Usefulness of some primary anti-human antibodies in immunohistochemistry in guinea pig

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Abstract

The commercially available antibodies for immunohistochemical purposes, though numerous and diversified, are mostly designed to detect human antigens. This study was undertaken to evaluate the usefulness of anti-human antigen antibodies in guinea pig tissues. The subjects of our interest were mostly skin and striated muscles and it was important for us to stain vascular and neural elements and to visualize separate cell kinds, including also skin Langerhans cells and the cells, contributing to inflammatory infiltrations.

Key words: guinea pig, immunohistochemistry, skin, skeletal muscles.

Introduction

Though human tissues are difficult to be obtained for research from diseased and, even more, from healthy people, animal derived biopsy/section material is relatively easily available, allowing for a proper selection of controls and for experiment planning. On the other hand, the commercially available antibodies for immunohistochemical purposes, though numerous and diversified, are mostly designed to detect human antigens. Additional difficulties arise when the antibodies are to be applied to paraffin sections. Thus, a researcher, performing animal experimental studies, apparently has a limited access to some research areas, potentially relevant for the elucidation of human disease mechanisms. This report summarizes several years of our studies on guinea pig tissues and it presents the adversities, overcome in the process. The reports, published by us to date, contain illustrations of immunohistochemical reactions to neuropeptides: β-endorphin [1], neuropeptide Y (NPY), calcitonin gene related peptide (CGRP), protein gene product 9.5 (PGP-9.5) [2], protein S-100, T lymphocytes and macrophages in guinea pig tissues [3, 4]. The panel of all primary antibodies and their used dilutions has been listed in Table 1. Out of the antibodies, used by us, only the one against pan-T-cell antigen (Serotec, Oxford, UK) was specially designed for studies in guinea pigs. The subjects of our interest were mainly the guinea pig skin and striated muscles and we considered staining of all possible cell kinds in those tissues, including epidermal Langerhans cells (LC), as well as the infiltrating elements, vascular and neural components. Neuropeptides are phylogenetically old protein compounds, occurring in humans and in animals with a high degree of identity among vertebrate species [5]. PGP 9.5 has been reported to be a marker for neurons and neuroendocrine cells [6]. Langerin (CD207), an antigen of human epidermal LC, is known to distinguish LC from other dendritic cell subsets and is characteristic for immature LC [7]. Fascin - an actin-binding protein is characteristic for different cell types, for example, for mature dendritic cells and endothelial cells [8]. CD56, neural cell adhesion molecule (NCAM) is a marker of myoblasts and regenerating muscle fibres [9]. CD59 (protectin) protects cells from lysis by complement compounds and is present on the surface of many cell types [10]. The proliferating cell nuclear antigen (PCNA) is a marker of proliferation.

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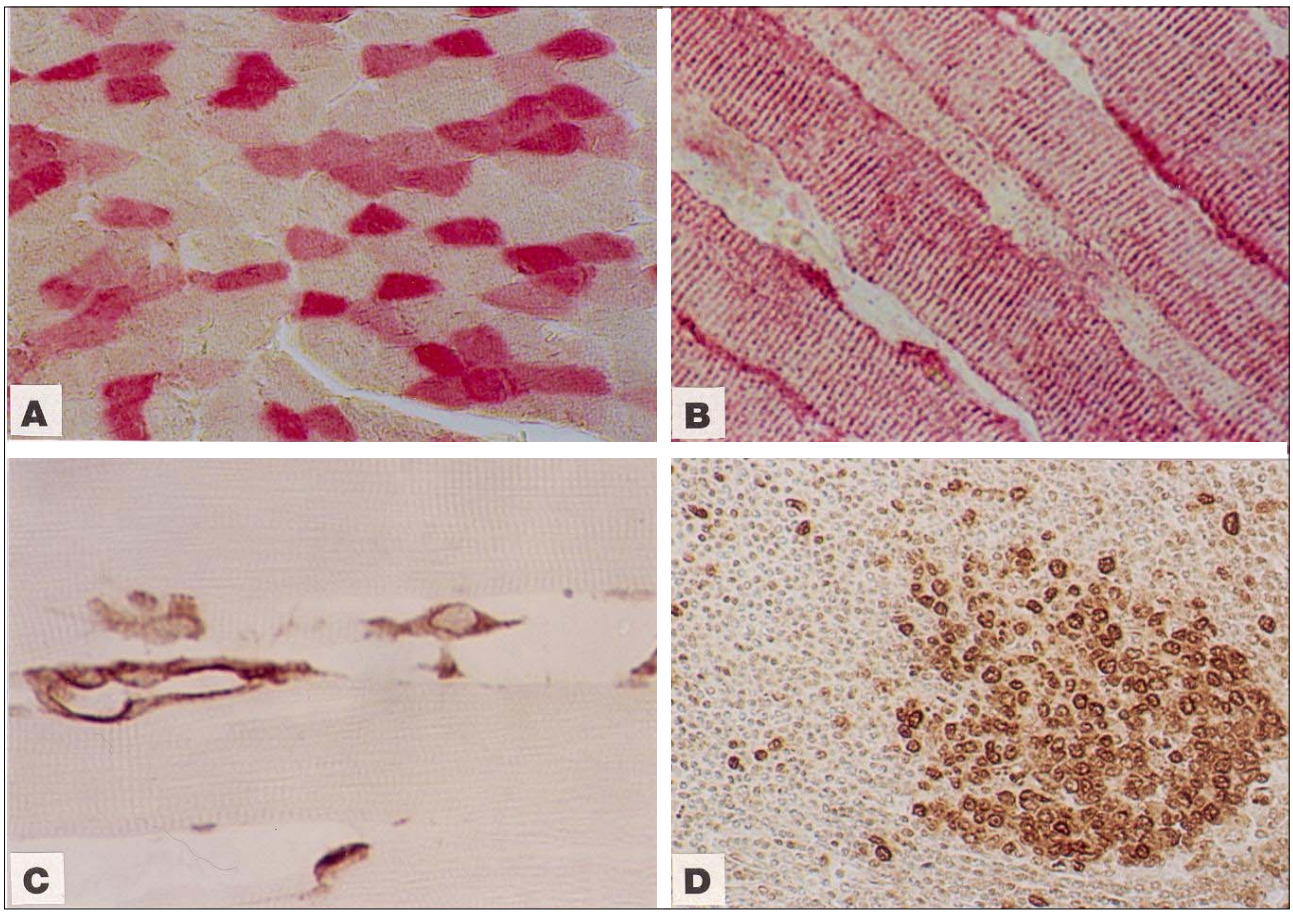
Material and methods

