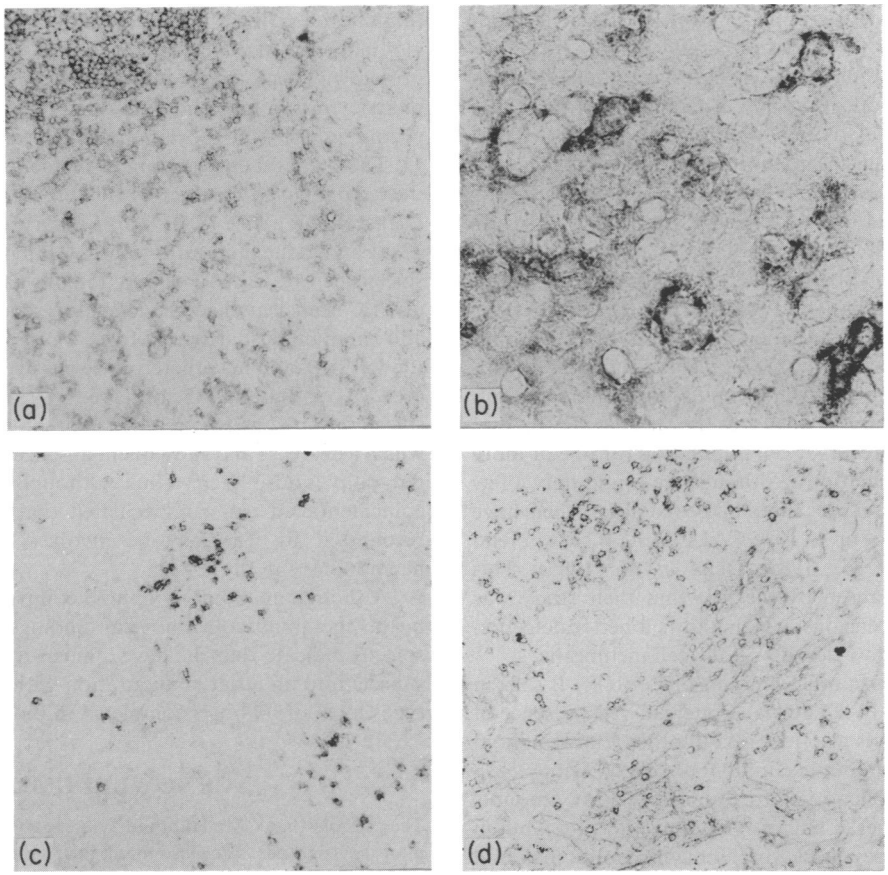

Figure 4. (a) Leu3 staining of fetal spleen, 28 gw, showing staining of lymphocytes, and in the red pulp of histiocytes (magnification × 112). (b) Higher magnification of Leu3 staining of histiocytes in the red pulp (magnification × 448). (c) T8 staining of 23 gw spleen: few scattered positive lymphocytes; no staining of sinusoid lining cells is observed (magnification × 112). (d) T8 staining of adult spleen: staining of lymphocytes as well as red pulp sinusoid lining cells (magnification × 112).

OKT9 reactivity, probably representing erythroblasts. In several, but not all, cases, globular cytoplasmic staining for IgG was observed in large cells in the red pulp.

Leu3 and T10 stained, in addition to T cells, variable amounts of large cells in the red pulp, most probably of myeloid lineage (monocytes/histiocytes) (Fig. 4a and b). After 28 gw this particular reactivity of Leu3 decreased to a pattern comparable to the adult situation, and the reactivity of T10 was reduced or absent.

Staining of sinusoid lining cells (SLC) in the red pulp by T8, as observed in adult spleen (Timens & Poppema, 1985), was not observed in fetal spleens before 32 gw (Fig. 4c and d). After 32 gw most fetal and neonate spleens showed this specific T8 staining of SLC.

Apart from B cells and FDC, B2 also stained the endothelial cells of small vessels. These endothelial cells did not stain with HB5 or aC3bR.

Immunohistology of infant spleen

The immunophenotypes of the different cell subsets and lymphoid compartments of the three infant spleens (of children aged 1, 1·5 and 4 years, respectively) were all similar to the findings in adult spleen (Timens & Poppema, 1985).

