

Immunohistochemical Detection of Heparin-Binding Lectin in the Development of the Bovine Thymus

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Abstract: Heparin is a heterogeneous mixture of highly charged glycosaminoglycans that can exert a variety of biological and biochemical effects. Increasing attention is being paid to its role in cellular processes such as angiogenesis, cellular attachment, growth modulation and smooth muscle cell proliferation. The characterisation of cellular receptors for heparin-binding proteins is a rational step in the quest to elucidate the biochemical basis of specific binding and heparin-mediated responses.

We studied the distribution of heparin-binding lectin at the optical level during bovine thymus development on sections of fixed tissue using antibodies raised against this lectin. The presence of ligands accessible to the antibody which possesses binding activity can be inhibited by the addition of an excess heparin detected immunohistochemically. The reactive cells were present in both the cortical and medullary zones of the thymus. Heparin-binding lectin was localised mainly in the migrating macrophages and Hassall's corpuscles. Furthermore, the nucleus membranes of thymocytes and epithelial cells were weakly labelled. Binding sites were also present in some blood vessels.

Key Words: Thymus, heparin-binding lectin, development

Gelişim Evresindeki İnek Timuslarında Heparin-Bağlayan Lectin'in İmmunohistokimyasal Olarak Belirlenmesi

Özet: Heparin birçok biyolojik ve biyokimyasal işlevi bulunan yüklü glikozaminoglikanların oluşturduğu bir moleküldür. Anjiyogenez, büyümenin kontrolü, düz kas hücrelerinin proliferasyonu ve hücre adezyonu gibi birçok biyolojik rolünün bulunduğu bildirilmiştir. Heparin-bağlayan proteinin hücrelerdeki lokalizasyonunun tespiti heparinin aracılık ettiği proseslerin anlaşılmasında rasyonel bir adım olacaktır. Kuşkusuzdur.

Biz bu çalışmada Heparin-bağlayan proteine karşı üretilmiş antikorlar kullanarak bu proteinin gelişmekte olan inek fetal timuslarındaki dağılımını ışık mikroskop düzeyinde araştırdık. Antikor tarafından bağlanabilen ligandların varlığı immunohistokimyasal olarak belirlenirken heparin ile preinkübe edilen antikorların dokudaki proteine bağlanma yeteneğini yitirdiği görüldü. Antikorla reaksiyona giren hücrelerin kortekste sınırlı kalmayıp medullada da bulunduğu saptandı. Heparin-bağlayan proteinin özellikle göç halindeki makrofajlarda ve Hassall cisimciklerinde lokalize olduğu tespit edildi. Ayrıca, timositlerin ve timus epitel hücrelerinin nükleus membranlarıyla bazı kan damarlarının da zayıf bir reaksiyon gösterdikleri tespit edildi.

Anahtar Sözcükler: Timus, heparin-bağlayan lektin, gelişim

Introduction

In the development of the animal embryo, cells undergo a series of cellular relocations that culminate in the organisation of the primary cell layers and the establishment of organ primordia. These relocations, termed morphogenetic movement, have been the subject of intensive research, the results of which indicate that the carbohydrate-containing macromolecules at the cell surface and in the extracellular matrix play an important role in this process (1-6). The foetal bovine thymus was chosen for these studies, since this period corresponds to the major ontogenetic events and there is no information on the heparin-binding lectin destination in the bovine

thymus (7). The demonstration of the presence of diverse families of endogenous lectins in animal tissues has opened a new branch in the field of glycosciences and engenders a host of questions with regard to their actual functions in cells and organs in normal and diseased states (8-11)

Heparin-binding lectin is composed of four heteromers with a relative molecular weight of 14,400, 15,000, 16,200 and 16,700 (12). This protein, apparently involved in the inhibition of smooth muscle cell proliferation, also exhibits no substantial binding to other glycosaminoglycans besides heparin. The interaction of this lectin with naturally occurring heparin may be

physiologically involved in the modulatory regulation of heparin-mediated processes. The function of this molecule is largely unknown, but several lines of evidence support the idea of a role in cell adhesion, angiogenesis and smooth muscle cell proliferation (12). Excellent work has been performed on the molecular structure of heparin-binding lectin. However, the tissue destination of this lectin is unknown. Our aim is to demonstrate the possible changes in the distribution of this lectin throughout the foetal development of the bovine thymus. We suggest that the identification of binding sites for heparin-binding lectin on the bovine thymus will further our understanding of the biological roles of this molecule.

Materials and Methods

Specimens of adult bovine pancreas and of three stages of foetal development, grouped on the basis of crown-rump length into distinct age categories, F1 = 7-12 cm (week 7-11), F2 = 13-23 cm (week 12-16) and F3 = 24-38 cm (week 17-21), were freshly obtained from a slaughterhouse. Specimens were fixed in 4% paraformaldehyde for 24-72 h at 4 °C and embedded in paraffin. Embedding was carried out with Paraplast at 55 °C.

