Expression of tissue transglutaminase in blood and lymphatic vessel endothelia and in mesothelium

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Abstract

This study was undertaken to evaluate tissue transglutaminase (TG2) expression in guinea pig tissues, using an antibody against the guinea pig liver TG2. The tests were performed by means of a few immunohistochemical methods in specimens from: gut, skin, the lungs, lymph nodes, the heart, the thymus, the spleen, skeletal muscles of control guinea pigs and from inflamed skeletal muscles and skin. TG2 expression was found in artery, vein and lymphatic vessel endothelia, in mesothelia of pleura, pericardium and peritoneum, and in smooth muscle cells. Spleen deserves a special attention, since this organ, especially rich in TG2, may be much more involved in celiac disease and in liver diseases than it has been accepted so far. The subject calls for further elucidation.

Key words: tissue transglutaminase, guinea pig, immunohistochemistry.

Introduction

Type II transglutaminase (TG2) is an enzyme, the activity of which requires Ca^{2+} ions presence. It catalyzes reactions, producing cross-linking amide bonds between proteins, but also between proteins and polyamines. Tissue transglutaminase, or transglutaminase 2 (TG2, EC 2.3.2.13), belongs to the superfamily of Ca^{2+}-dependent enzymes. It catalyzes a variety of post-translational modifications of proteins. The most common among them is the formation of isopeptide bonds, producing crosslinks between proteins [1, 2]. During skeletal muscle development, there is a transient expression of this protein [3]. The TG2 is the main, if not the sole, target of the endomysium celiac disease-specific autoantibodies; assigned to this enzyme is the role of master regulator of celiac disease [4]. In experimental mice, TG2 plays a protective role in hepatic injury [5]. Thus, TG2 is physiologically constantly expressed in some regions, while in others, it may be induced. This study was undertaken to perform immunohistochemical detection of TG2 in guinea pig different organs to evaluate its expression in normal guinea pig tissues and in inflamed skeletal muscles and skin, using an antibody against the guinea pig liver TG2.

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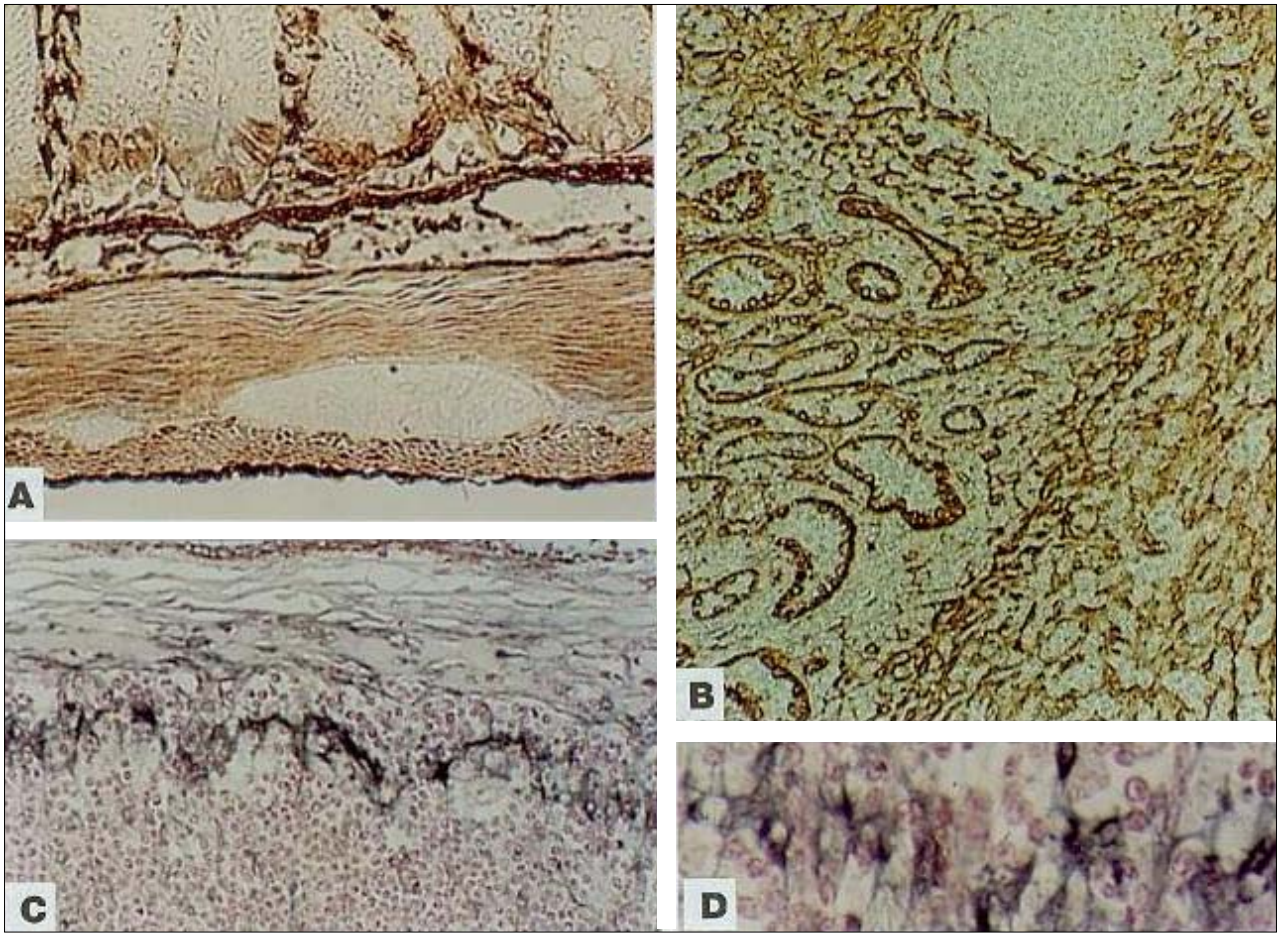
Material and methods

Normal 6 male guinea pigs of Dunkin-Hartley line, weighing 350-400 g each, were used throughout the study. Additionally, we used a material, derived from our experiments on the induction of skin and muscle inflammation by intradermal/intramuscular injections of dermatomyositis patient sera, as described earlier [6]. Experimental animal tissue specimens were taken at different time points after the sera injections (3 to 72 hrs). Tissue specimens were taken after pentobarbital intraperitoneal anaesthesia. The experiment was approved by the Local Ethics Committee.

One half of each sample was fixed in Bouin's solution, while the other one in Carnoy's solution; they were paraffin embedded and used for immunohistochemistry. The tests were performed by means of a few immunohistochemical methods in specimens from: gut, skin, the lungs, lymph nodes, the heart, the spleen, the thymus, skeletal muscles of control guinea pigs and from inflamed skeletal muscles and skin. Five (5) μm sections were used throughout the study. The mouse anti-guinea pig liver TG2 monoclonal IgG1 antibody, clone CUB 7402 (DakoCytomation), in dilution of 1:50 - 1:150, was used as a primary antibody. Stainings were performed, using a number of techniques: the two-step indirect immunohisto-

Figure 1. Immunolocalization of transglutaminase 2 in guinea pig organs. A. Fragment of normal intestine. Positive immunostaining is visible in mesothelial cells of peritoneum, in myocytes, forming longitudinal and circular layers of intestinal wall, in endothelia of blood and lymphatic vessels and in single cells of intestinal glands; intestinal nervous ganglia are completely negative. Immunoperoxidase method, DAB, used as a chromogen (brown). Magnification 240x. B. Spleen of normal guinea pig. Endothelia of capillaries and lymphatic vessels show very strong immunostaining; peripheral zone of lymph follicle shows intensive staining, too. Immunoperoxidase method, DAB, used as a chromogen (brown). Haematoxylin counterstain. Magnification 240x. C. In the lymph node, interdigitating cells of the subcapsular region form a zone, showing positive immunostaining. Immunoperoxidase method, Vector SG, used as a chromogen (dark blue). Nuclear fast red counterstain. Magnification 240x. D. Fragment of "C" micrograph . Magnification 600x.



chemical method with peroxidase-labelled rabbit F(ab')2 anti-mouse IgG (Serotec, Oxford, UK), the labelled streptavidin biotin procedure, using an LSAB2 System-HRP kit (DakoCytomation) and the enhanced polymer procedure, using the DAKO EnVision System with alkaline phosphatase (DakoCytomation). Primary antibodies were incubated for 1 h with tissue sections after blocking endogenous peroxidase with 2% hydrogen peroxide in peroxidase employing procedures. Slides were washed with PBS and incubated with the proper secondary antibody, depending on the used system, according to prescriptions. A negative control was obtained by omitting the primary antibody. After thorough washing in PBS, the antibody, bound to the antigen, was revealed by staining with 3.3'-diaminobenzidine substrate solution (Sigma) or Vector SG (Vector) for peroxidase and Fast Red TR/Naphthol AS-MX (Sigma) for alkaline phosphatase-labelled antibody.

Results

TG2 expression was found in artery, vein and lymphatic vessel endothelia, in mesothelia of pleura, pericardium and peritoneum, and in smooth muscle cells. In the gut, besides the intestine reaction in mesothelium and in smooth myocytes, an intensive staining was present in endothelial cells of blood and lymphatic vessels of villi (Fig. 1A). Sporadically, epithelial cells of the gut were positive, mainly in the intestinal glands (Fig. 1A). In the spleen, both lymphatic and blood vessel endothelia were intensively stained, but also in lymph follicles, especially at their periphery, the reaction was pronounced (Fig. 1B). In the thymus, the strongly positive cells were large, scattered mainly in cortical parts of this organ and vascular endothelia were positive. A positive reaction to the antigen was also observed in sinuses of lymph nodes; positive interdigitating cells formed a zone in the subcapsular region of lymph nodes (Fig. 1C, Fig. 1D). In normal capillaries of the skin and skeletal muscles, the reaction was negative. In tissue specimens from experimental myositic muscles, significantly dilated lymphatic vessels with strongly immunoreactive endothelium were found and some of them seemed to surround (squeeze) blood vessels. The endothelia of capillaries in endomysium were positive, too.

Discussion

In the literature, different functions of TG2 are pointed out (including: protein crosslinking, GTP-ase activity, acting as G protein, kinase activity), implying an opinion that still the knowledge on this protein is far from being complete, but a progress in it seems very promising.

In the present study, a strong TG2 expression was shown in endothelia and in mesothelia, what can be considered as an indication of some role of this protein in creating barriers, regulating molecular flow (infiltration). The used by us antibody seems to be especially useful and valuable for lymphatic vessel detection, which are often overlooked in histologically examined tissues. The spleen deserves a special attention, since this organ, especially rich in TG2 (Fig. 1B), may be much more involved in celiac disease [4] and in liver diseases [5] than it has been thought so far. The subject calls for further elucidation.

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