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Measurement of interleukin 1 alpha and 1 beta (IL-1 alpha and IL-1 beta) in human cystic lesions of the jaw. Implications for the pathogenesis of radicular cysts

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SUMMARY

Human radicular cystic tissue of jaws was found to contain between 0.823 pg/mg to 18.026 pg/mg interleukin 1 beta and from 0.34 pg/mg to 0.708 pg/mg interleukin 1 alpha.
No IL-1 beta and alpha could be found in specimens from healthy patients. A finding which may be extremely relevant in cystic growth and episodes of alveolar bone resorption around the cystic lesion.

KEY WORDS:

IL-1 alpha and beta, radicular cysts, human jaw.

RÉSUMÉ

Les tissus des kystes radiculaires chez l'homme contiennent 0,823 à 18,026 pg/mg d'interleukines 1 beta et 0,34 à 0,708 pg/mg d'interleukines alpha. Il n'a pas été trouvé d'IL-1 beta et alpha dans des prélèvements effectués chez des individus sains. Cette constatation peut avoir des conséquences importantes en relation avec la croissance du kyste et la résorption de l'os alvéolaire avoisinant.

MOTS CLEFS:

IL-1 alpha et beta, kystes radiculaires, maxillaires.

INTRODUCTION

Pathologic mechanisms responsible for the growth of odontogenic cysts are not yet fully clarified.

Cystic expansion, characterized by local bone resorption, was first attributed to hydrostatic pressure (James and Connsell, 1932; Toller, 1970) but new sophisticated theories support the importance of soluble mediators inducing resorptive processes of bone cells.

Several bone resorbing factors have been identified, including prostaglandin E 2 (Raisz and Klein, 1970), leukotriens (LTs) and hydroeicosatetraen acids (HETEs) (Meghji et al., 1987), all of which could be isolated from dental cysts (Harris et al., 1973; Matejka et al., 1985). However, bone resorption induced by these cytokines could only be partially inhibited by the inclusion of cyclooxygenase - (Sato et al., 1986) and lipoxygenase (Thomson and Chambers, 1985) inhibitors. This indicates that in addition to arachidonic acid products other mediators may account for the bone resorbing activity found in dental cysts.

Interleukin 1 is a key mediator of various immunological and inflammatory processes (Dinarello, 1988). Moreover, interleukin 1 is one of the most potent

factors known to stimulate bone resorption and IL-1 beta is identical to «osteoclast activating factor» (OAF) (Dewhirst et al., 1985).

Radicular cysts are infiltrated by chronic inflammatory cells (Harvey et al., 1984) and in view of the fact that interleukin 1 is particularly associated with chronic inflammatory lesions such as rheumatoid arthritis (Nouri et al., 1984) and periodontal disease (Hönig et al., 1989), the purpose of the present study is to demonstrate interleukin 1 activity in tissue specimens derived from radicular cysts. For quantification and single determination of the interleukin 1 alpha subclasses and beta a new developed two-site directed Elisa was used.

MATERIAL AND METHOD

10 radiologically verified dental cysts from different patients were enucleated under local anaesthesia and the specimens divided into two parts (Fig. 1).

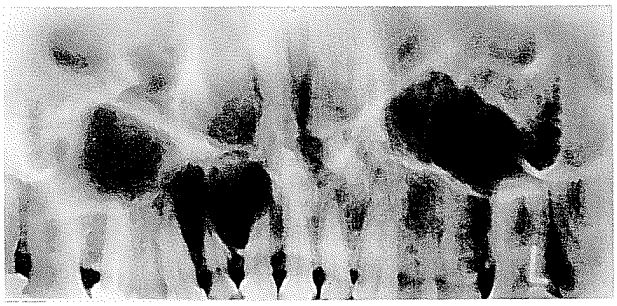

Fig. 1: X-ray of an clinical example of radicular cyst, where tissue specimens was taken. Note the bone resorption.

Fig. 1: Radiographie d'un kyste radiculaire. Noter la résorption osseuse.

