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Research paper

Immunohistochemical detection of soluble immunoglobulins in living mouse small intestines using an in vivo cryotechnique

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ABSTRACT

Some morphological changes are inevitable during immersion- or perfusion-fixation and following alcohol-dehydration for tissue preparations. Common immunostaining techniques for these specimens have some limitations to capture accurate localizations of soluble proteins in cells and tissues. In this study, to examine *in situ* distributions of immunoglobulins (Igs), small intestinal tissues of living mice were prepared with our "*in vivo* cryotechnique" (IVCT). Thin sections were first stained with hematoxylin-eosin for morphology, and then some immunostainings were performed on serial sections for IgA, Ig kappa light chain, IgG1 heavy chain (IgG1), and IgM. Living morphological states of small intestinal tissues, including flowing erythrocytes and opening blood vessels, were observed on paraffin sections prepared with IVCT. IgA was immunolocalized in many plasma cells of the lamina mucosa propria, intestinal matrices, and also in epithelial cells of the intestinal villi and crypts. Both IgG1 and IgM immunoreactivities were mainly detected in blood vessels, whereas only IgG1 was also immunolocalized in interstitial matrices of mucous membranes. By perfusion-fixation and alcohol-dehydration, however, IgA immunoreactivity was observed in plasma cells, but not in epithelial cells or the lamina mucosa propria. Thus, IVCT was more useful to examine *in vivo* immunolocalizations of soluble Igs in small intestines.

1. Introduction

The animal intestinal mucosa is well known to maintain highly developed immunological systems with circulating immunoglobulins (Igs), which contribute to some defensive barriers against foreign antigens such as pathogenic viruses and bacteria (Wines and Hogarth, 2006; Tezuka et al., 2007). Recently, *in vivo* imaging with intravital microscopy and fluorescence-imaging techniques has revealed dynamic mucosal immunity in the gastrointestinal tracts of animals (Hosoe et al., 2004; Zinselmeyer et al., 2005). Therefore, preservation of soluble Igs *in situ* for immunohistochemistry is essential to examine the mucosal immunity in living animal intestines without morphological artifacts during the specimen preparation steps.

Conventional preparation techniques, such as perfusion- or immersion-fixation followed by alcohol-dehydration, have

Abbreviation: ABC, avidin-biotin-horseradish peroxidase complex; Cr, crypt; DAB, diaminobenzidine; FS, freeze-substitution; FT-QF, quick-freezing of fresh resected tissues; HE, hematoxylin-eosin; HRP, horseradish peroxidase; IgA, immunoglobulin A; IgG1, immunoglobulin G1 heavy chain; IgM, immunoglobulin M; Igs, immunoglobulins; Igκ, immunoglobulin kappa light chain; IM-DH, immersion-fixation followed by alcohol-dehydration; IP, isopentane-propane; IVCT, *in vivo* cryotechnique; MAS, Matsunami Adhesive Slide; MI, mouse immunoglobulin A; MM, molecular marker; ML, muscle layer; PB, phosphate buffer; PBS, phosphate-buffered saline; PFA, paraformaldehyde; PF-DH, perfusion-fixation followed by alcohol-dehydration; PF-QF, perfusion-fixation and quick-freezing; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; Si, small intestine; Vi, villi.

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been broadly used for histological analyses and immunohistochemistry of Igs and secretory component in animal and human intestinal tissues (Brown and Burns, 1973; Taylor and Mason, 1974; Nagura et al., 1980). However, these prepared specimens were always accompanied by artificial changes in histology, such as tissue shrinkage, antigen-masking and diffusion of soluble proteins, during the preparation steps (Ohno et al., 2008; Saitoh et al., 2008). Since 1996, we have been developing an "*in vivo* cryotechnique" (IVCT) to clarify the functional histology and immunohistochemistry of soluble proteins in living animal organs under normal and experimental conditions (Ohno et al., 1996; Li et al., 2005; Zhou et al., 2008). In the present study, living mouse small intestines under normal blood circulation were directly cryofixed with IVCT, and analyzed for immunolocalizations of soluble Igs.

