Time-dependent cytokine expression in bone of experimental animals after hydroxyapatite (Hap) implantation

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Abstract. Proinflammatory cytokines mediate bone loss around the implants in patients with peri-implant disease. However, there is no complete data about the expression of cytokines into the bone around the implants. The aim of this work was to investigate the distribution and appearance of inflammatory cytokines and anti-inflammatory proteins in the bone of jaw of experimental rabbits in different time periods after HAp implantation. Material was obtained from 8 rabbits in lower jaw 6 and 8 months after HAp implants were placed. Tissues were processed for immunohistochemical detection of tumor necrosis factor alfa (TNFα), Interleukin 1, 6, 8, 10 (IL-1, IL-6, IL-8, IL-10) and defensin 2. Results demonstrated practically unchanged expression of IL-6 and IL-10 between both – experimental and control side 6 months after implantation, while IL-1 and IL-8 notably increased in control side. IL-1 and IL-10 expression did not change in either the experimental side nor the controle side after 8 months HAP implantation, but IL-6 and IL-8 demonstrated a decrease in the control sites. Only IL-8 was elevated with time in experimental sites, while IL-10 showed individual variations in 2 cases.

1. Introduction

Inflammation followed by bone loss after the implantation is one of the complications after biomaterial implantation into the hard tissues. Proinflammatory cytokines are known to increase in crevicular fluid and mediate bone loss in patients with peri-implant disease.

Levels of IL-6 and IL-8 in the peri-implantant disease patients is reported to be initiated by the interleukins like IL-17 [1, 2]. Other authors have reported elevation in the levels of interleukins in patients with peri-implant disease [3]. IL-1 is suggested to increase bone loss, while IL-10 mainly displays anti-inflammatory properties and inhibits the bone resorbtion [4]. Very little is known about the interactions between the cytokines and defensins – small cationic peptides that are expressed by cells and provide antimicrobial defence by usuration of microbial membranes [5]. Despite the two main groups of defensins, mammalian cells mainly demonstrate beta defensin expression blocking inflammatory reactions by chemotactic, immunomodulating and cytotoxic activity [6].

There is controversy over the effect of interleukins in relation to their appearance in bone in different time periods after the implants and the ability of the same bone cells to express antimicrobial proteins that provide the anti-inflammatory defence. Thus, the aim of this experiment was to research

the changes in distribution and appearance of inflammatory cytokines and anti-inflammatory proteins in the bone of jaw of experimental rabbits after HAp implantation in different time periods.

2. Materials and methods

The Animal Ethics Committee of Latvian Food and Veterinary Administration approved the use of 8 New Zealand male rabbits for this morphofuncional study. The rabbits received general anaesthesia with Ketamini 15 mg/kg and Midazolami 0.5 mg/kg i/m and additional local anaesthesia with Sol. Lidocaini 2% (4 mg/kg). Intraoseal implantation of HAp granules in rabbit lower jaw was performed.

After raising a mucoperiosteal flap in the angular part of right side lower jaw a channel was drilled using 2.0 mm diameter trephine and HAp granules were inserted. HAp granules were produced in Riga Technical University Biomaterial Innovation and Development centre. We used 0.1 g 1000 – 1400 μm HAp granules with porosity 53 %. The subdermal layer was closed with 4-0 Vicryl and the mucosa with 5-0 prolene sutures. The control site was similarily prepared and closed but no HAp granules were placed. After 6 and 8 months euthanasia by air embolisation was performed.

Blocks of bone and soft tissue from experimental and control side were harvested with diamond disc, fixed in Stefanini solution. Then tissues were embedded into the paraffin, cut in 5 μm thick slides and prepared for detection of the interleukins: Interleukin 1 (IL-1, mouse, working dilution, 1: 1000, abcam, UK), Interleukin 6 (IL-6, mouse, working dilution 1:50, Santa Cruz Biotechnology, Inc), Interleukin 8 (IL-8, goat, Santa Cruz Biotechnology, Inc), Interleukin 10 (IL-10, rabbit, working dilution 1:400, abcam, UK), tumor necrosis factor alfa (TNFα, working dilution 1: 100, abcam, UK) by use of Hsu et al. [7] biotin-streptavidin immunohistochemistry. Innate immunity in rabbits' bone was detected by β defensin 2 (working dilution 1: 100, R and D systems, UK). Routine staining for haematoxylin and eosin was performed for each case. Structure quantification using a semi-quantitative counting method was made [8].

