MORPHOLOGICAL ASPECTS OF MIGRATION OF LYMPHOCYTES WITH BROAD CYTOPLASM THROUGH THE EPENDYMAL CELL OF THE MAMMALIAN VASCULAR PLEXUS

V. A. Shakhlamov and E. G. Bitner

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KEY WORDS: brain; vascular plexus; ependymal cells; lymphocytes

There have been many morphological studies of the vascular plexus of the brain, dealing with different aspects of its organization and function [1, 3-5, 8, 9, 11]. Permeability of the blood-CSF barrier for substrates and cells contained in the plasma is a subject of particular interest. Whereas the mechanisms and pathways of transport for noncellular components of the plasma and CSF are mainly known, the pathways along which the blood cells pass from the blood stream into the CSF and vice versa have not yet been adequately studied [3, 6, 11].

Under normal conditions the cerebrospinal fluid has the quantitative and qualitative characteristics of the blood cells, lymphocytes, monocytes, plasma cells, macrophages, neutrophils, and eosinophils present in it [6]. The leading component of the blood-CSF barrier, responsible for selectivity of the processes of permeability, CSF formation, and migration of white blood cells, is undoubtedly the ependymal cells with tight junctions in their apical part [3, 8-11].

In general two main methods of penetration of white blood cells from the blood stream into the tissues are known: penetration along intercellular spaces and through the cell. Several stages can be distinguished in this process on the basis of the morphological picture of interaction between migrating cell and endotheliocyte [7]. In publications dealing with migration of white blood cells through a layer of epithelium (epithelium of the intestine and other organs [2]), mention is made of the intercellular pathway, but nothing is said about the method of migration through the ependymal cell in the accessible literature. Accordingly, we have attempted to study this process by light-optical and electron-microscopic methods, taking as our example the epithelium of the vascular plexus of the lateral ventricles of the mammalian brain.

EXPERIMENTAL METHOD

Mature cows and goats weighing 500-600 and 30-40 kg respectively, 16 of each, were used as the experimental objects. The vascular plexuses of the lateral ventricles of the brain were removed from the animals in the abattoir within 5-7 min of decapitation, and fixed in 2.5% glutaraldehyde solution in phosphate buffer, pH 7.3, for 2 h, followed by rinsing in phosphate buffer with the same pH for 2 h and postfixation in 1% osmium tetroxide solution in phosphate buffer, pH 7.3. The material was dehydrated in alcohols of increasing concentration and embedded in Epon–Araldite mixture. Semithin and ultrathin sections were cut on an LKB Ultrotome (Sweden). The semithin sections were stained by a combined method (methylene blue – azure II – fuchsine). Ultrathin sections were stained with uranyl acetate and lead citrate. The specimens were examined and photographed on a light microscope and LEM 100 CX II electron microscope (from "JEOL," Japan), with accelerating voltage of 80 kV.

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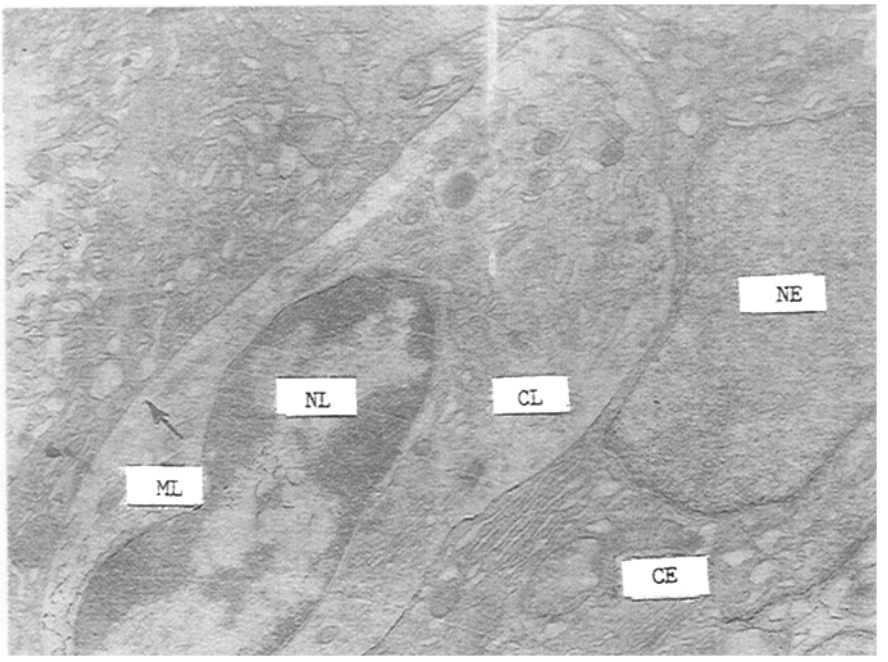