DISCUSSION

The finding that all lymphocytes in the different developmental stages of fetal spleen are of intermediate size (i.e. clearly larger than the small B and T lymphocytes observed in adult tissues) indicates that no separate lymphoid compartments can be distinguished on morphological grounds. Follicular dendritic cells, staining for B2, aC3bR, a-DRC and OKIa, but not for immunoglobulin, and strongly OKIa-positive interdigitating cells of T-cell area, are present in early developmental stages. Localization and immunophenotype of these cells are similar as observed in adult spleen (Timens & Poppema, 1985). T-lymphocyte subsets in fetal spleens have adult-type immunophenotypes, and the appearance of a more or less organized T-cell compartment, the primitive PALS, takes place at relatively advanced gestational age. Impaired cellular immunity in neonates (Olding, 1979; Andersson *et al.*, 1981; Toivanen *et al.*, 1981) cannot simply be explained by the absence of one or more T lymphocyte subsets. However, it cannot be excluded that this reduced immunity is due to functional immaturity or inadequate quantities of certain fetal T-cell subsets (Andersson *et al.*, 1981). Leu7 generally shows reactivity in very few cells, which is in accordance with a low natural killer activity in neonates (Toivanen *et al.*, 1981; Abo *et al.*, 1982; Baley & Schacter, 1985)

and with the absence of germinal centre development (Poppema, Visser & de Ley, 1983; Pizzolo *et al.*, 1984).

The conclusion that in rats the earliest B cells in fetal spleen are the marginal zone cells (Dijkstra & Dopp, 1983) cannot be confirmed in humans. Although we do observe intermediate-sized cells in human fetal spleen, the immunophenotype of these cells, determined by positive staining for B1, Leu14, aC3bR, T10, sIgM, sIgD, and strong staining for BA1, while B2 staining, as well as HB5 reactivity, in most cases is very weak, and in some (early) cases absent, does not at all resemble that of adult marginal zone cells [IgM(+) IgD(−) B1(+) BA1(+) Leu14(+) aC3bR(+) T10(−) and strongly B2(+)]. Lymphocytes of intermediate size with this fetal spleen immunophenotype do not comprise a significant population in human adult lymphoid compartments (Timens & Poppema, 1985), and probably represent a precursor cell population for one or more of the adult B-cell populations. Although we found only a few weakly CALLA(+) B cells localized in the red pulp, others have reported the presence of up to 10% CALLA(+) B cells in fetal spleens (Rosenthal *et al.*, 1983; Delia *et al.*, 1985). This discrepancy may be caused by the fact that their study was performed on cell suspensions, which allows a better detection of antigens present in low density on the cell membrane.

The finding of a substantial amount of Leu1(+) B cells in early fetal life (mainly 15–25 gw) is of interest. The presence of Leu1(+) IgM(+) cells is also reported in other fetal lymphoid tissues, often in large amounts (Bofill *et al.*, 1985; Antin *et al.*, 1986). B cells with this phenotype are found in very low amounts in adult lymphoid tissues (Caligaris-Cappio *et al.*, 1982), but in large amounts in centrocytic and immunocytic lymphomas, and in B-CLL (Harris, Nadler & Bhan, 1984; Anderson *et al.*, 1984; Harris & Bhan, 1985). Therefore, fetal Leu1(+) IgM(+) IgD(+) B cells may represent the normal counterpart of these lymphoma subtypes and/or B-CLL (Bofill *et al.*, 1985; Antin *et al.*, 1986).

The most striking feature in human B-cell immunity in neonates is a defective response against polysaccharide antigens (Andersson *et al.*, 1981; Gathings *et al.*, 1981; Hayward, 1981). The immune response against the group of thymus-independent antigens type 2 (TI-2), which includes polysaccharide antigens, is thought to take place mainly in the spleen, as determined in animal experiments (Amlot & Hayes, 1985; Amlot *et al.*, 1985; Gray *et al.*, 1985; Claassen, Kors & van Rooijen, 1986). One particular aspect of this response is the presentation of the TI-2 antigens by specialized marginal zone macrophages to B cells (reviewed by Amlot *et al.*, 1985; Gray *et al.*, 1985; Claassen *et al.*, 1986). Whether this immune response is restricted to marginal zone B cells and maybe T cells (Amlot *et al.*, 1985) is not yet clarified. The total absence of an ('adult type') marginal zone in fetal and neonatal spleen may be at least a partial explanation for the defective immunity against TI-2 antigens in newborns.