3. Results

Routine histological examination showed thickening of endomysium and sclerotisation of arteries (Figure 1) around the implant – in 6 and 8 months after the HAp implantation. The border between the bone and biomaterial was uniform and filled by proliferative connective tissue. However, detachment of tissue from the HAp at some sites was observed (Figure 2).

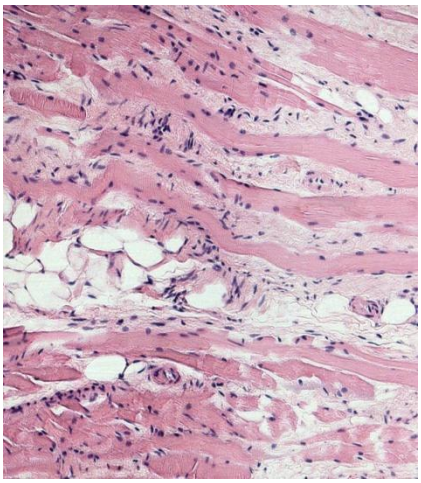

Figure 1. Note sclerotisation of small arteries and thickening of endomysium in tissue around the 6 month HAp implant. Haematoxylin and eosin, X 250

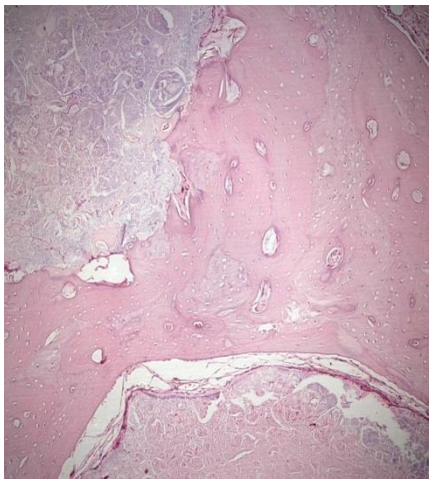

Figure 2. Border between the 8 month HAp implant and connective tissue showing irregular detachment. Haematoxylin and eosin, X 160

TNFα-containing structures were not detected in any of bone of experimental and control side. Number of IL-1 positive bone cells in bone 6 and 8 months at control sites showed no increase, while the experimental side of 8 months demonstrated notable increase of positive cells (Figure 3). IL-6-containing bone cells were seen in moderate numbers in experimental bone at all implantation times, but in control side this cytokine expression varied from few positive osteocytes to an abundance of patchy distributed cells (Figure 4).

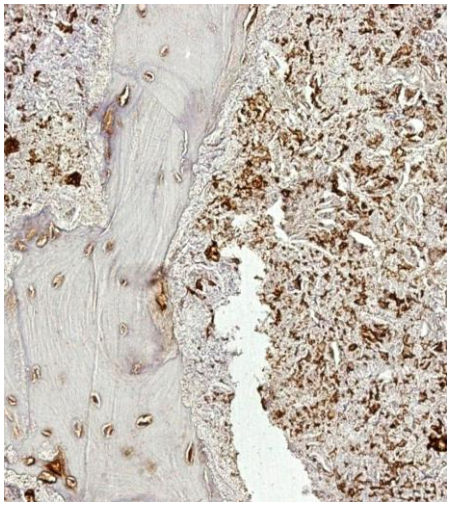

Figure 3. Moderate number of IL-1-containing osteocytes in bone of experimental side 8 months after HAp implantation. IL-1 IMH, X 250

