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Kupffer cell numbers during human development

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SUMMARY

Immunohistological assessment of Kupffer cells was made using the antibody MAC387 and an antibody to lysozyme. Autopsy liver samples from 13 fetuses aged from 17 weeks gestation to term, and from 10 neonates and children aged 1 day to 18 months, were studied. For comparison, 10 normal adult autopsy liver specimens were included. The number of positively staining cells per unit area was counted for periportal sinusoids (zone 1) and centrilobular sinusoids (zone 3). No difference was found between zone 1 and zone 3 macrophage numbers with either antibody at any stage of development. Hepatic sinusoidal macrophage numbers were low during early gestation but increased during intra-uterine life to reach approximately normal adult values in the neonatal period. The numbers of cells staining with MAC387 or lysozyme were similar in each case except for hepatic sinusoidal macrophages in fetuses of less than 30 weeks gestation. Here anti-lysozyme stained significantly fewer cells, suggesting that lysozyme production may be low in immature fetuses. No difference was found between infants of similar maturity who had died immediately or had lived for more than 48 h and hence been exposed to gut antigens.

Keywords immunohistology Kupffer cells liver lysozyme macrophages

INTRODUCTION

Kupffer cells are macrophages lining the hepatic sinusoids which are important as phagocytic cells ingesting and degrading particulate and soluble material. In adults, hepatic macrophage phagocytic function is impaired in cirrhosis and may be a factor contributing to the increased incidence of acute bacterial infections in cirrhotics (Rimola *et al.*, 1984). Similarly, in patients with chronic liver disease, high titres of antibodies to gut-associated bacteria but not to non-gut-associated bacteria could be explained by failure of Kupffer cells to degrade antigens absorbed from the gut (Triger, Alp & Wright, 1972).

In intra-uterine life, by week 6 of development, the principal source of blood to the liver is through the left umbilical vein coming directly from the placenta and hence rich in nutrients. At birth, flow ceases through the umbilical vein, the ductus venosus closes and the adult circulation is adopted with the chief source of nutrients being from the gut through the portal vein. The gut is sterile during intra-uterine life; after birth, however, it receives a variety of dietary antigens and is also colonized by bacteria. Thus blood passing through the hepatic sinusoids after birth contains a selection of new antigens, making hepatic phagocytic and antigen-presenting mechanisms likely to be important in the neonatal period.

An immune response is not essential for normal fetal growth and development but becomes essential for survival after birth. Premature infants are, therefore, at increased risk of infection because of immaturity of many aspects of their defensive immune function. We investigated this subject by studying the number and distribution of hepatic macrophages in second and third trimester fetuses, in neonates and infants and in adults. We also compared those surviving for more than 48 h with those dying immediately after birth.

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MATERIALS AND METHODS

Clinical details

Liver tissue was obtained from fetuses or children aged under 2 years at routine post-mortem examination. The causes of death were spontaneous abortion (five), premature lung disease (five), birth asphyxia (five), infections (three), cardiac failure (three), bronchopulmonary dysplasia (one) and sudden infant death syndrome (one). Ages ranged from 17 weeks gestation (from last menstrual period) to 18 months after birth.

Liver tissue from 10 adults was examined for comparison. The causes of death were bronchopneumonia (three), myocardial infarction (three), bronchial asthma (one), acute heart failure (one), acute epilepsy (one) and bronchial carcinoma (one). Ages ranged from 31 to 87 years.

All subjects had no congenital abnormalities and full histological assessments of other organs were included.

Immunohistology

Liver tissue was formalin-fixed and dehydrated through alcohol to xylene, prior to embedding in paraffin wax. Sections (4 μm)

placed on poly-L-lysine-coated slides were dried and treated with 0·05% trypsin before blocking endogenous peroxidase using 1% hydrogen peroxide in methanol. Then the sections were stained by an avidin–biotin-conjugated peroxidase technique. Primary antisera were MAC387 (kindly donated by Dr D. Jones, Southampton; but available commercially from Dako), or rabbit anti-human lysozyme (Dako). Appropriate biotinylated secondary antiserum was applied followed by avidin–biotin-conjugated peroxidase complex and then visualization with diaminobenzidine. Sections were counter-stained with haematoxylin. Negative controls consisted of replicate sections handled identically except for omission of primary antisera. Haematoxylin-and-eosin-stained sections were also examined to confirm normal histological appearance.