The observed clusters of large OKT9 stained cells probably represent erythroblasts, expressing large amounts of transferrin receptors (Omary, Trowbridge & Minowada, 1980; Sutherland *et al.*, 1981).

Leu3 was reported to react weakly with cells of monocyte/histiocyte lineage (Wood, Warner & Warnke, 1983; Stewart, Fujimoto & Levy, 1986), and T10 is known to be reactive with myeloblasts and myelocytes, but not with mature myeloid cells (Janossy *et al.*, 1981). Large cells in the fetal splenic red pulp, positive for Leu3 and/or T10, are therefore likely to represent (immature) cells of myeloid and/or monocytic origin. The globular IgG staining in the cytoplasm of large cells, most probably of histiocyte/macrophage lineage, is likely to represent phagocytosis of maternal IgG, since during fetal life hardly any production of fetal IgG is found (Hayward, 1981).

The T8 and Leu2 staining of sinusoidal lining cells (SLC) in the red pulp in adult spleen (Timens & Poppema, 1985; Buckley, Dickson & Walker, 1985) is also observed in fetal spleens after 32 gw and in the three infant spleens, but is totally absent in fetal spleens before 32 gw. The function of this T8-reactive molecule on SLC is unclear. The T8 molecule is reported to be specifically and spontaneously released from T cells (Fujimoto, Stewart & Levy, 1984), and may therefore be passively acquired by certain cells. In our material, the observed T8 reactivity of SLC in fetal spleen (after 32 gw) is present in the same gestational period in which a decrease and subsequent absence of haemopoiesis in the red pulp is also seen. Thus, although these events may be coincidental, it can be speculated that the antigen on SLC, recognized by T8, may be involved in the regulation of haemopoiesis in the spleen.

Although no 'adult' lymphoid compartments are present at birth, the immunohistological findings in the three infant spleens indicate that the development of the splenic lymphoid subsets into an adult configuration with adult immunophenotypes most probably is completed in the first year after birth.

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REFERENCES

ABO T., COOPER M.D. & BALCH C.M. (1982) Postnatal expansion of the natural killer and killer cell population in humans, identified by the monoclonal HNK-1 antibody. *J. exp. Med.* **155,** 321.

ABO T., MILLER C.A., GARTLAND L. & BALCH C.M. (1983) Differentiation stages of human natural killer cells in lymphoid tissues from fetal to adult life. *J. exp. Med.* **157,** 273.

AMLOT P.L., GRENNAN D. & HUMPHREY J.H. (1985) Splenic dependence of the antibody response to thymus-independent (TI-2) antigens. *Eur. J. Immunol.* **15,** 508.

AMLOT P.L. & HAYES A.E. (1985) Impaired human antibody response to the thymus-independent antigen, DNP–Ficoll, after splenectomy. Implications for post-splenectomy infections. *Lancet,* **i,** 1008.

ANDERSON K.C., BATES M.P., SLAUGHENHOUPT B.L., PINKUS G.S., SCHLOSSMAN S.F. & NADLER L.M. (1984) Expression of human B cell-associated antigens on leukemias and lymphomas: a model of human B cell differentiation. *Blood,* **63,** 1424.

ANDERSSON U., BIRD A.G., BRITTON S. & PALACIOS R. (1981) Humoral and cellular immunity in humans studied at the cell level from birth to two years of age. *Immunol. Rev.* **57,** 5.

ANTIN J.H., EMERSON S.G., MARTIN P., GADOL N. & AULT K.A. (1986) Leu1+ (CD5+) B cells, a major lymphoid subpopulation in human fetal spleen: phenotypic and functional studies. *J. Immunol.* **136,** 505.

