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ORIGINAL ARTICLE

Macrophage in periodontal inflammation

Makrofagi w zapaleniach przyzębia

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Abstract

***Aim of the study**. To investigate the expression of CD68 and HSP90AA1 in periodontal inflammation. **Methods**. A total of twenty-seven patients (giant-cell epulis) and thirty patients (acute and chronic inflammations) have been examined for expression of CD68 and HSP90AA1 by hematoxylin – eosin and immunohistochemistry. **Results**. Strong giant-cell positivity for CD68 was observed in 100% of patients whereas only 85.31% of giant-cell was positive for HSP90AA1 ($p<0.05$). Strong macrophage immunoreactivity for CD68 was figured in acute 23.2±1.3%, and in chronic 83.1±5.6% ($p<0.05$) inflammation. Immunoreactivity for HSP90AA1 was fixed in acute and chronic inflammation 98.2±1.79%. **Conclusion**. Inflammatory macrophages are a group of cells with protective functions, macrophages being more set up in chronic inflammation. Central giant cell epulis is the multinucleated macrophages, formed as a result of chronic inflammation. Population of macrophages resulted in bone resorption.*

Streszczenie

***Cel pracy.** Celem tego badania było zbadanie ekspresji CD68 i HSP90AA1 w zapaleniu przyzębia. **Metody.** 27 pacjentów (nadziąślak olbrzymiokomórkowy) i 30 pacjentów (ostre i przewlekłe stany zapalne) zostało zbadanych pod kątem ekspresji CD68 i HSP90AA1, hematoksyliną – eozyną i immunohistochemicznie. **Wyniki.** Silne oddziaływanie nadziąślaka dla CD68 zaobserwowano u 100% pacjentów, natomiast dla HSP90AA1 ($p<0,05$) tylko u 85,31%. Wysoka immunoreaktywność makrofagów dla CD68 została oznaczona w ostrym stanie zapalnym na 23,2±1,3%, a w przewlekłym 83,1±5,6% ($p<0,05$). Immunoreaktywność dla HSP90AA1 została ustalona w ostrych i przewlekłych stanach zapalnych jako 98,2±1,79%. **Wniosek.** Zapalne makrofagi są grupami komórek o funkcjach ochronnych, makrofagi występują częściej w przewlekłym zapaleniu. Nadziąślak olbrzymiokomórkowy to wielojądrowe makrofagi powstałe w rezultacie przewlekłego zapalenia. Populacja makrofagów skutkuje resorpcją kości.*

KEYWORDS:
CD68, HSP90AA1, immunohistochemistry, oral giant-cell epulis, inflammation, macrophages

HASŁA INDEKSOWE:
CD68, HSP90AA1, immunohistochemia, nadziąślak olbrzymiokomórkowy, zapalenie, makrofagi

Introduction

Central giant cell granuloma was first described by Jaffe in 1953. It is an uncommon, benign and proliferative non-neoplastic process.[1] It occurs more frequently in women than in men, with a slightly higher prevalence in the 30- to 70-year-olds.[2]

CD68 is a glycoprotein (monocyte and macrophage) but is relatively non-specific. It also can express in Langerhans cells, myeloid cells, dendritic cells, fibroblasts and others.[3] Morphologically, inflammation has observed as area with monocytes and macrophages. Some

inflammation has a low density of CD68 positive immunoreactivity.

Heat shock proteins (HSPs) or stress proteins (SPs) are a series of important molecular chaperones, which are expressed in the cell membranes, nucleus and cytoplasm.[4] Polypeptide chain must be protected during inflammation[5] in the case of: biosynthesis process (polypeptide chain attached to the ribosome), after the separation from the ribosome (protein not yet acquired tertiary structure), in a compact intermediate state and if "false" folding takes place.

Jennifer A Onyimba et al.[6] found that Hsp90 mRNA was more highly expressed in males. Among the genes expressed with high amplitude, *Maren Keller* et al.[7] found the member of the stress response protein Hsp90aa1.

