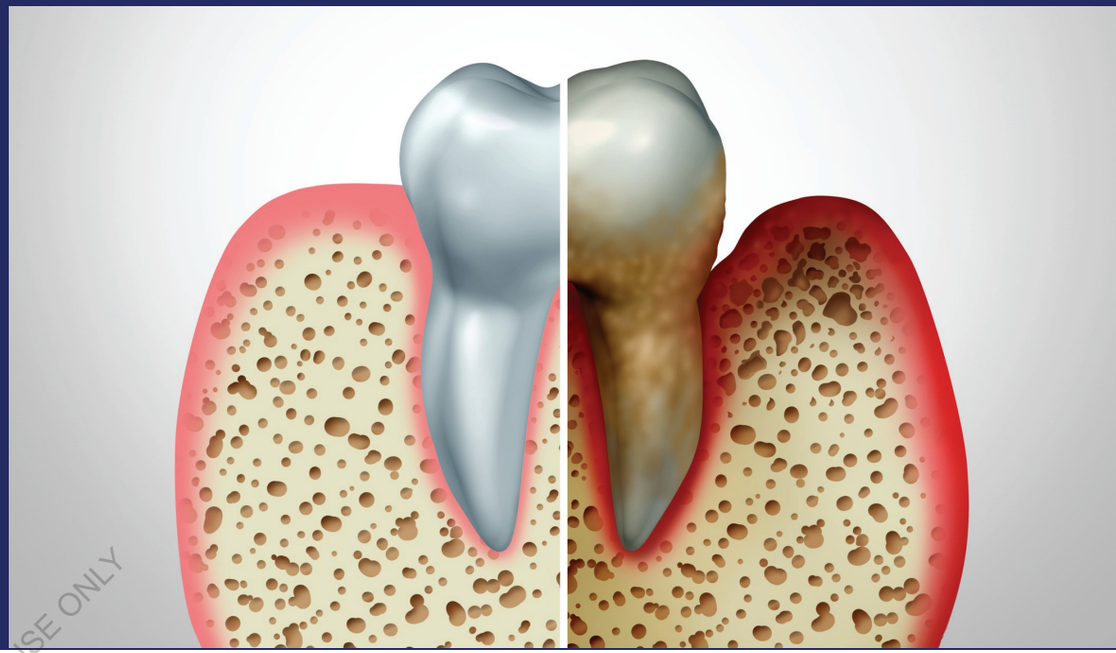
This book is a comprehensive guide to anti-inflammatory cytokines and their role in periodontics. It covers information on inflammatory processes involved in causation of periodontal disease, including the interplay between pro- and anti-inflammatory cytokines, description of anti-inflammatory cytokines specific to periodontics and their applications in periodontal disease activity. This book will help both graduate and post-graduate students in dentistry in familiarizing with the molecular processes behind the periodontal disease.



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LIST OF ABBREVIATION

IL-1β	Interleukin-1 beta
IFN-γ	Interferon-gamma
TNF-α	Tumor necrosis factor- alpha
TGF-β	Transforming growth factor- beta
IL-1ra	Interleukin-1 receptor antagonist
OPG	Osteoprotegerin
LPS	Lipopolysaccharides
GM-CSF	Granulocyte macrophage-colony stimulating factor
ELISA	Enzyme linked immunosorbent assay
NF-κB	Nuclear factor-kappa beta
RT-PCR	Reverse trancriptase- polymerase chain reaction

INTRODUCTION

Periodontitis is an inflammatory disease affecting the supporting tissues of teeth, where mainly the plaque bacteria are necessary to initiate disease and drive the chronic inflammatory response in the periodontal tissues. At the same time, there is strong evidence that destructive processes occurring as part of the host inflammatory response are responsible for the majority of the hard- and soft-tissue breakdown leading to the clinical signs of periodontitis.

