X-Ray Microprobe Analysis of Foreign Body Granulomas with Special Reference to Cellular Responses to Metal Implants

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Introduction

Tissue response to implanted biomaterials consists of granulomatous inflammation with an accumulation of epithelioid cells and giant cells around the foreign body. The release of metal elements from metal implants is one of the most serious problems with regard to biocompatibility of implanted biomedical materials. However, the mechanisms of such release of metal elements remain to be elucidated. In this study, we used an analytical electron microscope equipped with an energy-dispersive X-ray microprobe (EDX) analyzer to examine the distribution and localization of aluminum (Al) released from Al implants inserted subcutaneously in rats.

Materials and Methods

A piece of the aluminum foil (3 mm in width, 8mm in length, and 0.3 ìm in thickness) was implanted into the subcutaneous loose connective tissue in the dorsal thoracic region of 6-week-old Wistar rats under pentobarbital anesthesia. After 1-24 weeks, the rats were anesthetized with pentobarbital and were perfused transcardially with physiological saline followed by 4% paraformaldehyde in 0.05 M phosphate buffer. After fixation, tissue blocks were taken and embedded in paraffin for light microscopy or in Spurr·s resin for analytical electron microscopy. Subcellular microprobe analysis was carried out in a JEOL 2000FX analytical electron microscope equipped with a TN 5500 EDX probe (Noran Instruments Inc., Middleton, Wisconsin). Histochemical demonstration of aluminum at a light microscopic level was undertaken using aluminon (the triammonium salt of aurin tricarboxylic acid). The production of hydrogen peroxide was histochemically investigated by the cerium method at an electron microscopic level.

Results and Discussion

Light microscopy of aluminum foil implants

Granulomatous inflammation was a distinct pattern of early responses to the aluminum foil after 1 to 2 weeks of the implantation. In two weeks after the operation, the implanted specimen was surrounded by epithelioid cells characterized by a modified epithelial-like appearance and frequently fused to form giant cells. Clusters of macrophages with cytoplasmic pigment granules were distributed around the implants. Multinucleated giant cells associated particularly with colloid-like structures, were also distributed in the granulomas. In contrast to acute responses to the implant, inflammatory cells decreased in number around the implant after 16-24 weeks.

Electron microscopy and Elemental analysis

The epithelioid cells encompassed the Al foil after two weeks of the implantation fused to form the multinucleated giant cells. However, phagosomes containing Al did not identified in the multinucleated giant cells. The other type of multinucleated giant cells appeared in the granulomas. These giant nucleated cells were characterized by the engulfment of large amorphous inclusions. In the amorphous inclusions, S was detected by EDX analysis but not Al. Therefore, these giant multinucleated cells may ingest the damaged tissues during inflammation after the implantation. Two types of mononuclear phagocytic cells were found in the granulomas surrounding the aluminum foil implant. One consisted of outstretched cells containing numbers of cytoplasmic electron-opaque phagosomes in which a large amount of Fe and trace amounts of P, S, Cl, and Ca were detected by the EDX analysis. The other type consisted of variable rounded-to-elongated cells containing cytoplasmic electron-opaque, fragment-like phagosomes. Because Al was detected predominantly in phagosomes, these cells are believed to phagocytize the fragments of the implanted aluminum foil.

Histochemical staining for hydrogen oxide and aluminum

One of the mechanisms by which the implanted aluminum foils are collapsed may be the production of free radicals, superoxide anions, and hydrogen peroxide. H_2O_2 is visualized by Ce deposits by electron microscopy. Ce deposits were not localized in the multinucleated giant cells and epitheloid cells surrounding the implant. However, electron opaque Ce deposits were localized on the cell membrane of the young mononuclear macrophages indicating the production of H_2O_2 with decomposition of the Al foil into pieces small enough to be engulfed by phagocytic cells. Aluminon reacts with Al^{3+} forming the reddish lake. The positive reaction was found only in the mononuclear macrophages in the granuloma around the implant. No positive reaction was observed in the multinucleated giant cells surrounding the implant. The mononuclear macrophages reacted with aluminon may be able to change the metal into the ionized state.

Conclusions

1. Foreign body granulomas were induced in rats by the implantation of Al foil into the subcutaneous layer in the dorsal thoracic region. Within 2 weeks, the implanted Al foil encompassed by epithelioid cells and giant multinucleated cells in which no phagosomes containing Al were found by EDX analysis. Therefore, the epithelioid cells and multinucleated cells encompassed the implant may not be associated with phagocytosis of the Al foil.
2. Many multinucleated giant cells were present in the granulomas and were associated particularly with electron-lucent, colloid-like structures in their cytoplasms. However, Al was detected in neither the giant cells nor the colloid-like structures. Because a prominent S peak was detected in the colloid-like structures by EDX analysis, these structures may represent degenerated tissues engulfed by the multinucleated giant cells in the granulomas. There were two

types of mononuclear macrophages in the granulomas: histiocyte-type macrophages and activated macrophages. The histiocyte-type macrophages were flat and contained many electron-dense phagosomes in which EDX analysis detected a high amount of Fe but no Al. Therefore, the histiocyte-type macrophages may be old cells which engulfed the degenerated erythrocytes after bleeding during the operation. The other activated macrophages were round with many cytoplasmic protrusions and an abundance of phagosomes. Because the fragments of Al foil were found only in the activated macrophage, these cells may play an important role in removing dissolved Al.

3. The H_2O_2 production was detected on the mononuclear macrophages, but not the multinuclear giant cells surrounding the implant. Ionized aluminum also localized in the mononuclear macrophages, while the multinuclear giant cells were devoid of reaction. From these histochemical experiments, the mononuclear macrophage may dissolve the metallic implant.