We have tested 27 giant cell granuloma cases and 30 various types of inflammation cases on a couple of markers such as CD68 and HSP90AA1 to assess the origin of stromal cells and multinucleated giant cells.

Methods

The study samples included the periodontal and epulis tissues of patients. The subjects were divided into two equal groups:

Patient Group (Group 1) consisted of 27 subjects who had a morphological diagnosis of giant cell granuloma.

Control Group (Group 2) consisted of 30 patients who had died in Sumy Regional Hospital. The patients had various diagnoses (not atherosclerotic ones).

The study population included 27 patients with giant-cell epulis. Only the patients with the available tissue represented a subset of the overall study cohorts.

Hematoxylin and eosin (H&E) stains have been used for at least a century and are still essential for recognizing various tissue types and the morphologic change.

Immunostainings for CD68 and HSP90AA1 have been performed on formalin-fixed (pH 7,4), paraffin-embedded thyroid tissue sections using mouse monoclonal anti-CD68 and anti-HSP90AA1 (Thermo Fisher Scientific, UK). Briefly, 4μm thick tissue sections were dewaxed in xylene and were brought to water through graded alcohols. Antigen retrieval was performed by microwaving slides in 10mM citrate buffer (pH 6.2) for 30 min at high power, according to the manufacturer's instructions. To remove the endogenous peroxidase activity, sections were treated with freshly prepared 1.0% hydrogen peroxide in the dark for 30 min at 37° C temperature. Non-specific antibody binding was blocked by means of blocking serum. The sections were incubated for 30 min, at 37°C temperature, with the primary antibodies against CD68 and HSP90AA1 diluted 1:100 in phosphate buffered saline (PBS) pH 7.2, a triple washing with PBS. Anti-(mouse IgG)–horseradish peroxidase conjugate (1:40 000 dilution) was used for the detection of the CD68 and HSP90AA1 primary antibodies, then the sections were incubated for 20 min, at 37°C temperature. The colour was visualized by DAB.

The appearance of the positive factors was detected semiquantitatively by counting positive giant cells in the field of vision.

The data were analysed using STATISTICA 8.0 software, user version STA862D175437Q. The results were presented as average values (±SD). The K-S test was used in order to estimate the normality of the data. Also, the Student method was used to perform a simple comparative analysis. The value of $p<0.05$ was considered significant.

Results

Groups 1 and 2 of men and women consisted mostly of 30- to 70-year-olds. Group 1 giant cells occurred in the lower jaw (55%) more frequently than in the upper jaw. In group 2 patients were divided into 13 with acute and 17 with chronic inflammations

We have observed low-size cell infiltration in acute inflammation (Fig. 1-I). Significant cell infiltration and proliferating epithelium (Fig-II) appeared more intensive in the chronic inflammation.

Peripheral giant cell epulis have shown in Fig. 1-III. Microscopic examination revealed the tissue with the abundance of giant-cell (Fig. 1-III F), fibrous connective tissue, areas of haemorrhages

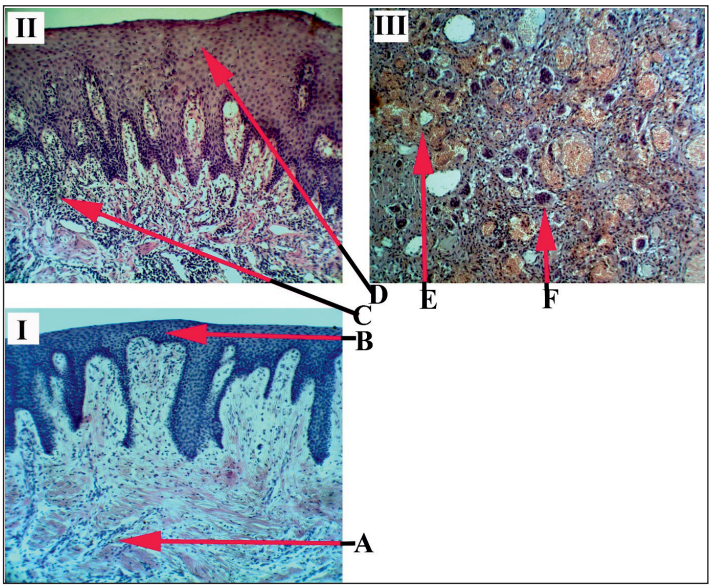

Fig. 1. *Haematoxylin- and Eosin-stained periodontal tissues (x100 magnification) A – pint-size cells infiltration with superimposed edema, B – layers of the epithelium, C – great cells infiltration with superimposed edema, D – epithelial proliferation, E – hemorrhage zone, F – giant cells.*

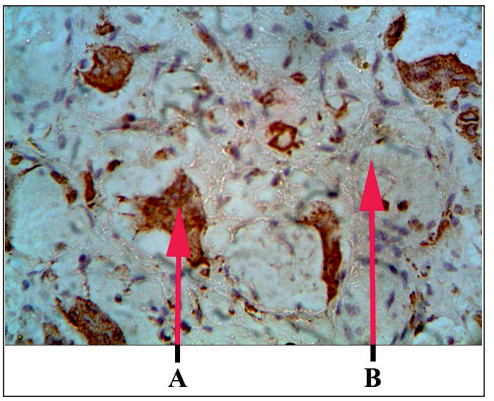

Fig. 2. *Expression of CD68 proteins in giant-cell epulis (x400 magnification) A – giant cells CD68 "+", B – Fibroblastic stroma CD68 "-".*

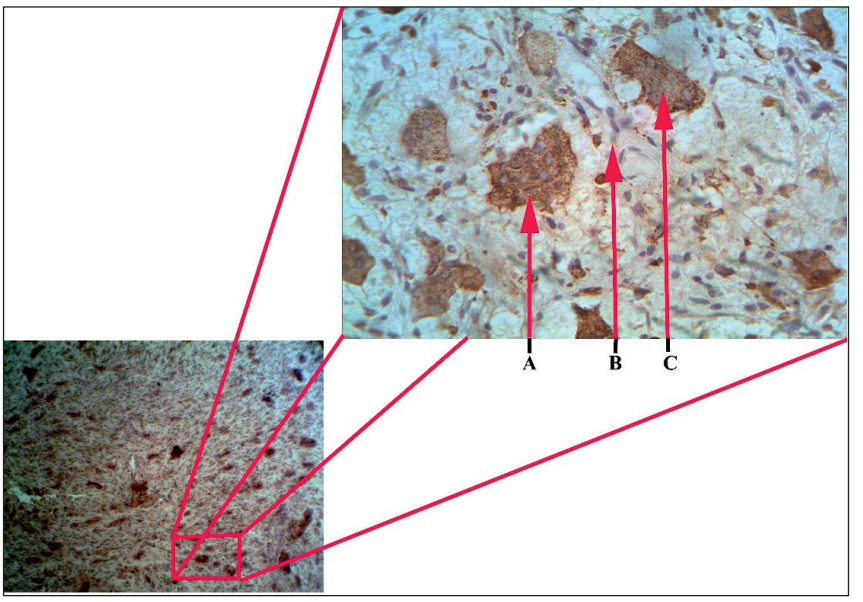

Fig. 3. *Expression of HSP90AA1 proteins in giant-cell epulis, (x100 -x 800 magnification) A – giant cells of HSP90AA1cytoplasmic "+", B – fibroblastic stroma HSP90AA1 "+, -", C – giant cells of HSP90AA1 nucleus "+".*