ASMA G.E.M., LANGLOIS VAN DEN BERGH R. & VOSSEN J.M. (1984) Development of pre-B and B lymphocytes in the human fetus. *Clin. exp. Immunol.* **56,** 407.

BALEY J.E. & SCHACTER B.Z. (1985) Mechanisms of diminished natural killer cell activity in pregnant women and neonates. *J. Immunol.* **134,** 3042.

BOFILL M., JANOSSY G., JANOSSA M., BURFORD G.D., SEYMOUR G.J.,

WERNET P. & KELEMEN E. (1985) Human B cell development. II. Subpopulations in the human fetus. *J. Immunol.* **134,** 1531.

BUCKLEY P.J., DICKSON S.A. & WALKER W.S. (1985) Human splenic sinusoidal lining cells express antigens associated with monocytes, macrophages, endothelial cells, and T lymphocytes. *J. Immunol.* **134,** 2310.

CALIGARIS-CAPPIO F., GOBBI M., BOFILL M. & JANOSSY G. (1982) Infrequent normal B lymphocytes express features of B-chronic lymphocytic leukemia. J. *exp. Med.* **155,** 623.

CLAASSEN E., KORS N. & VAN ROOIJEN N. (1986) Influence of carriers on the development and localization of anti-2, 4, 6-trinitrophenyl (TNP) antibody-forming cells in the murine spleen. II. Suppressed antibody response to TNP–Ficoll after elimination of marginal zone cells. *Eur. J. Immunol.* **16,** 492.

DELIA D., CATTORETTI G., BONATI A., VILLA S., DE BRAUD F. & BUSCAGLIA M. (1985) Detection of the common acute lymphoblastic leukemia antigen (CALLA) on B cells from human fetal fetuses. A multiple phenotypic characterization. *Clin. exp. Immunol.* **59,** 305.

DIJKSTRA C.D. & DOPP E.A. (1983) Ontogenetic development of T and B lymphocytes and non-lymphoid cells in the white pulp of the rat spleen. *Cell Tiss. Res.* **229,** 351.

FOON K.A., SCHROFF R.W. & GALE R.P. (1982) Surface markers on leukemia and lymphoma cells: recent advances. *Blood,* **60** 1.

FUJIMOTO J., STEWART S. & LEVY R. (1984) Immunochemical analysis of the released Leu2 (T8) molecule. *J. exp. Med.* **160,** 116.

GATHINGS W.E., KUBAGAWA H. & COOPER M.D. (1981) A distinctive pattern of B cell immaturity in perinatal humans. *Immunol. Rev.* **57,** 107.

GERDES J., NAIEM M., MASON D.Y. & STEIN H. (1982) Human complement (C3b) receptors defined by a monoclonal antibody. *Immunology,* **45,** 645.

GRAY D., CHASSOUX D., MACLENNAN I.C.M. & BAZIN H. (1985) Selective depression of thymus-independent anti-DNP antibody responses induced by adult but not neonatal splenectomy. *Clin. exp. Immunol.* **60,** 78.

HARRIS N.L., NADLER L.M. & BHAN A.K. (1984) Immunohistologic characterization of two malignant lymphomas of germinal centre type (centroblastic/centrocytic and centrocytic) with monoclonal antibodies: follicular and diffuse lymphomas of small-cleaved cell type are related but distinct entities. *Am. J. Pathol.* **117,** 262.

HARRIS N.L. & BHAN A.K. (1985) B cell neoplasms of the lymphocytic, lymphoplasmacytoid, and plasma cell types: Immunohistological analysis ands clinical correlation. *Human Pathol.* **16,** 829.

HAYWARD A.R. (1981) Development of lymphocyte responses and interactions in the human fetus and newborn. *Immunol. Rev.* **57,** 39.

JANOSSY G., TIDMAN N., PAPAGEORGIOU E.S., KUNG P.C. & GOLDSTEIN G. (1981) Distribution of T lymphocyte subsets in the human bone marrow and thymus: an analysis with monoclonal antibodies. *J. Immunol.* **128,** 1608.

KAMPS W.A. & COOPER M.D. (1982) Microenvironmental studies of pre-B and B cell development in human and mouse fetuses. *J. Immunol.* **129,** 526.