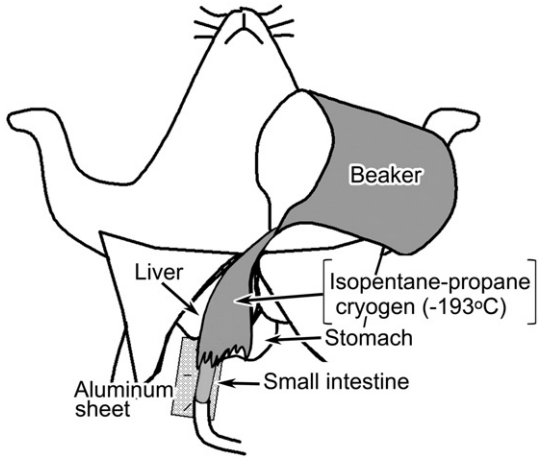

Fig. 2. A schematic drawing shows how to perform the "*in vivo* cryotechnique" (IVCT) for living mouse small intestines. The small intestine of an anesthetized mouse is exposed in the abdominal cavity, which is immediately cryofixed *in vivo* by pouring the isopentane-propane cryogen (−193 °C) from a beaker prepared with liquid nitrogen.

2. Materials and methods

2.1. Animals

The present animal experiments were approved by the University of Yamanashi Animal Care and Use Committee. Ten C57BL/6 mice, aged 2–3 months, were anesthetized with an intraperitoneal injection of 50 μl Nembutal (50 mg/ml). Their small intestinal tissues, 2–3 cm from the end of the stomach, were prepared for immunohistochemical studies by five different preparation methods (Fig. 1) and also for immunoblotting analysis.

2.2. Tissue preparation methods

2.2.1. "In vivo cryotechnique" (IVCT) followed by freeze-substitution (FS) fixation

Small intestines of three anesthetized mice were exposed under normal blood circulation and carefully put on aluminum sheets in their abdominal cavities. The IVCT was immediately performed by directly pouring isopentane–propane (IP) cryogen (−193 °C) precooled in liquid nitrogen over the small intestines from the outer side of the serous membrane (Figs. 1a and 2). The frozen intestinal tissues were removed with a dental electric drill in liquid nitrogen, and commonly processed for FS in acetone containing 2% paraformaldehyde (PFA) at −80 °C for 48 h, and then serially kept at −30, −10, and 4 °C for 2 h each. The freeze-substituted specimens were routinely treated with pure acetone and xylene, and finally embedded in paraffin wax, as reported before (Ohno et al., 2004).

2.2.2. Immersion-fixation followed by alcohol-dehydration (IM-DH)

Small intestines of two anesthetized mice were removed with a pair of scissors, and cut into small pieces with razor blades. They were routinely immersed with 2% PFA in 0.1 M phosphate buffer solution, pH7.4 (PB), at 4 °C overnight (Fig. 1b). The fixed specimens were then dehydrated in a graded series of ethanol, immersed in xylene, and finally embedded in paraffin wax.

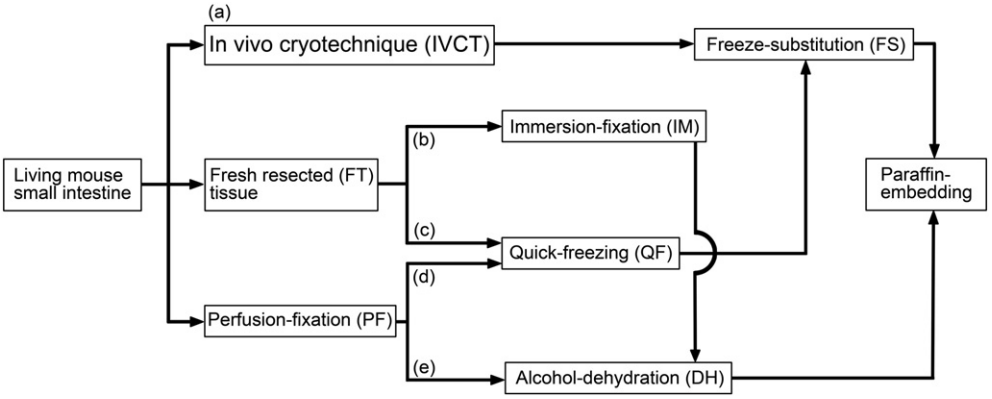

Fig. 1. A flow chart of the five different preparation procedures, including cryotechniques (a, c, d), and conventional fixation and dehydration (b, e) for living mouse small intestines. The letters correspond to the procedures described in Materials and methods.