Morphometry

For each section, counts of positively staining cells were made for perivenous and periportal regions of the lobule. Within sinusoids, only cells of spindle shape adherent to the wall were counted to exclude any circulating cells. Measurement of area was made using an eyepiece graticule with at least 2·0 mm^2 examined for each section. The area at the periphery of the block was ignored to avoid spurious edge staining. The areas counted were not deliberately selected and, hence, for practical purposes were random. The results were analysed statistically using the Wilcoxon rank sum test.

RESULTS

Histology

All of the cases had liver sections stained with haematoxylin and eosin to confirm that they had normal light microscopial appearance without any evidence of inflammation or hepatocyte damage. In the fetal group, extramedullary haemopoiesis was common with erythropoiesis most marked within hepatic lobules. Granulopoiesis occurred principally at the peripheries of the portal tracts and was less frequent than erythropoiesis. Occasionally megakaryocytes were also present. In the early post-natal period, extramedullary haemopoiesis was sparse and at later stages was entirely absent.

Immunohistology

The subjects were divided into three groups: those of age less than 30 weeks gestation (group A); those of age greater than 36 weeks gestation but less than 2 years (group B); and adults (group C). In group A there were 10 cases (median age 24 weeks gestation) and in group B there were 13 cases with median age $3\frac{1}{2}$ months. Table 1 gives the number of cells staining with both antibodies in each group.

First, the number of positively staining macrophages in the periportal part of the lobule was compared with the number in the perivenous part. There was no difference for either antibody in any group. With MAC387, there were fewer positive cells in group A than in group B ($P < 0·01$). For lysozyme, group A had

Table 1. Positively staining cells in hepatic sinusoids (values per mm^2)

		Group A		Group B		Group C		
Area	Antibody	Median	Range	Median	Range	Median	Range	Significance
Periportal	MAC387	40	6–91	96	25–234	46	16–205	A < B*
	Lysozyme	7·5	3–27	36	4–246	55·5	18–148	A < B, A < C*
Perivenous	MAC387	29	3–96	54	8–136	28·5	13–153	A < B*
	Lysozyme	6	2–9	23	3–207	47·5	13–105	A < B, A < C*

Group A < 30 weeks gestation; group B, > 36 weeks gestation, < 2 years old; group C, adult. *$P < 0·01$.

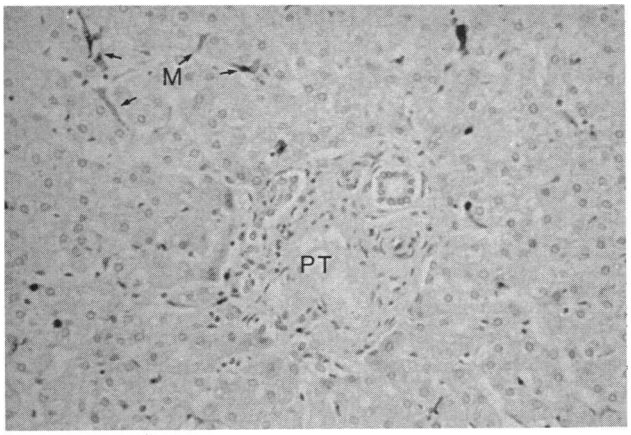

Fig. 1. Lysozyme staining of sinusoidal macrophages (M) in a liver from a child aged 10 weeks. PT, portal tract.

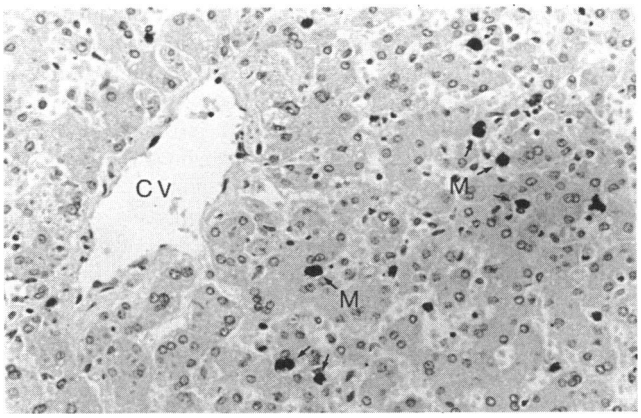

Fig. 2. Macrophage staining of the same liver with MAC387. CV, central vein.

fewer sinusoidal macrophages than had either group B ($P < 0.01$) or group C ($P < 0.01$) (Figs 1, 2).