NAIEM M., GERDES J., ABDULAZIZ Z., STEIN H. & MASON D.Y. (1983) Production of a monoclonal antibody reactive with human dendritic reticulum cells and its use in the immunohistological analysis of lymphoid tissue. *J. clin. Pathol.* **36,** 167.

NAMIKAWA R., MIZUNO T., MATSUOKA H., FUKAMI H., UEDA R., ITOH G., MATSUYAMA M. & TAKAHASHI T (1986) Ontogenetic development of T and B cells and non-lymphoid cells in the white pulp of human spleen. *Immunology,* **57,** 61.

OLDING L.B. (1979) Interactions between maternal and fetal/neonatal lymphocytes. In: *Current Topics in Pathology* (eds E. Grundmann and W. H. Kirsten), Vol. 66, p. 83. Springer-Verlag, New York.

OMARY M.B., TROWBRIDGE I.S. & MINOWADA J. (1980) Human cell surface glycoprotein with unusual properties. *Nature (Lond.).* **286,** 888.

PIZZOLO G., SEMENZATO G., CHILOSI M., MORETTA L., AMBROSETTI A., WARNER M., BOFILL M. & JANOSSY G. (1984) Distribution and heterogeneity of cells detected by HNK-1 monoclonal antibody in blood and tissue in normal, reactive and neoplastic conditions. *Clin. exp. Immunol.* **57,** 195.

POPPEMA S., VISSER L. & DE LEY L. (1983) Reactivity of presumed anti-natural killer cell antibody Leu7 with intrafollicular T lymphocytes. *Clin. exp. Immunol.* **54,** 834.

ROSENTHAL P., RIMM I.J., UMIEL T., GRIFFIN J.D., OSATHANONDH R., SCHLOSSMAN S.F. & NADLER L.M. (1983) Ontogeny of human hematopoietic cells: analysis utilizing monoclonal antibodies. *J. Immunol.* **131,** 232.

RUBEN F.L., HANKINS W.A., ZEIGLER Z., NORDEN C.W., HARRISON A., WINKELSTEIN A. & HERRMANN D.J. (1984) Antibody responses to meningococcal polysaccharide vaccine in adults without a spleen. *Am. J. Med.* **76,** 115.

SCHWARTING R., STEIN H. & WANG C.Y. (1985) The monoclonal antibodies aS-HCL1 (aLeu14) and aS-HCL3 (aLeuM5) allow the diagnosis of hairy cell leukemia. *Blood,* **65,** 974.

STEWART S.J., FUJIMOTO J. & LEVY R. (1986) Human T lymphocytes and monocytes bear the same Leu3 (T4) antigen. *J. Immunol.* **136,** 3773.

SUTHERLAND R., PELIA D., SCHMEIDER C., KEMSHEAD R. & GREAVES M. (1981) Ubiquitous cell surface glycoprotein on tumor cells is proliferation associated receptor for transferrin. *Proc. natl. Acad. Sci. U.S.A.* **78,** 4515.

TIMENS W. & POPPEMA S (1985) Lymphocyte compartments in human spleen. An immunohistologic study in normal spleens and noninvolved spleens in Hodgkin's disease. *Am. J. Pathol.* **120,** 443.

TOIVANEN P., UKSILA J., LEINO A., LASSILA O., HIRVONEN T. & RUUSKANEN O. (1981) Development of mitogen responding T cells and natural killer cells in the human fetus. *Immunol. Rev.* **57,** 89.

WEIS J.J., TEDDER T.F. & FEARON D.T. (1984) Identification of a 145,000 Mr membrane protein as the C3d receptor (CR2) on human B lymphocytes. *Proc. natl. Acad. Sci. U.S.A.* **81,** 881.

WIGGLESWORTH J.S. (1984) Perinatal pathology. In: *Major Problems in Pathology* (ed. J. L. Bennington), Vol. 15, p. 28. W. B. Saunders, Eastbourne, U.K.

WOOD G.S., WARNER N.L. & WARNKE R.A. (1983) Anti-Leu3/T4 antibodies react with cells of monocyte/macrophage and Langerhans lineage. *J. Immunol.* **131,** 212.