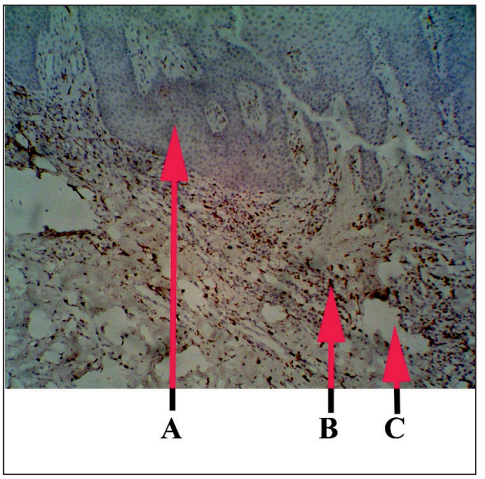

Fig. 4. *Expression of CD68 in gingiva, group 2, acute inflammation (x100 magnification) A – cells of epithelium, B – neutrophil and macrophage infiltration, C – pericellular and perivascular edema.*

(Fig. 1-III E) and capillaries. There was no sign of malignancy.

CD68 and HSP90AA1 have expressed in giant-cell. Expression of CD68 and HSP90AA1 in giant-cell epulis can be seen in Fig. 2 and 3. By immunohistochemistry, 100% of giant-cells appeared to be positive for CD68, whereas only 85.31% of giant-cells were positive for HSP90AA1 ($p<0.05$). Induction of the enzymatic activity of HSP90AA1 was increased by the stressor. This could be explained by the fact that HSP90AA1 is physiologically expressed by the giant-cells, HSP90AA1 expression being weak or absent in the connective tissue.

The immunoexpression of CD68 (Group 2) was confirmed by the presence of the brown stained

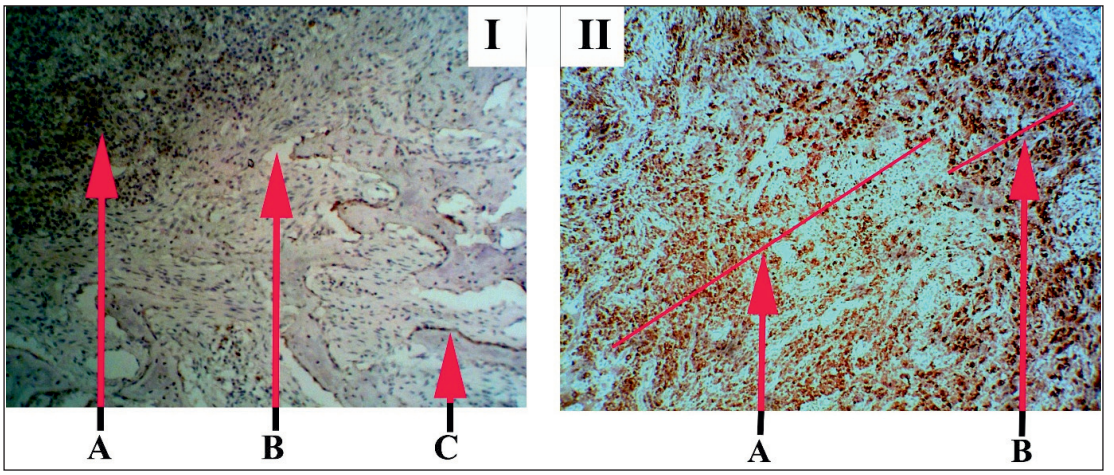

Fig. 5. *Expression of CD 68 in gingival tissue, group 2, chronic process (x100 magnification) I – A - Blood cells infiltration, B – pericellular and perivascular edema, C – macrophage bone resorption. II A – zone of neutrophil differentiation B – under epithelial zone of macrophage.*