Portions of each lesion were immediatelly sealed in plastic screw-top vials and placed in a container with dry-ice and kept frozen until the time of assay.

For diagnosis, the other parts of the specimens were embedded in paraffin wax after fixation in formalin, stained with haematoxylin-eosin and examined histologically using routine techniques.

Samples of tissue from the cyst wall were minced with a scalpel into small pieces and subjected to a sonification step followed by three cycles of freeze-thawing in order to extract extracellular as well as cell-associated interleukin 1.

Eleven specimens from healthy control subjects were obtained during surgical extraction of retained but non inflammed impacted third molars and were analysed for IL-1 alpha and IL-1 beta by the same procedure used for cystic materials.

IL-1 alpha and IL-1 beta concentrations were determined with a two-site directed ELISA. Measurement were done blindly on coded samples.

SPECIMEN HANDLING

In order to extract the IL-1 contained in the specimen (extracellular as well as cell-associated IL-1) tissue samples were subjected to a sonification step followed by three cycles of freeze-thawing. Tissue specimens were first thawed, then weighed and suspended in 0.5 ml sterile phosphate buffer (PBS, pH 7.2, 13 mM phosphate, 0.15 M NaCl) for 30 min at + 4° C and centrifuged at 2000 g for 10 min. The supernatant was collected for IL-1 beta determination. The tissue pellet was resuspended in 0.5 ml PBS and sonicated for 2 min at + 4° C using a Branson sonifier cell disruptor B15 set at 40 watt. The tubes were centrifuged, the supernatant collected for IL-1 determination and the pellet was resuspended in 0.5 ml PBS and taken through a cycle of freeze (−20° C) thawing (+37° C) and then centrifuged. The supernatant was collected for IL-1 determination. Two further cycles of freeze-thawing were performed. The amount of IL-1 beta contained in samples A to E was summed and is referred to as total IL-1 beta and expressed as fg per mg wet weight of the original tissue specimen.

Antibodies to human IL-1 beta

A mouse monoclonal anti-human IL-1 beta antibody (antibody 2D8, IgG1 kappa) was obtained from a fusion of the myeloma line P3x63-Ag 8.653 with spleen cells of a Balb/c mouse previously immunized with recombinant human IL-1 beta (rhIL-1 beta, Biogen, Switzerland). A polyclonal anti-rhIL-1 beta was obtained by immunizing a rabbit (Rabbit 203) with 12.5 ug rhIL-1 beta in complete Freund's adjuvant whereafter 11 booster injections of the same material in incomplete Freund's adjuvant were given 4 wk. Both antibodies were checked for their ability to inhibit the biological activity of IL-1 beta in various assays, (IL-1 induced IL-2 production in the EL 4 cells (Dinarello and Savage, 1989), IL-1 receptor binding (Dinarello, 1988) and for their specificity. They were shown to react with the secreted mature 17 kD as well as with the cellassociated 31 kD prescursor forms of human IL-1 beta.

ELISA for the quantification of IL-1 beta

IL-1 beta concentrations were determined in a two-site-directed ELISA. Measurements were performed

blindly on coded specimens. Polystyrene Immulon flat-bottomed plates (Dynatech) were coated with the monoclonal antibody 2D8 (100 µl/well, 10 µg/ml in 0.3 M NaHC03) by overnight incubation. Plates were washed with water and 100 µl of samples or known amounts of rhIL-1 were added in duplicate to the wells. Samples were tested at a 1/2 dilution and rhIL-1 beta was added at concentrations between 7.5 -15 pg/ml to 1000 pg/ml. Dilutions were carried out in ELISA buffer (PBS) supplemented with 0.5% bovine serum albumin, 0.05% brij, 0.05% thimerosal). After 3 h of incubation at room temperature, the plates were washed with water and incubated for a further 3 h at room temperature with 100 µl/well of a 1/5000 dilution in ELISA buffer of the rabbit anti-human IL-1 beta antibody. The plates were washed with water and incubated overnight with 100 µl/well of a peroxidase conjugated donkey anti rabbit immunoglobulins antiserum (1/5000 in ELISA buffer, Amersham). The plates were washed with water and developed with ophenylene diamine in citrate buffer. The reaction was stopped with 2.5 M H_2SO_4 and the color measured at 492 mm. Absorbance values of the samples were transformed into concentrations (pg/ml) by comparison with the absorbance values given by the various dilutions of the rhIL-1 beta using a four-parameter logistic fit equation (Immunosoft, Dynatech).

