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Inflammatory and reparative tissue reaction in developing human central nervous system

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Abstract. Morphologic features of inflammatory reactions in the immature central nervous system (CNS) develop in the second half of pregnancy. The cells composing the infiltrations arise early during development but their presence in circulation and final localization in fully mature inflammatory reactions is prolonged in time. The aim of this work was to compare the picture of inflammatory infiltrations in a group of fetal brains following various infections and aseptic injuries. It was found that numerous granulocytes appeared in bacterial infections, but not in aseptic lesions of the brain. The young maturing blood cells and granulocytes demonstrate the subsequent stages in the development of the inflammatory reaction. The changes depend on the character of the injurious factor and the level of maturation of the CNS. The topography of maturing brain lesions due to infections and anoxic/ischemic damage was similar and localized most often in the periventricular white matter.

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INTRODUCTION

The actual study was performed with the intent of analyzing the character of inflammatory reaction in human fetal and neonatal brains coinciding with tissue lesions and resulting often in scarring. The morphological picture of meningo-encephalitis observed in these types of cases allows observation of the differences in its presentation during the long-lasting process of CNS maturation (Dąmbska and Laure-Kamionowska 1998). The differences in responses of fetuses and small infants to infection and aseptic lesions have also to be analyzed in order to help the diagnosis of brain damage in children (Grether and Nelson 1997, Shida et al. 1996).

METHODS

Formalin fixed samples of the brain hemispheres (dissected at the frontal and parietal levels) cerebellum and brain stem were embedded in paraffin. Sections 8 μm thick were stained with cresyl-violet, haematoxylin-eosin or were used for immunohistochemistry.

Antibodies

For identification of astrocytes, the polyclonal rabbit antibody generated against glial fibrillary acidic protein (GFAP; Dako, Glostrup, Denmark) was used (dilution 1:500). Macrophages were demonstrated using monoclonal antibodies: mouse anti-human CD 68 and mouse anti-human macrophage. All of these, and the secondary antibodies biostinylated goat anti-rabbit IgG and goat anti-mouse IgG, were purchased from Dako, Glostrup, Denmark.

The three-stage immunohistochemical technique was applied. In brief, rehydrated tissue sections were treated for endogenous peroxidase (using 0.3% hydrogen peroxide in absolute methanol for 15 min) and incubated with non-immune goat serum for 30 min at room temperature (RT). Then, tissue sections were incubated overnight at 4°C with one of the primary antibodies to macrophage markers or GFAP. After washing in PBS (3x10 min) the goat anti-mouse or goat anti-rabbit immunoglobulin conjugated to biotin (secondary antibodies) was introduced for 45 min at RT. After further washing in PBS, StreptABComplex horseradish peroxidase conjugate (Dako) was applied for 45 min. Afterwards, tissue sections were washed again with PBS and horseradish peroxidase was developed using 3,3'diaminobenzidine (DAB) as the chromogen. Tissue sections were counterstained with Mayer's haematoxylin.

For negative controls, primary antibodies were replaced with an appropriate normal mouse or rabbit immunoglobulin fraction at matched protein concentration.

Table I

Clinical data	
Infections	
21 WG + 1 hour	Premature labor
31 WG + 12 days	Premature. Extremity and facial defects
30 WG	Premature
10 days	Toxoplasmosis. Hydrocephalus
14 days	Hydrocephalus
2 months	Perinatal asphyxia. Sepsis
3 months	Congenital hydrocephalus. Pneumonia
6 months	Thoraco-lumbal.Meningomyelocele. Meningitis
Anoxic-ischemic lesions	
27 WG	Immaturitas
33 WG	Pulmonary oedema
32-34 WG	Premature. Cerebral hemorrhagia susp. RDS III
16 days	Congenital heart defect
2.5 months	Congenital heart defect
4 months	Ileus. Hirschprung's disease
33 WG + 6 months	Pneumonia. Hyponatremia
9 months	Congenital heart defect

(WG) week of gestation

These were included for the examination of each specimen and consistently produced negative results.