Sections were deparafinised according to a conventional procedure using xylene. Endogenous peroxidase activity was inhibited by incubation with 3% H₂O₂ in ethanol. After rehydration in graded ethanol solutions, the sections were washed twice with PBS and then incubated with the antibody raised against the heparin-binding lectin at a concentration of 5 µg/ml for

16 hr, followed by quick washes and further incubation with a biotinylated secondary antibody, i.e., goat anti-rabbit IgG (Camon, Burlingame), at a 1:200 dilution for 1 h. The specificity of the antibody was determined by Kohnke-Godt and Gabius (12) using the method of immunoblotting with the indicator horseradish peroxidase conjugated protein-A, and the antibody was kindly provided by Prof. Gabius (LMU, Munich, Germany). Sections were carefully washed with PBS, incubated for 1 h with Vectastain ABC kit reagents (Camon, Wiesbaden, FRG), and careful washing was repeated. The binding of probes was visualised using a heavy metal-mediated enhancement of the colour development with the peroxidase substrates 3,3'-diaminobenzidine 4-HCl/hydrogen peroxide, as described in detail elsewhere (13,14). After rinsing in tap water for 20 min, the sections were covered with eukitt (Riedel-de-Haen, Seelze, FRG). Control reactions were carried out in the absence of the antibody. Controls were negative, revealing no non-specific binding.

Results

The immunohistochemical analysis revealed a non-uniform pattern of intensity levels for the bovine thymus. As shown in Figures 1 and 2, the binding sites for the antibody raised against heparin-binding lectin were detected at the level of light microscopy. Binding sites were present in the cytoplasm of the macrophages of adult and F3 stages (Figs. 1.A and B). Specific epitopes for the antibody were present in significant amounts in the nucleus of the migrating macrophages at the adult

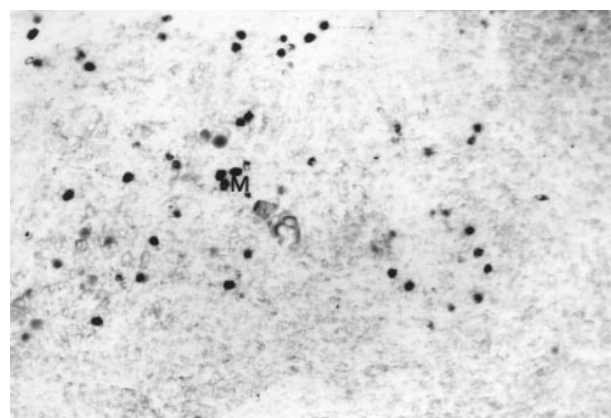
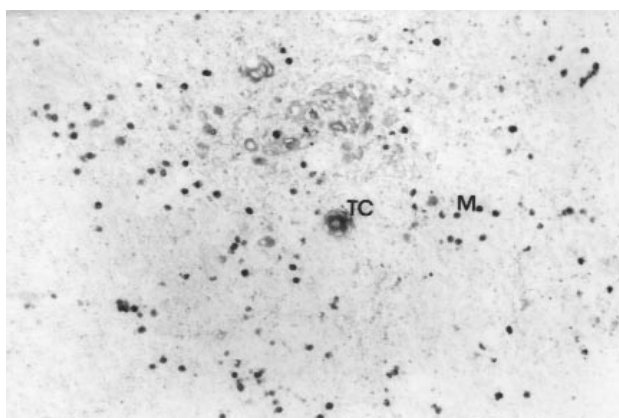


Figure 1. Localisation of binding sites for the antibody raised against heparin-binding lectin in paraffin sections of adult (A x 150) and F₃ stages (B x 185). The reactive cells were present in both the cortical and medullary zones of the thymus.

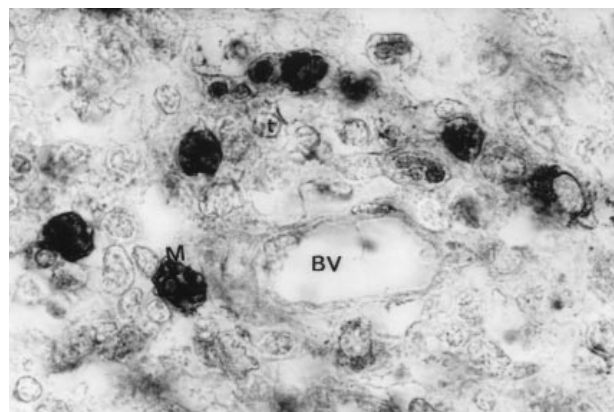


Figure 2. Immunohistochemical localisation of binding sites for the antibody raised against heparin-binding lectin in paraffin sections of adult (A x 1320) and F_3 stages (B x 1280). A very strong staining of heparin-binding lectin was observed in the cytoplasm of migrating macrophages (M) localised around the blood vessels (BV).

