SHORT COMMUNICATION

The action of human peripheral monocytes on synthetic hydroxyapatite in vitro
—Electron microscopic observations—

Iwao Sato, Yoshinobu Iwakawa, Kiyohiko Hobo
and Katsumi Ikeda

Department of Periodontology, Meikai University
School of Dentistry, Sakado, Saitama 350-02, Japan

[Accepted for publication: December 3, 1988]

Key words: Human monocyte/synthetic hydroxyapatite/electron microscopy

Introduction

Recently, we established a new in vitro bone resorbing system using \(^{45}\text{Ca}\)-labelled synthetic hydroxyapatite (\(^{45}\text{Ca-HA}\))\(^1\). In this system, monocytes induce the release of \(^{45}\text{Ca}\) from \(^{45}\text{Ca-HA}\) and activity is enhanced by supernatants from cultures of peripheral blood leukocytes which are stimulated by phytohemmaglutinin or dental plaque.

Reports\(^2\)\(^-\)\(^6\) have been published on bone resorption or involvement in bone resorption by human monocytes or the monocyte-macrophage system. This involvement is one aspect for elucidating the mechanism of bone resorption.

Since morphological observations on the experimental system (synthetic-HA destruction by human monocytes) have not been reported, they were performed by electron microscopy in the present study.

Materials and Methods

Purified human peripheral monocytes and synthetic hydroxyapatite (synthetic-HA) were used.

Monocytes were separated by plastic adherence\(^7\) from human peripheral blood leukocytes isolated by differential centrifugation using Ficoll-Conray and cultured in RPMI-1640 medium with 10\% fetal calf serum. Synthetic-HA was made by the wet method\(^8\)\(^-\)\(^1\): \(18.3\% \text{CaCl}_2\) was slowly added to 38.5\% NaHPO\(_4\) and 53.3\% NaOH, then stirred for 2 hrs at room temperature. The precipitate was washed with deionized water. Synthetic-HA particles were filtered by a mesh screen (40 \(\mu\text{m}\) in diameter).

Monocytes and synthetic-HA were cultured in 35 mm plastic dishes for 48-120 hrs, and the mixture was observed with an electron microscope.

In preparation for scanning electron microscopy (SEM), the monocyte and synthetic-HA-mixture was fixed with 2\% glutaraldehyde in 0.2M cacodylate buffer, pH 7.4. Specimens were dehydrated in ethanol and critical point-dried. They were evaporated with gold-paladium and examined with a high resolution SEM at 10 kV. In preparation for transmission electron microscopy (TEM), the mixture was fixed in glutaraldehyde buffered with cacodylate and the specimens were washed several times in preparation for the next steps. For thin sections following plastic embedding, the mixture was post-fixed in 1\% OsO\(_4\) for 1.5 hours, dehydrated in an ethanol series, embedded in Araldite and sectioned. Electron micrographs were taken at 80 kV with a JEM-100CX.

Results and Discussion

Figures 1 and 2 show adherence to synthetic-HA and the phagocytosis of synthetic-HA
Fig. 1 Monocyte in contact with synthetic-HA particle. The synthetic-HA particle is about twice as large in diameter than the monocyte.

Fig. 2 Monocyte adjacent to synthetic-HA particle. The structure of synthetic-HA is porous.

Fig. 3 By creating a phagosome (arrow), the monocyte phagocytoses synthetic-HA (higher magnification of Fig. 2).

Fig. 4 Monocyte with many filopodia.

Fig. 5 Surface of the monocyte is ruffled.

by purified monocytes after 84 hrs of culture. Since synthetic-HA was made without heat treatment, the surface is rough (shown on scanning electron microscopy, Fig. 1) and the structure is porous (shown on transmission electron microscopy, Fig. 2). Monocytes phagocytosed part of the synthetic-HA by making phagosomes (Fig. 3).

From these observations, it is suggested that human peripheral monocytes destroy (dissolves) or phagocytose synthetic-HA, which is the main component of bone mineral. Blair et al., in a study using a fluorescent dye, found that macrophage-mediated bone resorption occurs at pH a below 6.0 at the cell-bone interface. We previously reported that a positive correlation between the increase of $^{45}$Ca from $^{45}$Ca-HA and the secretion of citric acid by monocytes was shown. But, it was not demonstrated that citric acid was produced on the contact surface between monocytes and synthetic-HA. However, taking the reports of Blair et al. into consideration, along with our findings, the data seem to indicate synthetic-HA destruction on the surface to which monocytes adhered.

In the present experimental system, mono-
cytes co-cultured with synthetic-HA developed many processes and showed floriform structures (Figs. 4 and 5), presenting a morphologically active condition. This phenomenon is considered one step in the contact with bone components in which the cell functions in bone resorption.

There is no denying the possibility that the peripheral monocytes used in the present experimental system might have differentiated into macrophages during incubation. It is therefore necessary to take into account macrophage function in the results of the present experiment. An experimental system that includes this aspect is required for future studies.

Acknowledgements

We are grateful to Associate Professor Hiroshi Nakahara (The First Department of Oral Anatomy) and Chief Engineer Shiro Kunii and Yumiko Kanda (Electron Microscopy Laboratory) for their kind assistance in carrying out the present study.

References