In the presence of the microbial challenge, the susceptible host responds with an immediate inflammatory and immune response in order to control the challenge. Perpetuation of the host response further disrupts homeostatic mechanisms and results in recruitment of neutrophils, macrophages, and release of inflammatory mediators, such as eicosanoids[1], reactive oxygen species[2], matrix metalloproteinases (MMPs)[3], chemokines[4] and cytokines[5], which are directly responsible for periodontal disease pathogenesis.

Cytokines are defined as regulatory proteins controlling the survival, growth, differentiation, and functions of cells. The term cytokine, meaning "cell protein," is reserved for molecules which transmit information or signals from one cell to another. It is part of a fundamental, cell-to-cell communication network. They are produced not only by immune cells such as lymphocytes (especially T cells), monocytes, macrophages, and granulocytes, but also by epithelial cells, endothelial cells, and fibroblasts.

Cytokines generally fall under two categories-

Pro-inflammatory cytokines are: IL-1β, IL-2, IL-6, IL-8, IL-12, IL-17, IFN-γ, TNF-α

Anti-inflammatory cytokines are: IL-4, IL-5, IL-6, IL-10, IL-11, IL-13, TGF-β, IL-1ra

Periodontal health has been described as a dynamic state in which the pro and anti-inflammatory responses of the host are in balance to prevent unwarranted

inflammation and subsequent tissue destruction[6]. Under pathologic inflammatory conditions, such as periodontitis, the balance between pro- and anti-inflammation has been converted into pro-inflammatory activity.

The pro-inflammatory cytokines such as IL-6, TNF-α, and IL-1 enable the recruitment of cells towards infection sites, promote bone resorption, and stimulate prostaglandin release by monocytes, fibroblasts and release of metalloproteinases that degrade extracellular matrix proteins[7]. The anti-inflammatory cytokines IL-10 and IL-4, on the other hand, tend to attenuate the inflammatory response[8] and present a protective role toward tissue destruction by production of a group of endogenous proteins named tissue inhibitors of metalloproteinases (TIMPs), which are capable of inhibiting almost every member of the MMP family in a nonspecific way[4,9].

It is probable that the balance between pro-inflammatory and anti-inflammatory cytokines, together with the regulation of their receptors and signaling cascades, determines the level of periodontal tissue loss and regulates the homeostasis of the immune system. Therefore, future research is warranted to determine the causes disturbing this balance & therapeutic procedures have to be targeted to regain this balance.

GENERAL REVIEW

Cytokines are hormone-like small molecular weight peptides or glycopeptides. They regulate all of the important biological processes, such as cell proliferation, cell growth, cell activation, inflammation, immunity and repair. Members of the cytokine family include:

- Interleukins (formerly "lymphokines")
- Cytotoxic factors (tumor necrosis factor α and β)
- Interferons (anti-viral IFNα and β, "immune- IFNγ")
- Colony stimulating factors (CSF)
- Growth factors (GF)

There is abundant evidence that cytokines, which are secreted by fibroblasts[10], endothelial cells, and epithelial cells, play a crucial role in tissue homeostasis.

During the inflammatory reaction, which is a component of natural immunity, the pro-inflammatory cytokines (IL-1β, IL-8, TNF-α, IFN-γ) do battle with the inhibitory "immune-regulating" anti-inflammatory cytokines (IL-1ra, IL-10, TGF-β).

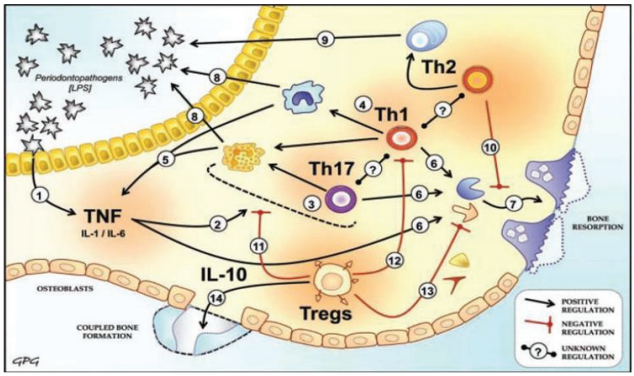
The balance between pro- and anti-inflammatory mediators determines the outcome of resorption in tissue destructive diseases, including periodontitis. As a general rule, pro-inflammatory mediators have been associated with tissue destruction, while anti-inflammatory mediators can counteract and attenuate disease progression. Thus it is suggested that similar immune response patterns (i.e., involving similar cytokines) considered harmful in the context of tissue destruction may play important roles in the control of periodontal infection[11,12].

While pro-inflammatory responses include production of pro-inflammatory cytokines such as IL-6, TNF-α, and IL-1, which are able to enhance both local and systemic inflammation[13], the anti-inflammatory cytokines IL-10 and IL-4, on the other hand, tend to attenuate the inflammatory response[8]. These cytokines, among other

inflammatory mediators, guide further immune responses including innate and adaptive responses.

Pro-inflammatory cytokines, such as TNF-α and IL-1β, initiate tissue destruction through the generation of proteases that degrade extracellular matrix, mainly matrix metalloproteinases (MMPs). MMPs, a family of zinc- and calcium-dependent proteases, are usually found in balance with a group of endogenous proteins named tissue inhibitors of metalloproteinases (TIMPs), to keep matrix remodeling highly regulated[14]. In fact, MMPs and TIMPs are regularly expressed in healthy periodontal tissues, where they are supposed to control the extracellular matrix physiological turnover[15]. Also, pro-inflammatory cytokines activate mechanisms for bone resorption driven by the interaction between RANK (receptor activator for nuclear factor-κβ) and its ligand RANKL (receptor activator for nuclear factor κβ ligand), which leads to the differentiation and activation of osteoclasts[3,16].

Conversely, anti-inflammatory cytokines, such as IL-10, are widely expressed in diseased periodontal tissues and are associated with lower disease severity[17,18]. Indeed, IL-10 presents a protective role toward tissue destruction, inhibiting both MMPs and RANK systems[18,19]. IL-10 characteristically induces the up-regulation of a group of endogenous proteins named tissue inhibitors of metalloproteinases (TIMPs), which are capable of inhibiting almost every member of the MMP family in a nonspecific way[4,9]. In addition, IL-10 stimulates the production of OPG, a decoy receptor of RANKL, which strongly inhibits bone resorption by preventing RANK-RANKL engagement[20,21]. In agreement, a positive correlation between the levels of IL-10, TIMPs, and OPG was demonstrated in both human and experimental periodontal diseases[3,18](Figure 1).



Courtesy- Garlet GP 2010

Figure 1. The cytokine crosstalk and its destructive and protective roles in periodontal disease: a re-appraisal from host defense and tissue destruction viewpoints. The recognition of periodontopathogenic LPS by resident cells in the periodontal environment (1) leads to the initial inflammatory events, which include the production of the classic pro-inflammatory cytokine TNF-α. TNF-α is characteristically involved in the amplification of inflammatory immune reactions, due to its role in leukocyte chemo-attraction and phagocyte activation (2) The subsequent migration of T-cells into periodontal tissues includes Th17 (3) and Th1 (4) subsets, which act in the amplification of innate response through the activation of neutrophils and macrophages, and by the generation of an amplification loop that sustains the chronic inflammatory reaction (5). Taken together, innate immunity cytokines and products of Th17 and Th1 cells (6) are thought to result in increased levels of RANKL and MMPs in periodontal tissues, and consequently to contribute to disease evolution (7). Conversely, these mediators present a dual role, since they are also important mediators in the control of periodontal infection, mediated by phagocytes and their products (8). The production of antibodies driven by the Th2/B-cell axis also contributes to the control of periodontal infection (9), and Th2 cytokines are supposed to counteract the destructive events mediated by Th1 and Th17 cells (10). