Letter to the Editor

Post-embedding Immunoelectron Microscopy of Tissue Processed by Rapid Freezing and Freeze-substitution Without Chemical Fixatives

With great interest we read the recent article by Usuda et al. (1), which describes the immunoelectron microscopy of tissue processed by rapid freezing and freeze-substitution without chemical fixatives. In their article, Usuda et al. say that "our result . . . should (sic) be the first demonstration of immunostaining of rapidly frozen tissue processed without chemical fixation." We would like to point out that our paper, published in 1989 (2), reported the ultrastructural localization of an antigen in skin processed by rapid freezing and freeze-substitution without chemical fixation, using a post-embedding method. Bullous pemphigoid antigen, which localizes at the epidermal basement membrane zone, is recognized by a circulating antibody that occurs in patients with bullous pemphigoid, one of the major autoimmune skin-blistering diseases (3). The antigen is very labile and does not appear to tolerate any form of chemical fixation before immunolabeling. Therefore, after trying a variety of methods, we found that those mentioned above gave the best results.

Previously, we compared the results of cryofixation using either metal contact-freezing or plunging into liquid propane, and concluded that the liquid propane method was superior for skin tissue, which is structurally very different from liver—the tissue studied by Usuda et al. We think that this may account for the difference in results obtained using these two main forms of cryofixation. We found considerable variability of ultrastructural preservation among different blocks, which we attributed to the problems of rapidly freezing a complex tissue such as skin. Whereas Usuda et al. used Lowicryl K4M at −20°C, we adopted even lower temperatures (−80°C to −60°C) for freeze-substitution and embedding in Lowicryl K11M on the basis that improved structural and antigenic preservation can be obtained under these conditions (4).

We have since used these methods for immunogold studies on other intra- and extracellular skin antigens (manuscript in preparation), and have found them to yield consistently good results in terms of ultrastructural preservation and quality of immunolabeling.

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Literature Cited
4. Armbruster BL, Garavito RM, Kellenberger E. Dehydration and embedding temperatures affect the antigenic specificity of tubulin and immunolabeling by the protein A–colloidal gold technique. J Histochem Cytochem 1983;31:1380