P-D-3

Immunoelectron microscopic analysis of dendritic cells in the periapical region of the periodontal ligament of normal rat molars

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Aim: We have reported the presence of dendritic cell (DC)-like cells in the periapical region of normal rat periodontal ligament. This study was designed to further characterize these cells, employing CD11c, a previously untested DC marker. Methodology: Normal mandibular first molars of five-week-old male Wistar rats were subjected to TEM immunohistochemistry. ED1 (reactive to lysosomal structures of macrophages and DCs), OX6 (anti-class II MHC molecules), and CD11c were used as primary antibodies. Results: The three antibodies recognized DC-like cells, which showed the following features and thus were discriminated from macrophages: (1) presence of long cytoplasmic processes, (2) scarcity of lysosomal structures, and (3) lack of typical phagosomes. The DC-like cells were mainly seen in fibroblast-rich areas. OX6+ and CD11c+ DC-like cells were richly distributed near the alveolar bone surfaces, whereas fewer ED1+ DC-like cells were seen in this zone. Conclusions: Our findings may support the view that the DC-like cells are counterparts of DCs in other connective tissues. These cells might be composed of subpopulations that differ in the expression of cytochemical markers.

P-D-4

Immunofluorescence technique for 100 nm semi-thin sections of Epon-embedded tissues

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Previously we reported an immunoenzyme staining method for Epon sections (Histochemistry), in which epoxy resin of the sections was removed by sodium ethoxide, followed by trypsin digestion. In this paper, we invesigated an application of Epon sections to immunofluorescence staining. Rat kidney and liver were fixed by perfusion with 1% glutaraldehyde for 10 min. Tissues were cut into slices and embedded in Epon. Semi-thin sections with thicknesses ranging from 1000 nm to 100 nm were cut and mounted on clean glass slides. Epoxy resin was removed by treatment 10%with sodium ethoxide. Sections were treated with 0.05% trypsin and then with sodium borohydride. They were then immunostained for leucine aminopeptidase (LAP, plasma membrane), catalase (peroxisomes), 3-ketoacyl-CoA thiolase (mitochondria), cathepsin D (lysosomes), and LGP107 (lysosomal membrane) using Cy^2- or Alexa 546-labeled secondary antibodies. In 1000 nm thick sections, nonspecific fluorescence remained and such fluorescence decreased as the thickness of the sections became less. Clear specific fluorescence was obtained in the sections with thicknesses ranging from 250 nm to 100 nm using the Alexa 546-labeled antibody (red fluorescence) but not with Cy^2- or Alexa 430-labeled antibody (green fluorescence). Sodium borohydride greatly abolished autofluorescence of glutaraldehyde. The present method made possible to get signals in cross sections of biological materilas with a thickness of 250 nm – 100 nm, which is difficult to obtain as an optical sections by a confocal laser microscope.