Immunology Letters 168 (2015) 325–328

Contents lists available at ScienceDirect

Immunology Letters

journal homepage: www.elsevier.com/locate/immlet

Review

The discovery of the blood–thymus barrier

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ARTICLE INFO

Article history:
Received 2 October 2015
Received in revised form 16 October 2015
Accepted 23 October 2015
Available online 29 October 2015

Keywords:
Blood–thymus barrier
History of immunology
Thymus
Vascular permeability

ABSTRACT

The blood–thymus barrier is a functional and selective barrier separating T-lymphocytes from blood and cortical capillaries in the cortex of the thymus. The existence of this barrier was proposed for the first in time in 1961 by Marshall and White, and demonstrated in 1963 by Clark and Weiss. The most clear morphological evidence concerning the existence of the blood–thymus barrier may be attributed to the collaborative work published in 1972 by two scientists, Morris Karnovsky and Elio Raviola. Raviola and Karnovsky, using peroxidase as a permeability tracer, demonstrated that the venules at the cortico-medullary junction are the site of leakage for blood antigens, while the capillaries draining the cortex are largely impermeable. Other permeability studies have confirmed the existence of a blood–thymus barrier, which allow the access to low molecular weight tracers, while most exclude high molecular weight particles.

Contents

1. Introduction

Common examples for barriers are the blood–brain, the blood–placenta-, the blood–retina-, the blood–testis- and the blood–thymus-barrier. The barriers have a defined anatomic substrate: for the blood–brain-, the inner blood–retina and the blood–thymus-barrier it is the endothelium, for the blood–placenta-, the outer blood–retina-, the blood–testis- and the blood–thymus-barrier these are epithelial cells near to the capillary wall. Epithelia with barrier-function have dense intercellular junctions and few pinocytotic vesicles, and express many transporters for the selective transport and for the exchange of molecules.

The existence of a blood–thymus barrier was proposed for the first in time in 1961 by Marshall and White [1], and demonstrated morphologically by Clark [2] and Weiss [3]. Weiss studied the thymus of newborn and young adult mice by means of electron microscope. He demonstrated that in the thymus cortex a cellular pathway from the lumen outside the capillaries was established by means of cytoplasmic processes of the endothelium and the adventitial reticular cells. Moreover, after intravenous injection of thorium dioxide, the tracer was found in the vessel wall, and, to a limited degree, in the surrounding tissue. Weiss concluded that: "These vessels resemble both the fine vessels in the central nervous system where a blood–brain barrier is present, and the terminal arterial vessels in the spleen" [3].

The most clear morphological evidence concerning the existence of the blood–thymus barrier may be attributed to the collaborative work of two scientists, Morris Karnovsky and Elio Raviola (Figs. 1 and 2). Currently, Morris Karnovsky is "Shattuck Professor of Pathological Anatomy", Emeritus, and Elio Raviola, "Bullard Professor of Neurobiology", Emeritus, both at the "Harvard Medical School", Boston, USA. Other areas of interest of Karnovsky are the oxidative metabolism of activated leucocytes and osteoclasts and lipid domains in cell membranes, and the reaction of blood vessels to injury and in transplants. The main focus of Ravi-

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http://dx.doi.org/10.1016/j.imlet.2015.10.014
0165-2478/