RESULTS

Eight cases aged from 21 weeks of gestation (WG) up to 9 months post-natal constituted the group with infection (Table I). In three of them generalized sepsis with changes in the CNS were observed. In another two disseminated septic encephalonecrosis was found, and in three further cases toxoplasmosis with its destructive sequelae was observed. The youngest of the observed cases with generalized infection presented meningitis with macrophages accompanied by lymphocytes, some granulocytes, and monocytic cells with particularly large nuclei. The cases around 30 WG revealed in the periventricular white matter many small necrotic foci filled with macrophages surrounded by proliferating vessels and multiplication of micro- and macroglial cells (Fig. 1), or presented only the nodules composed of hypertrophied microglial and glial cells in the form of young, still immature elements (Fig. 2). In the oldest of these cases some lymphocytes and granulocytes were seen around intraparenchymal vessels and macrophagic infiltrations while meninges contained few lymphocytes.

Generalized proliferation of young astrocytes with only minimal fibrillary changes was observed in the white matter. In cases 0-2 months old with generalized sepsis similar changes were observed, but progression in the maturation particularly of astroglial cells was noted. The cells were larger and fibrillary changes were often seen. Meningeal infiltrations were more abundant than in the youngest cases including macrophages, lymphocytes, many leukocytes and some large cells corresponding to elements of hematopoiesis foci. In the oldest cases diagnosed as purulent meningoencephalitis, including one with a small abscess in the parietal region, the type of infiltrations became similar to those observed in adults. The macrophagic compound was smaller and leukocytes often dominated (Fig. 3). The cases with toxoplasmosis in full-term newborns and older cases presented similar topography including parenchymal changes and cellular infiltrations; however, the appearance of plasmatocytic compounds in full-term newborns has to be mentioned. The prolonged

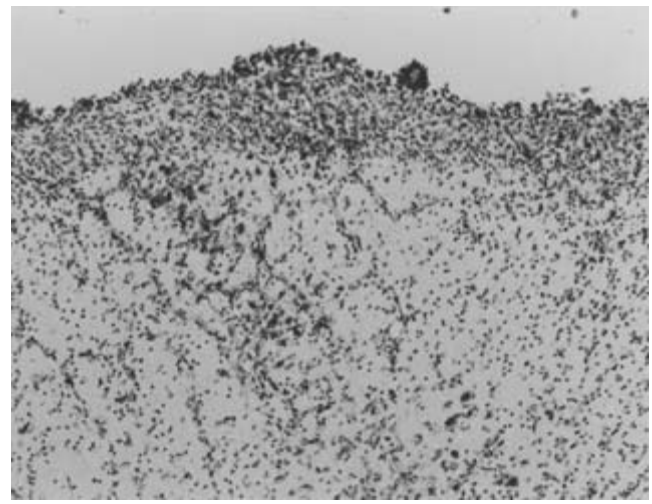

Fig. 2. Microcellular nodule in white matter, GFAP. Magnification, × 200.

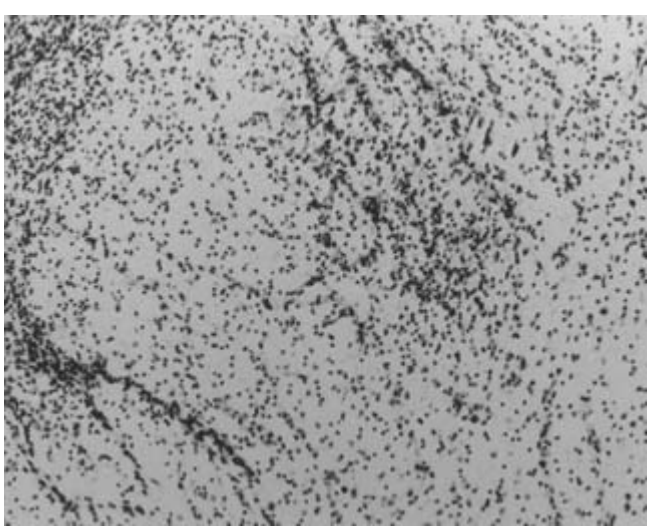

Fig. 1. Astroglial cells within small cellular nodules, GFAP. Magnification, × 200.

