O36-THE EXPRESSION OF GM-CSF AND OSTEOPONTIN IN IMMUNO-COMPETENT CELLS PRECEDES THE ODONTOBLAST DIFFERENTIA-TION FOLLOWING ALLOGENIC TOOTH TRANSPLANTATION IN MICE.

K. Saito¹, M. Nakatomi¹, H. Ida-Yonemochi¹, S. Kenmotsu¹, H. Ohshima¹.

¹Division of Anatomy and Cell Biology of the Hard Tissue, Department of Tissue Regeneration and Reconstruction, Niigata University Graduate School of Medical and Dental Sciences, 2-5247 Gakkocho-dori, Chuo-ku, Niigata 951-8514, Japan.

Key words

Cell differentiation, Dental pulp, Histocompatibility antigens class II, Granulocyte-macrophage colony-stimulating factor, Odontoblasts, Osteopontin, Transplantation, Mice (Inbred ICR).

Introduction

Dental pulp elaborates both bone and dentin under pathological conditions such as tooth replantation/transplantation. Once osteoclast-lineage cells appear at the pulp-dentin border, bone-like matrix deposition can be induced (Tsukamoto-Tanaka H et al. 2006), even beneath the pre-existing dentin, whereas the temporal appearance of dendritic cells there induces the tubular dentin formation (Shimizu A et al. 2000; Nakakura-Ohshima K et al. 2003). This study aims to clarify the expression of granulocyte macrophage colonv-stimulating factor (GM-CSF) and osteopontin (OPN) in the process of reparative dentin formation by allogenic tooth transplantation using in situ hybridization for OPN and immunocytochemistry for GM-CSF and OPN at the both level of light and electron microscope, and a double immunofluorescent staining for OPN and class II MHC molecule has been performed to confirm whether immuno-competent cells such as dendritic cells or macrophages express OPN.

Materials and Methods

CrIj:CD1 (ICR) mice, 3 weeks old, were used in this study. The upper-right first molar was extracted under anesthesia, and the roots and pulp floor were resected. The coronal portion of the sample without the periodontal tissue was immediately transplanted into the sublingual region after cutting the ventral side of the tongue of the littermates. Materials were collected in groups of animals at intervals of 1,3, 5, 7, and 14 days after allogenic tooth crown transplantation. The upper-left M1 of the same animal was used as control.

Results

In the control group, GM-CSF-positive reactions were not observed in either the dental pulp or the periodontal ligament, whereas OPN-positive reactions were observed in the dentinal tubules at the pulp horn in addition to the osteoblasts, cementoblasts, and the matrix of cementum and bone. On the other hand, nestin-immunoreactivity was exclusively expressed in the coronal and root odontoblasts, and the other types of cells lacked nestinpositive reactions in the dental pulp. On Days 1-3, immunocompetent cells such as macrophages and dendritic cells expressed both GM-CSF and OPN, and some of them were arranged along the pulp-dentin border and extended their cellular processes into the dentinal tubules. On Days 5-7, tubular dentin formation commenced next to the pre-existing dentin at the pulp horn where nestinpositive odontoblast-like cells were arranged. Although the expression of GM-CSF and OPN disappeared in the pulp tissue except for the prolonged expressions of OPN and rarely GM-CSF in the lesion lacking the regenerated odontoblasts, the OPN-immunopositive matrices were recognized between the pre-existing and postoperative dentin. Until Day 14, bone-like tissue formation occurred in the pulp chamber, where OPN-positive osteoblasts surrounded the bone matrix.

Conclusion

These results suggest that the secretion of GM-CSF and OPN by immunocompetent cells plays a role in the maturation of dendritic cells and the differentiation of odontoblasts, respectively, in the regenerated pulp tissue following tooth transplantation.

Acknowledgements

We are grateful to Dr. S. Nomura for providing riboprobe. This work was supported in part by Grantsin-Aid for Scientific Research (B) (no. 22390341 to H.O.) and Exploratory Research (no. 20659296 to H.O.) from MEXT and JSPS, and a Grant for Supporting Project for Strategic Research of Nihon Univ Sch Dentistry at Matsudo from MEXT, 2008-2012 (Team: Dental Morphogenesis).

References

Tsukamoto-Tanaka H et al. (2006) Cell and Tissue Research, 325 (2): 219-229. Shimizu A et al. (2000) Cell and Tissue Research 302 (2):

221-233.

Nakakura-Ohshima K et al. (2003) Journal of Electron Microscopy 52 (6): 581-591.