Statistical analysis

Correlation between the amount of IL-1 beta in a specimen and the gingival index or mean maximal attachment loss were assessed using the non-parametric Mann-Whitney U test and the Spearmann rank correlation coefficient. Results were considered statistically significant if $p < 0.05$.

RESULTS

Specificity, sensitivity and precision of the two-site-directed ELISA for the quantification of IL-1 beta.

As seen in Table I, the monoclonal antibody 2D8 and the polyclonal rabbit anti-human IL-1 beta antibody (Rabbit 203) used to develop the two-site directed ELISA were specific for IL-1 beta and did not react with IL-1 alpha. Although, as reported, the binding (Dewhirst et al., 1985) for I^{125}-labelled IL-1 alpha or IL-1 beta was displaced by a 100-fold excess of IL-1 beta (the 2 cytokines bind to the same receptor), only the binding of I^{125}-IL-1 beta to its receptor could be inhibited by the anti-human IL-1 beta antibodies 2D8 and 203. Conversely, the rabbit polyclonal anti-human IL-1 alpha antibody 201 only displaced the binding of I^{125} IL-1 alpha from its receptor.

Similar results were found in various bioassays. For example, the anti-human IL-1 beta antibodies 2D8 and 203 only neutralized the IL-1 beta-driven and not the IL-1 alpha-driven IL-2 production in the EL4 cells (Tab. II).

Furthermore neither human IL-1 alpha (up to 500 ng/ml) nor TNF alpha (up to 5 ng/ml) nor gamma interferon (up to 500 U/ml) nor IL-6 (up to 50 ng/ml) nor human immunoglobulins (up to 1 mg/ml) were detected in the two-site-directed ELISA for IL-1 beta. This assay is very sensitive and a concentration of IL-1 beta of 8 pg/ml (0,5 pM in a volume of 100 µl) was the lowest amount that could be repeatedly and accurately measured.

Within-assay precision was estimated at 10 different levels of rhIL-1 beta (range 8 pg/ml - 5 ng/ml) by 4 different experimenters. The coefficient of variation was found to be in the range of 1 to 10% and to increase with decreasing concentration of IL-1 beta. Inter-assay variability was estimated at 4 different levels of rhIL-1 beta (range 20 pg/ml - 5 ng/ml) and was in the range of 2% to 9%.

The linear part of the standard curve was from 50 pg/ml to 2000 pg/ml.

TABLE I:
Ability of the anti-hrIL-1 beta antibodies 2D8 and 203 or of the antihrIL-1 alpha antibody 201 to displace the binding of I^{125} IL-1 beta of I^{125} IL-1 alpha from their receptors
TABLEAU I:
Aptitude des anticorps anti-hrIL-1 beta 2D8 et 203 et des anticorps anti-hrIL-1 alpha 201 pour déplacer la liaison de I^{125} IL-1 beta ou de I^{125} IL-1 alpha de leurs récepteurs respectifs.