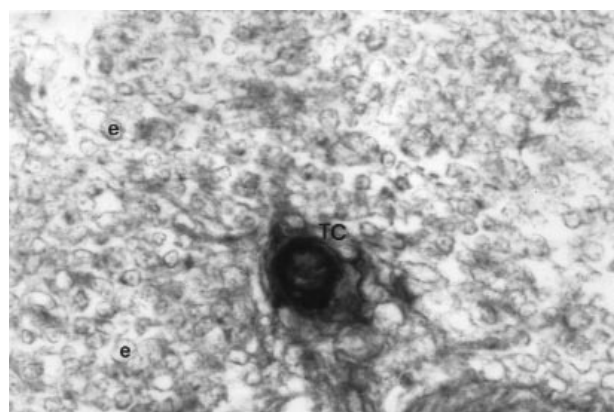
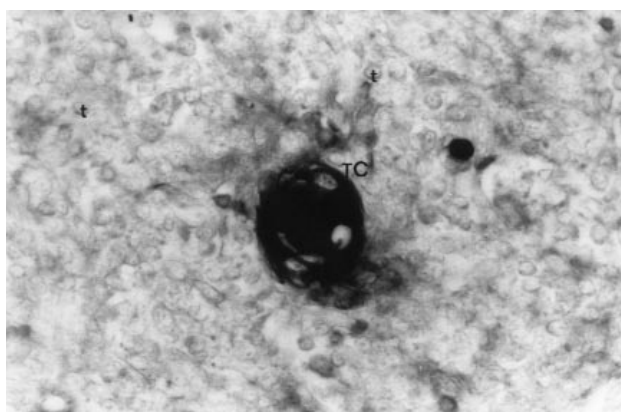


Figure 3. Immunohistochemical localisation of binding sites for the antibody raised against heparin-binding lectin in paraffin sections of F_2 (A x 540) and F_1 stages (B x 540). Hassall's corpuscles (TC) showed very strong cytoplasmic staining. However, thymocytes and epithelial cells showed a very weak labelling at the nucleus membrane (t, e).

(Fig. 2.A) and F_3 (Fig. 2.B) stages. However, in the early foetal stages no staining was observed in macrophages (Fig 3.A and B). Thymocytes and epithelial cells of all three foetal and adult stages showed a very weak staining at the nucleus membrane (t, e). There was no significant difference in staining patterns among the foetal stages and adult thymus. No labelling was observed in the mast cells, plasma cells and fat cells. The antibody also did not react with extracellular matrix components. Hassall's corpuscles showed very strong cytoplasmic staining at all three foetal stages (TC). In contrast, the binding sites in the adult thymic corpuscles were weak. Heparin-binding lectin was localised weakly in the blood vessels at the adult stage (BV).

Discussion

Raising antibodies against mammalian lectins offers an attractive available tool for the detection of endogeneous lectins. In this report, we have focused on the heparin-binding lectin of an apparent molecular weight of 78,000 (12). In order to address the question on the presence, localisation and developmental regulation of specific binding sites for heparin-binding lectin in the developing bovine thymus, an antibody raised against this lectin was applied.

Nuclear and cytoplasmic staining was observed in foetal and adult thymus macrophages. The nuclear labelling was also concerned with the nucleus membrane.

We confirmed the specificity of the macrophages with a well-known macrophage marker, CD68 (15,16). The reactive cells were present in both the cortical and medullary zones of the thymus. Antiserum which had been preadsorbed on heparin-binding lectin showed no reactivity with any thymic cells. It is certainly tempting to speculate on the role of the heparin-binding lectin in macrophages. It is already known that there is lymphatic death in the adult thymus. These data indicate that not only at the adult stage but also at the foetal stages there is lymphatic death in the thymus, and many macrophages may be there in response.

Only some endothelial cells have binding sites for the antibody raised against heparin-binding lectin, therefore the situation is very complex. The significance of these differences are not known. It may, however, be postulated that differences could essentially reflect the arterial-venous heterogeneity of the endothelial cells, as observed previously for β -galactoside-binding sites (16-21). The expression of heparin-binding lectin in lymphocytes is restricted to the nucleus membrane of these cells. This localisation pattern suggests that heparin-binding lectin is involved in the traffic between

the cytosol and the nucleus, with the actual function remaining to be determined.

Only in conjunction with other data can lectin studies and the influence of these molecules on embryonic development elucidate the nature and eventually the role of the carbohydrate-containing molecules present on the foetal cells. We previously immunohistochemically showed the existence and localisation of galectin-1 and galectin-3 in the bovine embryonic and adult thymus. These lectins were expressed mainly in the smooth muscle cells of blood vessels and macrophages (20). In particular, the binding pattern of biotin labelled galectin-1 was very similar to the binding sites of heparin-binding lectin. It may depend on the fact that galectin-1 binds the D-glucosamine unit of heparin.

Overall, these important issues warrant further studies, focusing on the properties of the ligands for the lectin and ultrastructural analysis of the lectin and lectin binding sites.

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