The tests were performed by means of a few immunohistochemical methods in specimens from different tissues and organs of control guinea pigs and from inflamed skeletal mus-

Table 1. A list of used antibodies. Abbreviations: Rb (rabbit), Mo (mouse), Sh (sheep), TBS (The Binding Site).

Antibody anti-	Type	Clone	Dilution	Source
beta-endorphin	Rb		1 : 400	Eurodiagnostica
neuropeptideY	Rb		1 : 400	Eurodiagnostica
CGRP	Rb		1 : 800	Eurodiagnostica
protein S-100	Sh		1 : 50	TBS
PGP 9.5	Sh		1 : 50	TBS
T cells	Mo	CT5	1 : 120	Serotec
macrophages	Mo	MAC387	1 : 100	Serotec
hsp90	Mo	JPB24	1 : 40	Novocastra
PCNA	Mo	PC10	1 : 80	DakoCytomation
CD59 (protectin)	Mo	MEM-43	1 : 10	Serotec
(CD56) NCAM	Mo	1B6	1 : 40	Novocastra
von Willebrand factor	Sh		1 : 40	TBS
desmin	Mo	DE-R-11	1 : 40	TBS
myoglobin	Sh		1 : 120	TBS
fascin	Mo	IM20	1 : 40	Novocastra

Figure 1. A - myoglobin immunostaining (red) in guinea pig skeletal muscle. Three types of myofibres are visible. Magnification 240x. B. Desmin immunostaining (red) in guinea pig skeletal muscle. Sharp striations and, probably, muscle satellite cells are strongly positive. Magnification 600x. C. Positive immunostaining for hsp90 is present in endothelial cells of capillaries and in some connective tissue cells of endomysium in guinea pig skeletal muscle, showing slight inflammatory changes. Magnification 600x. D. Positive immuno-staining for PCNA in guinea pig germinal centre of lymph follicle. Magnification 240x.



cles and skin. Normal, male guinea pigs of Dunkin-Hartley line, weighing 350-400 g each, were used throughout the study. Guinea pig tissue specimens, derived from the control animals and from the ones with experimentally induced skin and/or striated muscle inflammations, were collected by us during the years 1996-2003. Guinea pigs underwent pentobarbital intraperitoneal anaesthesia and decapitation. All the experiments had been approved by the Local Ethics Committee. The

material was fixed in Bouin's solution, in Carnoy's solution or in 10% formalin. The samples were paraffin embedded. Five µm sections were used throughout the study. Stainings were performed, using a number of techniques: a two-step indirect immunohistochemical method with peroxidase-labelled rabbit $F(ab')_2$ anti-mouse IgG (Serotec, Oxford, UK), labelled streptavidin biotin procedure, using LSAB2 System-HRP kit (DakoCytomation) and enhanced polymer procedure, using DAKO EnVision System with alkaline phosphatase (DakoCytomation); different antibody dilutions and unmasking antigen methods were applied. 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 or by using a properly diluted respective non-immune serum instead of the antibody. After thorough washing in PBS, the antibody, bound to the antigen, was revealed by staining, done with 3,3'-diaminobenzidine substrate solution (Sigma) or Fast Red TR/Naphthol AS-MX (Sigma) for alkaline phosphatase-labelled antibody.

Results and Discussion

Positive immunohistochemical staining on guinea pig tissues was obtained with anti-neuropeptide antibodies, anti-myoglobin (Fig. 1A), anti-desmin (Fig. 1B), anti-protein S-100, anti-PGP 9.5, anti-macrophages, anti-heat shock protein 90 (hsp90) (Fig. 1C), anti-PCNA (Fig. 1D), anti-von Willebrand factor and, of course, with anti guinea pig T-cells antigen. Out of the anti-human antigens antibodies, used by us, negative results were visible with anti-langerin, anti-fascin, anti-CD59 and anti-NCAM (CD56) antibodies; that fact reflecting substantial differences in those molecule structures between the man and the guinea pig. Differences in epitope structures in components, characteristic for dendritic cells, between the man and the guinea pig makes impossible the application of described here anti-langerin and anti-fascin antibodies on guinea pig tissues for detection of LC. Additionally, S-100 protein is known to be widely distributed, among others in LC and in melanocytes, so, this fact hinders the unquestionable identification of epidermal LC by anti-S-100 antibody application [11]. Additionally, neither Bouin's solution nor 10% formalin for fixation was useful for desmin detection; Carnoy's fixative was not suitable for hsp90 and PGP 9.5 detection (completely negative stainings).

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