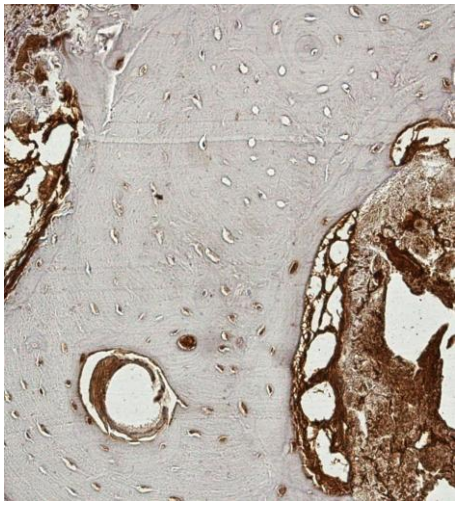

Figure 4. Moderate number of IL-6-containing osteocytes in bone of experimental side 8 months after HAp implantation. IL-6 IMH, X 250

The number of IL-8 expressing osteocytes was very similar at 6 and 8 months time intervals. However, experimental jaws showed increase of positive cells for this cytokine after longer HAp implantation in experimental side. Interestingly, main appearance of IL-8-containing cells was detected among the osteogenic cells. Finally, IL-10 positive cells were only few in bones after HAp implants of both implantation times in experimental and control side. However, with increase of implantation time number of positive cells expressing IL-10 showed also individual variations in 2 cases with sudden numerous patchy appearances of cells.

Defensin immunoreactivity was observed only in distance from HAp implants in experimental side 6 months after implantation and in few cells of control side after 8 months biomaterial implantation.

4. Discussion

Our experimental material showed a tendency in the increase in IL-1 and IL-8 expression in experimental side but an absence of TNFα. This may indicate the similarity of functions of both cytokines. These cytokines are known to stimulate osteoclast formation indirectly. Although IL-1 is absolutely essential for the bone resorbtion but IL-8 is suggested to be less potential for this purpose [1]. Some small variations in the distribution of cytokines, especially of IL-1 may be explained by earlier described polymorphism of IL-1 gene [9] and individual level of local defence mechanisms.

IL-6 showed a very slight elevated expression in both – experimental and control sites with an increase with time. This cytokine mainly affects the growth via altering of growth factor regulation systemically and locally [10]. The partially disturbed growth function in the tissue investigated by us may be due to the influence of surgical trauma rather than biomaterial effects as bone regeneration is reported to occur at least over 1 yr time interval [11].

IL-10 expression in bone was unchanged after different implantation times except in two samples. As IL-10 is true anti-inflammatory cytokine [12] its absence suggests the absence of inflammation in the bone (further supported by absence of TNFα-containing structures) and suggests good biocompatibility between the HAp and supportive tissue. However, any variation of IL-10 level should be evaluated as individual local response from host tissue.

Defensin 1-3 was first described in the osteoblast cell cultures [13] with dominance of defensin 1 and 2. However, the expression of β defensin 2 notably increases in case of bone infection like osteomyelitis [14]. The nature of antigens might play a role and change the expressed pattern of defensins, for instance, the response of bone to infection raised by Gram negative bacteria is based on the secretion of β defensin 3 [15].

We suggest a role of defensin 1 and 2 in traumatic injury could be important in case of biomaterial implantation in the bone. The combination of these two factors may suppress the secretion of defensin in bone (indirectly proved by distanced expression of defensin from osteoblast/osteocytes implant sites) that allows restoration of bone to presurgical norms. At control sites the regeneration may be as compensatory response of non-affected tissue.

5. Conclusions

Bone in the presence of HAp implants expresses mainly IL-8, IL-1 and IL-6, while IL-10 shows limited expression independently on implantation time that proves the absent anti-inflammatory (IL-10) and slightly increased anti-resorbtion (IL-1, -6, -8) action of bone around the implants realized on basis of various, but mainly indistinct presence of antimicrobial proteins.

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Acknowledgments

The European Social Fund within the project "Multidisciplinary Research in Biomaterials Technology of New Scientist Group", No.2009/0199/1DP/1.1.1.2.0/09/APIA/VIAA/090.