Inhibitor	Concentration	I^{125} IL-1 beta bound cpm (500 pM)	I^{125} IL-1 alpha bound cpm (50 pM)
I.Medium		900	1880
2D8	pure	62	1720
hybridoma	1/10	240	1810
(supernatant)	1/100		1890
	1/1280	400	
	1/2560	700	
IL-1 beta	50nM	62	270
II. Medium		1050	1130
203	1/500	100	1000
201	1/500	1125	110
IL-1 beta	50 nM	115	130

TABLE II: Ability of the anti-hrIL-1 beta antibodies 2D8 and 203 to inhibit the IL-1 beta-driven and not the IL-1 alpha-driven IL-2 production in the EL4 cells
TABLEAU II: Aptitude des anticorps anti-hrIL-1 beta 2D8 et 203 pour inhiber la production dans les cellules EL4 de IL-1 beta et IL-1 alpha

		Units/ml of IL-2 generated by the EL4 under stimulation with 3 pM of IL-1 beta or of IL-1 alpha	
Inhibitor	Concentration ng/ml	IL-1 alpha	IL-1 beta
I.Medium		100	70
2D8	0.050	100	70
	5	75	70
	50	50	—
	500	20	70
	5000	12.5	75
	50000	6	70
II.Medium		100	100
203	1/25	2	105
	1/50	2	85
	1/100	8	100
	1/200	56	100
	1/400	70	100

IL-1 DETERMINATION IN CYSTIC LESION TISSUE

The concentration of IL-1 alpha and IL-1 beta per sample are shown in Table III and IV. No IL-1 alpha and no IL-1 beta were detected in specimens from healthy subjects, irrespective of the original weight of the tissue sample.

In contrast, the two IL-1 subclasses were detected in all specimens derived from radicular cystic wall tissue. Concentrations ranged from 0.823 pg/mg to 18.026 pg/mg for IL-1 beta and from 0.134 pg/mg to 0.708 pg/mg for IL-1 alpha. There was a correlation between the amount of IL-1 alpha and IL-1 beta measured in each sample.

TABLE III: IL-1 alpha and IL-1 beta concentration for each healthy subject
TABLEAU III: Concentrations des IL-1 alpha et IL-1 beta pour chaque sujet sain

Subject	Total IL-1 alpha (Fg/mg of tissue)	Total IL-1 beta (fg/mg of tissue)	sample weight mg	tooth	sample	Sex	Age
SJ	undetected	undetected	95	11	1	m	22
FL	undetected	undetected	174	21	2	f	47
GG	undetected	undetected	190	22	3	f	34
PM	undetected	undetected	121	13	4	m	37
HL	undetected	undetected	271	11	5	m	21
HA	undetected	undetected	145	22	6	f	17
GW	undetected	undetected	149	21	7	m	42
WE	undetected	undetected	49	12	8	f	35
DE	undetected	undetected	618	11	9	m	19
HS	undetected	undetected	35	21	10	f	37
UK	undetected	undetected	31	22	11	f	24

TABLE IV: IL-1 alpha and IL-1 beta concentration for each diseased subject
TABLEAU IV: Concentration des IL-1 alpha et IL-1 beta pour chaque malade

Subject	Total IL-1 alpha (pg/mg tissue)	Total IL-1 beta (pg/mg tissue)	sample weight mg	sample	Sex	Age
HA	3.330	0.230	69	1	f	18
IL	2.730	0.352	67	2	f	22
KS	1.180	0.293	146	3	m	26
SJ	1.720	0.284	256	4	f	17
WW	0.823	0.134	185	5	m	14
HD	18.026	0.708	351	6	f	31
EB	1.982	0.320	120	7	f	21
LE	1.680	0.251	130	8	m	42
CI	2.501	0.342	70	9	m	27
AD	3.115	0.405	176	10	m	15

DISCUSSION

Increasing attention has been focused on the potential contribution made by inflammatory and immune cells, their soluble mediators and products to the pathogenesis of several bone and soft connective tissue diseases (Russel, 1987). One such cytokine, interleukin 1, is of special interest because this polypeptide can influence bone remodeling processes (Gowen et al., 1983; Evand, 1988). Two biochemically distinct but structurally related IL-1 molecules have been cloned and termed IL-1 alpha and IL-1 beta (March et al., 1985). They are coded by seperate genes and only share small stretches of amino acid homology but similar biological properties (Dinarello, 1988). In contrast to the precursor IL-1 beta cytokine (pro-IL-1 beta), pro-IL-1 alpha is active and comprises most of the membrane-bound form of interleukin 1, whereas IL-1 beta is the dominant extracellular form of interleukin 1 with parakine quality (Dinarello and Savage, 1989).