Fig. 1. Ultrastructure of lymphocyte with broad cytoplasm, migrating through cytoplasm of ependymal cell. CE) Cytoplasm of ependymal cell, NE) nucleus of ependymal cell, CL) cytoplasm of lymphocyte, NL) nucleus of lymphocyte, ML) membrane of lymphocyte (arrow). 18,500×.

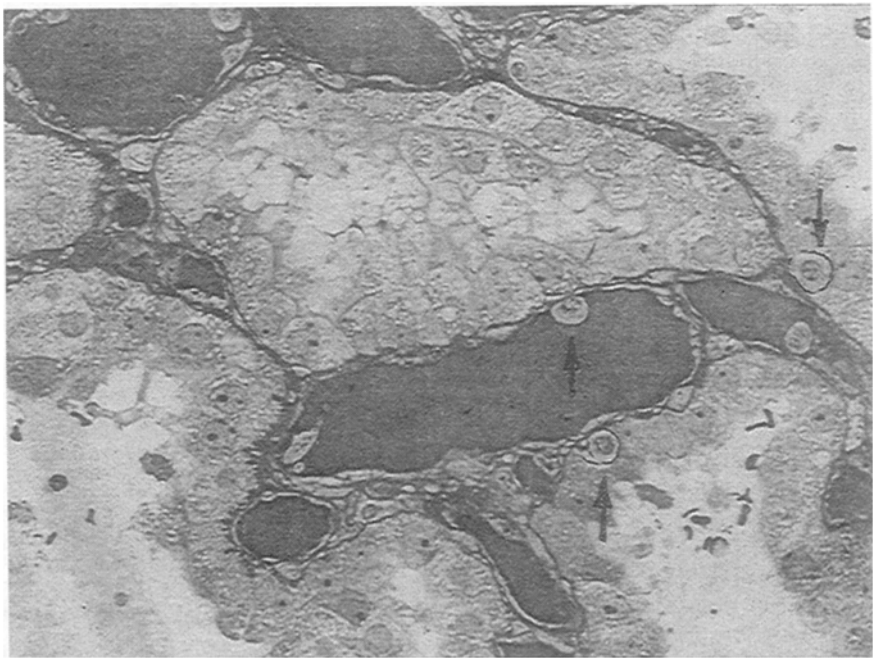

Fig. 2. Venous part of microcirculatory bed of a cow's vascular plexus. Lumen of vessel near endothelial cell contains lymphocyte with wide cytoplasm (phase 1, see diagram in Fig. 3) – indicated by arrow. 390×.

EXPERIMENTAL RESULTS

In semithin sections the layer of ependymal cells consisted of cubical and cylindrical ependymocytes living on a basement membrane, beneath which were located vessels of the microcirculatory bed in a connective-tissue stroma.

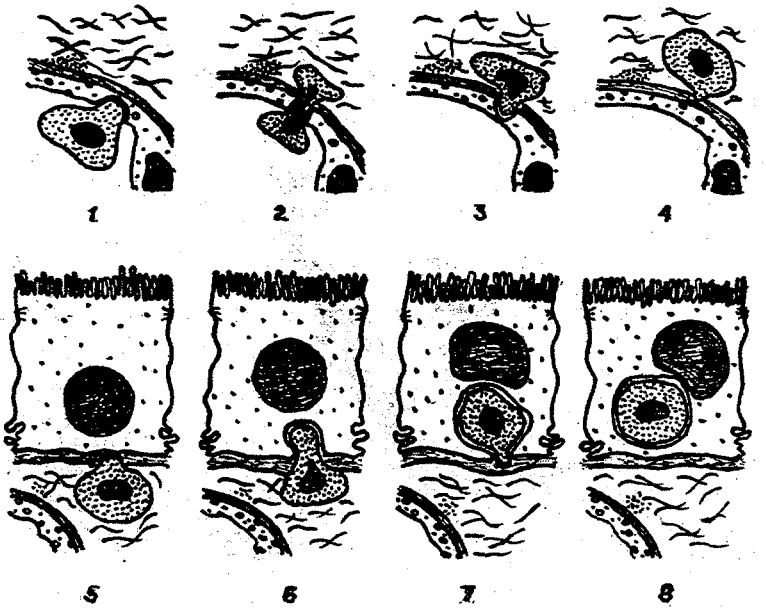

Fig. 3. Diagram of phases of development of emperipolesis (from 1 to 8) of lymphocyte with wide cytoplasm and ependymal cell.