In a comparison of fetuses and infants of gestational age 25 weeks to term, no significant difference in Kupffer cell number was found between those subjects who had lived for 48 h or more, and those who died within 48 h.

There was no significant difference between the numbers of cells stained with each antibody for groups B and C. However, for group A, MAC387 was found to stain significantly more cells than lysozyme ($P < 0.05$), whether the lobule was considered as a single entity or separated into zones 1 and 3.

DISCUSSION

This study demonstrates that there are fewer hepatic sinusoidal macrophages in foetuses of less than 30 weeks gestation (group A) than there are in infants (group B). Lysozyme-positive cells especially are reduced in group A, suggesting a functional immaturity in the macrophages. The number of macrophages in infants is similar to that in adults, although obviously similar cell density need not equate with similar functionl capability.

In fetal hepatic sinusoids, we observed a significant difference between the positive cell counts for the two antibodies. MAC387 labelled significantly more cells than did lysozyme ($P < 0.05$). MAC387 is an antibody which has been shown to react with L_1, a cytoplasmic protein present in most resting neutrophils and monocytes (Flavell, Jones & Wright, 1987; Brandtzaeg *et al.*, 1988). One possible explanation for this is that lysozyme levels are low during mid-gestation. Macrophage heterogeneity is also a possible explanation when different antibodies give contrasting counts. This has been demonstrated already in alcoholic liver disease using MAC387 and antibodies to lysozyme and α_1-anti-trypsin (Karakucuk, Dilly & Maxwell, 1989). However, in that study lysozyme consistently stained more sinusoidal cells than MAC387, further emphasizing how different our fetal findings are from the adult.

Granulocytes as well as macrophages stain with the antibodies used. This could conceivably lead to overestimating the macrophage numbers, particularly in fetal life when the liver supports extra-medullary haemopoiesis. However, it was not felt to be difficult to distinguish the poly-lobated nuclei of granulocytes from oval nuclei of macrophages. Also, our findings were of a reduction in macrophage numbers in the fetal group whereas this is the group which would be most susceptible to overestimation by inclusion of granulocytes.

The number of sinusoidal macrophages could be 'programmed' to increase during gestation to reach near-adult values just prior to delivery. Alternatively, the stimulus for increase in number may be the arrival of new antigens from the gut immediately after birth. In order to decide between these possibilities, we compared infants surviving for more than 48 h with those who died almost immediately. The subjects were of similar gestational ages. No significant differences in macrophage numbers was found. However, a survival time of 48 h may not be long enough for a difference to be shown, especially if the stimulus for increase is the influx of new antigens from the gut. Also, the infants who survived for 48 h or longer ultimately died in infancy and therefore may not have exhibited the same response to birth as healthy individuals.

Some work has suggested that in normal adult human liver (Mills & Scheuer, 1985) and in adult rat liver (Sleyster & Knock, 1982) there are higher numbers of macrophages in periportal sinusoids than in centrivenous zones. Karakucuk *et al.* (1989) found no significant difference between these areas in their adult surgical biopsy material and we also found no difference in fetal, infant or adult autopsy specimens.

Kupffer cells are capable of recognition and phagocytosis of a wide range of entities (e.g. bacteria, immune complexes) entering the liver from the portal circulation (Goresky, Huet & Villeneuve, 1982) and resistance to infection appears to be related to the functional activity of the Kupffer cells. Kupffer cells are also responsible for removing most, if not all of the endotoxin carried by portal venous blood (Jones, 1983), and activated Kupffer cells can respond to this type of stimulation by releasing enzymes and other tissue reactive factors. One of these is lysozyme, an enzyme continuously secreted by monocytes and macrophages in large amounts (Gordon, Todd & Cohn, 1974). In adults, raised lysozyme levels have been recorded during periods of increased resistance to infection (Cappuccino, Winston & Perri, 1964) when lysozyme's principal function is bacteriolysis, but it may also be involved in the enhancement of phagocytosis (Lasser, 1983). Our observation of a reduction in hepatic sinusoidal macrophage numbers and the suggestion of functional immaturity in fetuses aged less than 30 weeks gestation may be important factors increasing the premature infant's susceptibility to life-threatening infection and toxaemia.

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