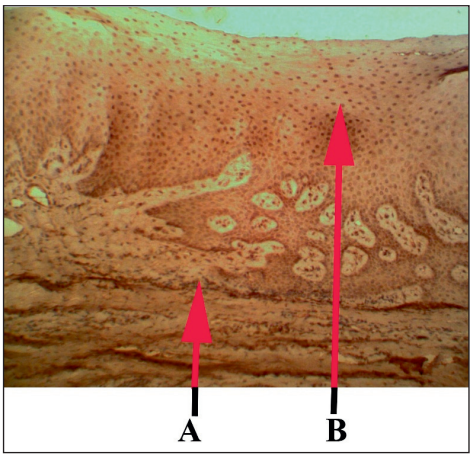

Fig. 6. *Expression of HSP90AA1, group 2 (x100 magnification) A – blood cells infiltration, B – layers of the epithelium (nuclear and cytoplasmic HSP90AA1 positive).*

cytoplasm in cell infiltration. In general, CD68 staining was more intensive in the monocytes and macrophages. In acute phase CD68 immunoexpression (Fig. 4) have only 23.2±1.3% of the cells being positive. Cell infiltration, immunoreactivity of CD68 was 83.1±5.6% ($p<0.05$) in the chronic process (Fig. 5-II A, B).

The results of the chronic process is shown in Fig.5-I. Widespread macrophages bone resorption (Fig. 5-I C) with the increasing width and depth of pocket which forms deep intrabony pocket. There were plenty of young vessels, loose edematous stroma (Fig. 5-I B) in the intrabone pockets.

There was no difference between levels in

HSP90AA1 in acute and chronic process of inflammation (Fig. 6). HSP90AA1 was expressed in group 2 at the primary invasion in all specimens examined. The immunoexpression of HSP90AA1 in epithelial layers has showed the result of 98.2±1.79% positive cells in acute and chronic process of inflammation. HSP90AA1 positive cells (63.34±2.84% $p<0.05$) in monocyte and macrophage infiltration during acute and chronic process of inflammation.

Discussion

The macrophages in periodontal tissues during inflammation are heterogeneous with respect to phenotype as well as function.[8]

Neutrophils and macrophages coexist at the site in periodontal tissues inflammation, yet the evidence has indicated that these cells interact to perform disparate functions. The neutrophils produce destructive oxidizing agents in the absence of other WBCs, such as macrophages.[9] This explains the number of macrophages in acute inflammation in our studies

Bone changes exhibit a chronic inflammatory and macrophage response in addition to recurrent membrane disruption and failed cellular repair. A bone has a fragile structure, resulting in frequent membrane disruptions after the slightest mechanical stress or strain. However, it is now becoming clear that chronic inflammation is an important aspect of the cyclic disruption of bone. In chronic infiltrate, we have observed a small number of dual-nucleus macrophages. This demonstrates the transition of chronic inflammations into giant cell granuloma.

The effect of the stimulation of the monocytes has induced into different subtypes: "M1" inflammatory macrophages and "M2" wound healing regulatory macrophages.[10] M2 macrophages show mostly pro-tumoral functions, promoting tumour cell survival, proliferation, and dissemination.[11] During embryogenesis, macrophage can express different of markers including lymphatic vessel endothelial hyaluronan receptor (LYVE-1) and the angiopoietin receptor Tie2.[12] The aforesaid explains the excessive vascularization of giant cell epulis. We can assume that giant cell epulis consists of giant cell type M2. M2 cells are working of the promote scavenging of debris, angiogenesis, remodeling and repair wounded/damaged tissues. Of note, M2 cells control the inflammatory response by down-regulating M1-mediated functions.[13,14]

We have found that HSP90AA1 are the most significant factors of prognosis in giant cell granuloma. As one of the most abundant proteins in non-malignant cells and a key factor that stabilizes proteins involved in giant cell granuloma growth, our results suggest that increased HSP90AA1 expression may play an important role in promoting the transition of chronic inflammation in giant cell epulis.

Conclusion

Inflammatory macrophages are a group of cells with protective functions, macrophages being more set up in chronic inflammation. Central giant cell epulis is the multinucleated macrophages, formed as a result of chronic inflammation. Population of macrophages resulted in bone resorption.

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