Our results clearly show the presence of increased amounts of IL-1 beta and IL-1 alpha, as measured by a sensitive immunoassay, in cyst wall samples and their absence in non-inflammed connective tissue specimens.

Interleukin 1 can be produced by virtually every nucleated cell type with the exception of red blood cells (Oppenheim, 1987). Although stromal fibroblasts and epithelial cells lining the cysts are possible sources of these cytokines, our data point to the macrophage/monocyte cell infiltrate as important producers of interleukin 1, since the quantitive distribution of the interleukin 1 subclasses is typical of activated mononuclear phagocytes (review in Dinarello and Savage, 1989). Additionally, it appears evident that despite seprate gene codation and activation, both genes are dependently transcribed which points to a virtually constant IL-1 beta to IL-1 alpha ratio in each sample. Since IL-1 beta is identical to OAF (Dewhirst et al., 1985) and is the more potent resorbing agent (Reynolds, 1988) and dominant form of interleukin 1 produced, the amount of IL-1 beta will be of great interest of the pathogenesis of odontogenic cysts. Once released, IL-1 beta induces several of direct and indirect local cellular interactions with osteolytic effects. Thomson and laboratory co-workers postulated that interleukin 1 needs the presence of osteoblasts to exert its activities on differentiated osteoclasts (Thomson et al., 1986). A cytokine stimulates osteoblasts to produce prostaglandin E_2 (PGE_2) (Tatakis et al., 1988) which in turn activates osteoclasts to resorb bone (Raisz and Martin, 1984). The use of the cyclooxygenase inhibitor indomethacin nevertheless revealed that interleukin 1 can also induce PGE_2 independent bone resorbing mechanisms (Thomson et al., 1986). Products of lipoxygenase transformation of arachidonic acid, the leukotriens and hydroeicosatetraenic acids are released by stimulated osteoblasts and were able to cause resorption in bone culture (Meghji et al., 1987); interleukin 1 induced bone resorption could only be partially reduced by lipoxygenase inhibitors (Thomson and Chambers, 1985).

In addition, interleukin 1 induces osteoblasts to produce collagenase (Heath et al., 1984) which is involved in the destruction of bone matrix and macrophage-colony-stimulating-factor (Sato et al., 1986) and which might be responsible for the recruitment of osteoclast precursor cells.

Gowen and Mundy showed in their study that interleukin 1 increases the number of activated osteoclasts (Gowen and Mundy, 1983), this enhances the bone resorbing rate. Similar results were published by Heath and colleagues (Heath et al., 1985).

Taken together, these facts point to different interleukin 1 dependent mechanisms resulting in loss of mineralized connective tissue. First, interleukin 1 induces osteoblasts to produce PGE_2 and perhaps LTs and HETEs which in turn stimulate differentiated osteoclasts and second, interleukin itself will induce the formation of multinuclear resorbing cells targets of osteoblast released cytokines.

Alternatively, factors other than interleukin 1 could possibly contribute to the bone pathology of radicular cysts (Reynolds, 1988). Furthermore, complex synergistic or antagonistic interactions between interleukin 1 and parathyroid hormone (PTH) and $1,25(OH)_2)D_3$ have recently been discribed (Tatakis et al., 1988; Muller et al., 1988), which have been known for a long time to play a pivotal role in the homeostasis of calcium and phosphate concentrations and in the maintenance of skeletal integrity. Although the mechanisms of the pathogenesis of radicular cysts are still under investigations, our study clearly points to a crucially important role of IL-1 beta in the complex process of pathological bone destruction associated with this type of cysts.

It is hoped that the availability of sensitive immunoassays for the quantification of cytokines and factors involved in the regulation of bone remodelling will shed more light on the complex interactions of these factors, and give way for the design of future drugs aimed at inhibiting the bone resorption characteristic of a number of chronic inflammatory diseases such as radicular cysts.

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