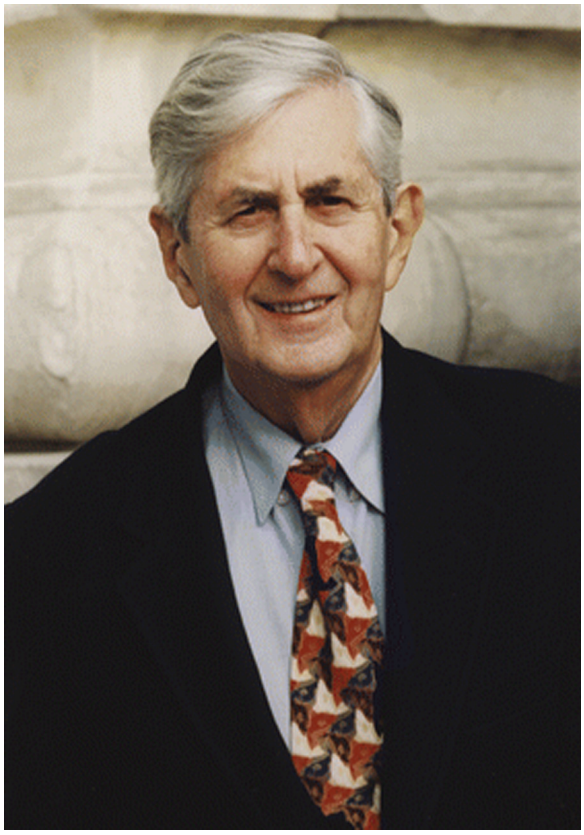

Fig. 1. A portrait of Morris Karnovsky.

Fig. 2. A portrait of Elio Raviola (on the left).

ola's laboratory is the biology of the eye, including research into the role of visual experience in postnatal eye development and studies of how the retina of mammals analyzes the visual world and encodes information about it for sending to the brain.

The blood–thymus barrier is a functional and selective barrier separating T-lymphocytes from blood and cortical capillaries. In the cortex, capillaries, which are rarely fenestrated, form the barrier together with perivascular lymphocytes, macrophages, and reticular epithelia cells. Barrier is complete in most of the cortex, where restricts access of circulating antigens to developing cortical lymphocytes. Otherwise, in iuxtamedullary cortex, around cortical venules, and in the medulla, barrier is not complete, allowing macromolecules and circulating antigens to penetrate from blood into thymic parenchyma. Barrier prevents antigens circulating in bloodstream from reaching thymic cortex where T-lymphocytes are formed [4].

2. Horseradish peroxidase as a marker of vascular permeability

Horseradish peroxidase (HRP) is a glycoprotein with a molecular weight of 40,000, which can be demonstrated at both light and electron microscopic level by cytochemical reactions. HRP injected intravenously into mice passed freely out of the capillaries in cardiac and skeletal muscle [5]. Since the introduction of the HRP as tracer, the number of hemoproteins used as enzymatic probes has been largely augmented towards both lower and higher molecular weight; the spectrum ranging from 1500 Da (heme-octapeptide) to 240,000 Da (catalase).

One of the most important contributions of Morris Karnovsky was the extension of HRP tracer method to both light and electron microscopic level, by introducing diaminobenzidine (DABI) as an electron donor. HRP oxides DAB in the presence of H_2O_2 and converts it into an insoluble polymer, which is detectable by light and electron microscope. When HRP is injected into the bloodstream, its pathways can be followed with DAB reaction.

As Karnovsky wrote in an his autobiographic paper: "Strauss [6] had used HRP to study endocytic electron uptake at the light microscopic level, but benzidine, the electron donor in the peroxidase reaction used at that time to reveal the site of the enzyme, did not yield a sufficiently electron–opaque reaction product suitable for electron microscopy. (. . .) I thought that a suitable substrate (electron donor) could be formulated, one that would yield an insoluble reaction product that would reduce osmium tetroxide and bind the reduced osmium. Modified benzidine, with additional amino groups, seemed to be a suitable candidate, 3,3′-diaminobenzidine (DAB) was the answer. (. . .) In the DAB-peroxidase reaction, HRP oxidizes DAB in the presence of H_2O_2 and converts it into an insoluble brown polymer; this polymer causes reduction of added osmium tetroxide, and the reduced osmium forms an insoluble electron–opaque precipitate at the site of the HRP. Thus, when HRP is injected into the bloodstream, the pathways that it takes to reach the tissues can be followed by fixing tissues at various times after injection and by performing the DAB reaction. Furthermore, HRP can be cross-linked to antibodies and other proteins for localization studies in vivo and in vitro [7]."

In the first paper introducing this technique, Karnovsky studied the passage of HRP through the glomerulus in the urine [8]. In 1967, Reese and Karnovsky showed for the first time at ultrastructural level that the endothelium of mouse cerebral capillaries constitute a structural barrier to horseradish peroxidase [9]. In cerebral capillaries, HRP entered the interendothelial spaces only up to, but not beyond the interendothelial tight junctions (*zonulae occludentes*). The most prominent feature of the blood–brain barrier is the presence of complex tight junctions between central nervous system