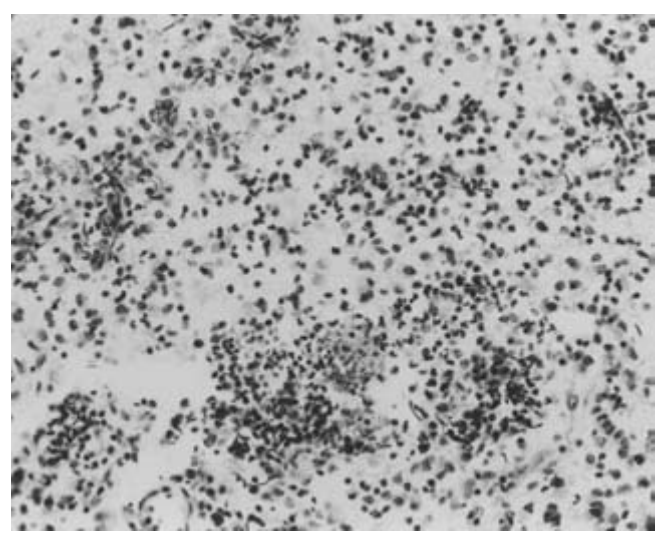

Fig. 3. Microabscess in periventricular white matter, HE. Magnification, × 200.

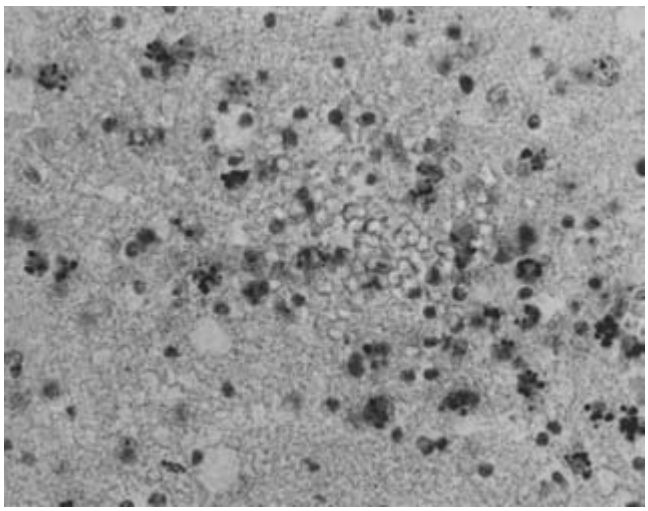

Fig. 4. Hypertrophied astrocytes around microabscesses, HE. Magnification, × 100.

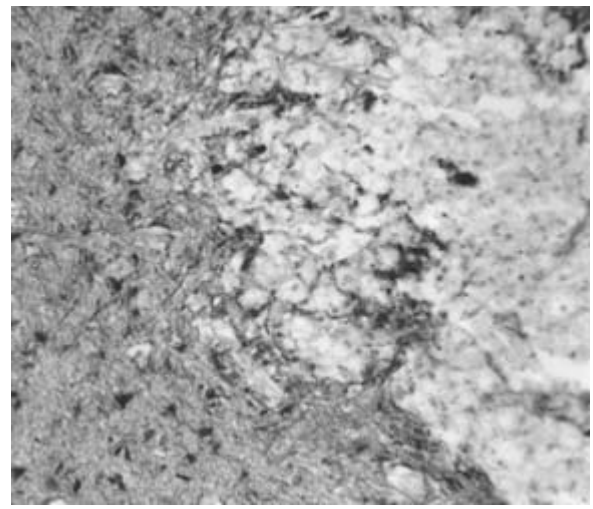

Fig. 6. Small glial reaction around the focal microcystic lesion, GFAP. Magnification, × 200.

duration of infection in one newborn with toxoplasmosis resulted in the destruction of brain hemispheres with proliferation of mesodermal tissue and the development of fibrillary astroglial changes (Fig. 4).