The ependymal cells in ultrathin sections had their typical structure. Their shape and size varied depending on their location on the villi. The apical part, facing the lumen of the ventricles, had microvilli and a small number (5-10) of cilia. The basal part of the plasmalemma was smooth and its outlines repeated the bends and invaginations of the basal layer. The nuclei lay in the basal zone and had 1-4 nucleoli. The karyoplasm in a homogeneous matrix contained finely granular heterochromatin, with varied electron density and granules of different sizes. Lysosomes were found in the cells, as homogeneous, uniform and also multivesicular bodies. The Golgi lamellar complex was located mainly in the perinuclear zone and consisted of a system of flattened cisterns and vesicles. The rough and smooth cytoplasmic reticula were distributed throughout the cytoplasm, but mainly in the upper two-thirds of the cell. The cytoplasm contained many free monosymes and polysomes. The mitochondria were oval or elongated in shape, and distributed mainly in the lower two-thirds of the cell. The basal ependymal layer varied in thickness throughout its length and could often follow a zigzag course, both toward the ependymal cells and toward the connective-tissue stroma.

In semithin and ultrathin sections, cells with pale cytoplasm similar in the ultrastructure of their organelles and nucleus to lymphocytes with wide cytoplasm (Fig. 1), and located inside the ependymal cells (emperipolesis) were found in the layer of ependymal cells. These lymphocytes with wide cytoplasm were located mainly in the center of the ependymal cells.

In serial sections of different thicknesses, we were able to trace the time course of migration of the cytoplasm and nucleus of these lymphocytes with wide cytoplasm into ependymal cells of the vascular plexus, and consequently, the migration of these cells from the wall of blood vessels of the microcirculatory bed to ependymal cells and into their cytoplasm. Once inside an ependymal cell the cytoplasm of such a lymphocyte is separated from the cytoplasm of the ependymal cell by two membranes, i.e., its own plasmalemma and the plasmalemma of the ependymal cell (Fig. 1). No morphological features of injury to the ependymal cells containing these lymphocytes with wide cytoplasm could be found.

We were thus able to demonstrate the successive stages of migration of lymphocytes with broad cytoplasm from the vascular bed through an endothelial cell (Fig. 2), the basal layer, the connective-tissue stroma, the basement membrane of the ependymal layer, and into the ependymal cells themselves (Fig. 3).

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MODULAR ORGANIZATION OF THE RAT HYPOTHALAMUS

V. B. Pisarev and V. P. Tumanov

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Some ideas on the modular organization of the nervous system have now been formulated. This is shown by the rapidly increasing volume of information devoted to functional and morphological aspects of this problem. An extensive survey of the literature, contained in a monograph by a group of authors [1], indicates the penetrating nature of morphological investigations of neuronal assemblies (modules) in the cerebral cortex both of animals and of man. However, a matter of special interest in the complex hierarchy (cortex – subcortex – brain stem) of brain structures is the diencephalic region, which plays a leading role in the development of psychoemotional stress, which is a generator of many pathological states. No data on modules of the hypothalamus could be found in the literature. Accordingly, it was decided to undertake a comprehensive neuromorphological study of the rat hypothalamus, with the aim of detecting modular systems and their role in the development of the stress reaction.

EXPERIMENTAL METHOD

The experimental study was carried out on noninbred male albino rats (24 animals) weighing initially 180-200 g. Emotional stress was produced by immobilizing the rats once or repeatedly by their limbs in the supine position for 3-4 h. In the case of repeated immobilization, the animals were fixed after 1-2 days. The mice were anesthetized with ether and killed after 1, 7, 20, 30, 45, and 60 days of the experiment. Intact rats (six) constituted the control group. The hypothalamic region was fixed in Bouin's fluid, 10% neutral formalin solution, and a 4% solution of paraform ("Fluka," Switzerland). made up in 0.1 M cacodylate buffer. Ultrathin sections were cut on an ultramicrotome, stained, and examined in ÉMV-100 LM and PÉM-100 electron microscopes.

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