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IMMUNOHISTOCHEMICAL INVESTIGATION OF CYTOARCHITECTONICS T-AND B-CELLULAR COMPARTMENTS OF THE SPLEEN

Key words: *spleen, immunohistochemistry, compartments of T- and B- leucocytes.*

Abstract. *Specific characteristics of cytoarchitectonics T-and B-cellular compartments of the rat spleen white pulp in health have been revealed by the methods of immunohistochemistry. Serial paraffin sections 5 mcm thickness of the rat spleen, fixed in formalin, were stained with immunohistochemically monoclonal antibodies against CD1a, CD3, CD4, CD8, CD20, CD23, as well as on myeloperoxidase in order to detect neutrophils and eosinophiles. It has been established that different populations of leucocytes have its definitely evident specific localization in multichamber spleen parenchyma.*

Introduction

At present immunophenotyping of leukocytes submultitudes is carried out by two main methods: flow cytometry and immunohistochemically (IHCh).

IHCh advantage is that the objects of investigation can be analyzed retrospectively, since it is possible to revert to the material, fixed in formalin and embedded in paraffin, and also to the completion of a definite part of the research.

It is especially important, as historically IHCh investigations were carried out only on frozen sections on which preservation of antigenic epitopes significantly suffered (K.J. Randall, Y. Pearse, 2008).

The purpose of the research: to reveal the specific characteristics of cytoarchitectonics T-and B-cellular compartments of the rat white pulp.

Material and Methods

The research has been carried out on six sexually mature rats. Serial paraffin spleen sections 5 mcm of thickness, fixed in formalin, were stained by immunohistochemically monoclonal antibodies against CD1a, CD3, CD4, CD8, CD20, CD23 as well as on myeloperoxidase in order to detect neutrophils and eosinophiles.

Decamouflage was carried out in buffer solution pH9,0 in apparatus PT LINK DAKO during 20 minutes at temperature +97C. The arrangement of the reaction was conducted in Autostainer Link 48DAKO using visualization system En vision Tlex+Mouse (Link).

Tincturing was in En Vision Flex Hematoxylin.

Dehydration was conducted in three ethanol changes and lucid interval in O-xylene. There was embedding into tegumental medium MOUNT-

QUICK. A part of sections were stained with hematoxyline and eosine for control.

The results of the research

White pulp of the mammals' spleen is subdivided into periarterial and lymphoid sheath, lymphoid nodes and marginal zone.

Clearly defined, when stained with hematoxyline and eosin, or hematoxyline and azure-II-eosin, portions of the white pulp - T and B - dependent zones, mantle zone (crown), as well as marginal sinus and marginal zone, are peculiar for the rat spleen in contrast to the spleen of man and other mammals.

On CD1a sections positive cells with intensive reaction antigen-antibody, localized on membranes and in cytoplasm, have been revealed in subcapsular zone of the spleen about periphery of the organ. They were presented by large cells of round, oval and polygonal forms with eccentrically situated nuclei having numerous ramified appendixes on membrane, so-called "dendritic cells".

Overwhelming majority of the cells, forming periarterial lymphoid sheath showed positive reaction to CD3, high density of which was observed along the inner side of PALS nearer to the wall of the central artery (fig. 1).

Detection of CD+cells using antibodies MoAb(SP35) showed, that all T-helpers with the evident reaction antigen-antibody on membranes were located along the outer side of marginal zone bordering with red pulp (fig. 2).

CD8 + (marker of T-killers), revealed by means of MoAb(SP16) antibodies, were observed in the form of single clusters in the red pulp (fig. 3). Antigen-antibody reaction, localizing both on

membranes and in cytoplasm, showed high intensity of cells' staining from brown to black. Particularly high intensity of CD8+ cells reaction was observed along venous sinuses of the red pulp, making cruciate plexuses, so-called "crisscross vessels".

When carrying out reaction using antibodies - MOAb(SP32) on Cd20+ a distinctly marked reaction antigen-antibody in the form of intensive staining of

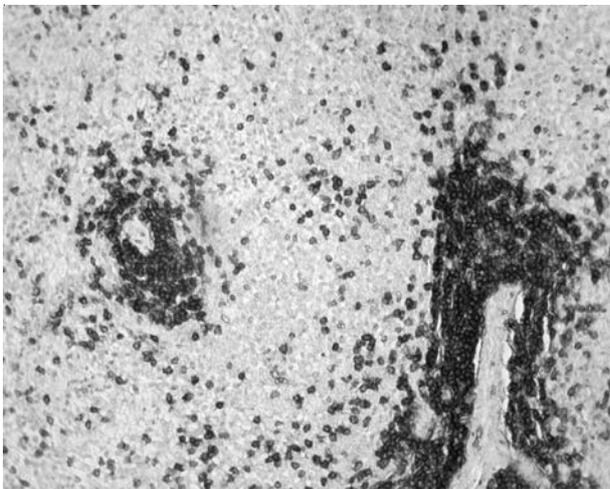


Fig.1. Rat spleen. IHCh. CD3+ lymphocytes in PALS. Vol.20, c.10.

mature B-lymphocytes membranes in a large quantity with high volume density filling B-zone and marginal zone of the lymphoid nodes has been detected. CD23+ follicular dendrite cells of germinal centers, revealed by means of antibodies MoAb(SP23), were observed in the red pulp only in one case as a single cluster of a spherical shape in the form of aggregate in the centre of forming lymphoid node.