The group of aseptic anoxic-ischemic lesions includes eight cases age-related to cases with generalized infection. This is a unique opportunity to examine the possible time-course of pathological processes in the CNS, particularly beginning prenataly when a pathological incident was not observed. The youngest 27-33 WG presented periventricular changes leading to necrotic lesions with formation of small cysts (Fig. 5). In older, mature newborns with a longer duration of the pathologic process the foci of leukomalacia were seen at various stages of tissue disintegration and organization. Macrophages were present in all younger and older cases within necrotic changes. Astrocytic proliferation was the second compound of cellular reaction within just-forming necrotic changes and around more advanced disintegration of tissue. In the youngest of the observed cases proliferating astrocytes were rather of protoplasmic type (Fig. 6). In newborns and older cases fibrillary gliosis was more evident, leading in several months or years to formation of thick glial scars. The multitemporal anoxemic necroses observed in our material did not reveal perivascular infiltrations around the necrotic changes even in a nine-months-old case. In younger cases the topography of anoxic lesions was most evident in the periventricular white matter, whereas in older cases the changes were more disseminated, also including lesions of gray structures. The review of lesions described according to neuropathological evaluation is summarized in Table II. The changes were divided into four morphological types and were estimated according to their intensity.

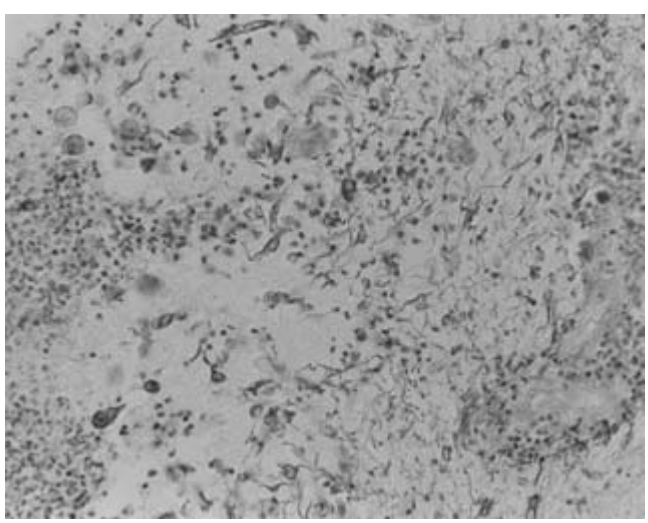

Fig. 5. Recent focal leukomalacia in white matter, cresyl-violet. Magnification, × 200.

DISCUSSION

The morphological picture of inflammatory reaction in fetal and neonatal brain, followed by the destruction and scarring of tissue lesions, presents some particularities observed for many years (Dąmbska 1968, Dąmbska and Laure-Kamionowska 1998). The morphological picture changes during maturation of the brain and for-

Table II

Review of neuropathologic changes

		Focal necrosis	Macrophagic-miroglial proliferation	Lympho-granulocytic infiltrations			Astroglia	
				Focal	P	M	GR	Scarring
Infection	prenatal	+	+++	++	++	+	++	+/-
	postnatal	++-+++	++	+++	++	++	++	+++
Anoxic lesions	prenatal	++	+++	+	-	+/-	++	+
	postnatal	+++	++	++	+/-	+	+	+++

(P) perivascular; (M) meningeal; (GR) generalizated reaction; (+/-) minimal changes; (+) small changes; (++) moderate changes; (+++) advanced changes

mation of fetal immune response. The changes are often very large, in many cases confirming that fetal inflammatory response contributes to neonatal brain injury and later developmental disability (Dammann and Leviton 2000). They may result in clinical syndromes observed relatively often in infants who survive fetal inflammatory lesions (Yoon et al. 2000).

Analyzing our cases of infections and anoxic--ischemic lesions we have observed that in a majority of these cases the topography of lesions is similar. In both groups the area most severely damaged is the periventricular white matter. This localization of the majority of fetal and neonatal brain lesions is attributed to the particular susceptibility of tissue with immature vascularization and blood-brain barrier, in the older among them also to intensive metabolic activity during the pre-myelination and myelination periods (Shida et al. 1996). Only in the few oldest cases from both groups does the topography of lesions look more similar to that observed in the adult brain.

In periventricular focal lesions in both the anoxic--ischemic and the infection groups, the reaction of microglia and the appearance of macrophages arising even in the youngest cases are most intensive. The abundance of infiltration seems to be dependent particularly on age and the degree of lesions. The number of macrophages seems to diminish with age when other cells start to participate in infiltrations. The question of origin of all macrophages within focal lesions is still open; the meningeal and perivascular ones may be considered as coming from circulating blood and those disseminated around necroses suggest microglial proliferation (Dąmbska and Wiśniewski 1999). It is worth mentioning that moderate but generalized activation of microglial cells is seen in the majority of both infected and ischemic cases.

Other cells characterizing the inflammatory reaction also are seen in our cases. It is worth remembering that hemopoietic activity starts very early during fetal development, during the second month of gestation. Particular types of cells appear consecutively in blood circulation, but their number increases in time. There are only a few lymphocytes in the blood during 8 WG and they are abundant in the second trimester of pregnancy. Among them maturation of lymphocytes T responsible for cellular reaction in pathologic conditions seems to be parallel with maturation of lymphocytes B, which are active in humoral reaction. Nevertheless they may change into plasmatic cells producing antibodies not earlier than around birth (Gajewska 1986). Granulocytes appear in blood after 10 WG and multiply during the third trimester. By this time the immature large cells with large nuclei are seen among them.

How do the pictures of inflammatory reaction in our cases reflect this history of blood cells' maturation? In cases with infection all cells characteristic for inflammatory reaction were present. Young large cells were seen in very early arising fetal meningitis. Lymphocytes and consecutively granulocytes became more abundant in the older cases. Only in one case with generalized infection were the granulocytes seen very early. In the young cases presenting the picture of *encephalonecrosis septica dispersa* (Głuszcz 1961, unpublished doctoral thesis) in the disseminated nodules, the macrophages dominated rather than the lymphocytic cells. In a 6-month-old case the presence of granulocytes was so

abundant that it was possible to recognize the micro-abscesses. Taking into account that in our material it was impossible to verify the time of infection, the structure of changes in younger and older cases permits only the observation of the intensity of reaction as more abundant in the older case (Dąmbska 1968). Plasmatocytes appearing in cases with toxoplasmosis around birth also confirm the manner of maturation of the inflammatory reaction. Those observations are in agreement with the previous findings of Norman and coauthors (1995) that inflammatory reactions develop in the CNS in the second half of pregnancy (Marshall-Clarke et al. 2000). The abundance of cells, particularly granulocytes, in infiltrations may even help to diagnose the character of changes.

In non-infected cases the primary reaction expressed by the multiplication of macrophages within periventricular changes and in meninges was dominant, and some microglial cells around necrotic foci were seen. The blood vessels even when proliferating were not surrounded by infiltration; this finding was observed not only in fetuses but even in several month old infants. This reminds us that inflammatory reaction is influenced by, among other things, the character of the primary damaging factor.

The last reaction to be discussed was that of astroglia. Astrocytes seen in the CNS since 15 WG (Roessman and Gambetti 1986) were multiplied and hypertrophied in both aseptic and infected cases when their multifunctional role developed. Around focal disintegration of nerve tissue and the formation of cystic changes the intensity of GFAP-positive large cells was similar to protoplasmic type of astrocytes. Their proliferation was seen as even more generalized in brain hemispheres. In older cases with longer duration of lesions the fibrillary changes of astroglia increased with time.

CONCLUSION

In the summarized observations presented above, we can say that the picture of inflammatory reactions in the developing CNS reflects the immune system maturation, the character of the damaging factor, its intensity, and the maturation of the nervous tissue.

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