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Light- and transmission-electron-microscopic investigations on distribution of CD44, connexin 43 and actin cytoskeleton during the foreign body reaction to a nanoparticular hydroxyapatite in mini-pigs

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ABSTRACT

Foreign body giant cells (FBGCs) are formed by fusion of mononucleated macrophages during the foreign body response to a nanoparticulate hydroxyapatite (HA) implanted in defects of mini-pig femura. The molecular mechanisms underlying the formation of FBGCs are still largely obscure. Here we propose connexin 43 (cx43) and CD44 as candidate molecules involved in the fusion process. Immunohistochemistry and ultrastructural immunogold labeling indicated that cx43 is present within the ruffled border of FBGCs and is the main component of gap junctions formed between fusing macrophages. CD44 was strongly expressed during clustering and fusion of mononucleated macrophages. FBGCs adhering apically at the implanted HA showed CD44 reactivity only along the basolateral aspects of the plasma membranes, while podosome formation was observed within the sealing zone and ruffled border. Taken together, these findings demonstrate that cx43 and CD44 are part of the fusion machinery responsible for the formation of FBGCs. Furthermore, the results of microfilament and cx43 labeling suggest a functional role for podosomes and hemi-channels in biomaterial degradation.

1. Introduction

The formation of multinucleated osteoclast-like foreign body giant cells (FBGCs) in response to implantation of medical devices, prostheses, or biomaterials is a common process occurring during the host response. The main causes of formation of FBGCs are the surgical procedure in the course of implantation and the subsequent cell reaction to the surface of the implanted foreign material. Since the biocompatibility of implanted materials depends on the degree of foreign-body response, it is essential to gain knowledge on the cellular mechanisms leading to FBGC formation after implantation of any medical device [1–3].

FBGCs derive from mononucleated precursors which are members of the monocyte–macrophage lineage [4]. The differentiation into multinucleated cells requires fusion of the mononuclear precursors. Although several molecules and key factors, such as M-CSF and RANKL, have been shown to be involved in the fusion process, the entire mechanism responsible for the formation of FBGCs remains to be elucidated [5–7].

In a previous study we determined a putative role of gap junctional communication in the formation of FBGCs by analyzing the expression pattern of cx43 in mononucleated macrophages and multinucleated giant cells [8]. For this purpose, we used an in vivo model where nanoparticulate hydroxyapatite (HA) was implanted into defects of mini-pig femura. The occurrence of cx43 m-RNA and -protein in FBGCs was attributed to the presence of gap junctions, which were identified at the ultrastructural level between adjacent FBGCs, as well as between neighboring mononucleated macrophages and FBGCs 20 days after implantation [8]. The present study is based on the same in vivo model and aims at further elucidating the ultrastructural distribution of cx43 in mononucleated macrophages and multinucleated FBGCs by means of immunogold labeling.

CD44, an integral membrane protein that binds to hyaluronic acid [9], has been shown to be responsible for cell fusion in multinucleated cells of the monocyte–macrophage lineage. However the mechanism by which this receptor regulates cell fusion is poorly understood [7,10,11]. It has been recently shown in vitro that CD44 is spatially associated with the podosome core [12,13], suggesting a role for both microfilaments and CD44 in cell adhesion [14,15] and in biomaterial-associated formation (i.e. fusion) of

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