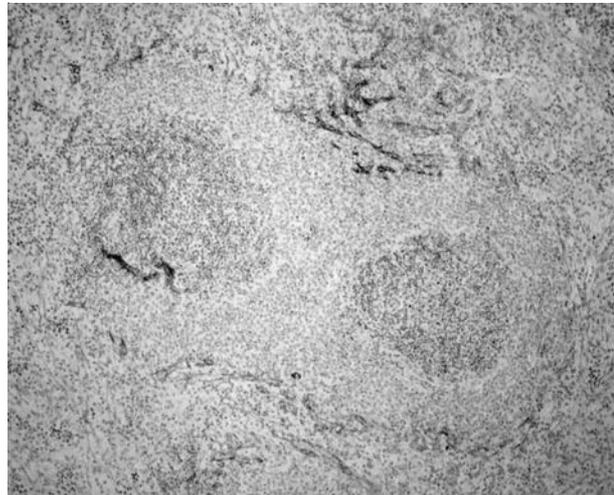


Fig.2. Rat spleen. IHCh. CD4+ lymphocytes (T-helpers) in marginal zone of the lymphoid nodes. Vol.10,c.10.

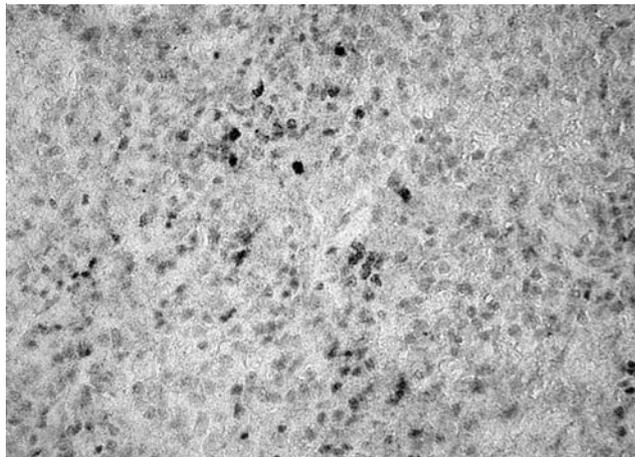


Fig.3. Rat spleen. IHCh. CD8+ lymphocytes (T-helpers) in the red pulp. Vol.20, c.10.

Reaction of MoAb (SP72) antibodies to myeloperoxidase, staining neutrophils and eosinophiles discovered their presence in a significant quantity in peripheral blood of the vessels' lumens, whereas in parenchyma only single neutrophils and eosinophiles, intensively stained from dark-brown to black colour, were revealed between lymphoid nodes of the spleen.

Thus, various populations of lymphocytes have its definitely marked specific localization in multichamber spleen parenchyma.

Perspectives of further investigations

Using data in immunoarchitectonics of different populations of lymphocytes in multichamber spleen parenchyma in health, as the basic ones, inves-

tigations of immunoarchitectonics and, later on, reactions of different populations T- and B-cellular compartments of the spleen in experiment and pathology are planned.

Literature. K. Randall, G. Pearse. A Dual-label Technique for the Immunohistochemical Demonstration of T-lymphocyte Subsets in Formalin-fixed, Paraffin-Embedded Rat Lymphoid Tissue//Toxicolog Pathology,36:795-804,2008

ИММУНОГИСТОХИМИЧЕСКОЕ ИССЛЕДОВАНИЕ ЦИТОАРХИТЕКТониКИ Т- И В- КЛЕТочНЫХ КОМПАРТМЕНТОВ СЕЛЕЗЕНКИ

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Резюме. Методами иммуногистохимии выявлены особенности цитоархитектоники Т- и В- клеточных компартментов белой пульпы селезенки крысы в норме. Серийные парафиновые срезы толщиной 5 мкм

фіксованої формаліном селезенки окрашивались иммуногистохимически моноклональными антителами против CD1a, CD3, CD4, CD8, CD20 CD23, а также на миелопероксидазу для выявления нейтрофилов и эозинофилов. Установлено, что различные популяции лимфоцитов имеют четко выраженную свою специфическую локализацию в многокамерной паренхиме селезенки.

Ключевые слова: селезенка, иммуногистохимия, компартменты Т- и В- лейкоцитов.

**ІМУНОГІСТОХІМІЧНЕ ДОСЛІДЖЕННЯ
ЦИТОАРХІТЕКТОНІКИ Т- ТА В- КЛІТКОВИНИХ
КОМПАРТМЕНТІВ СЕЛЕЗІНКИ**

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Резюме. Методами імуногістохімії виявлені особливості цитоархітектоніки Т- та В- клітковинних компартментів білої

пульпи селезінки криси в нормі.

Серійні парафінові зрізи товщиною 5 мкм фіксованої формаліном селезінки зафарбовувалися імуногістохімічними моноклональними антитілами проти CD1a, CD3, CD4, CD8, CD20, CD23, а також на міелопероксидазу для виявлення нейтрофілів і еозинофілів. Встановлено, що різні популяції лімфоцитів мають чітко виражену свою специфічну локалізацію в багатокамерній паренхімі селезінки.

Ключові слова: селезінка, імуногістохімія, компартменти, Т- і В- лейкоцити.

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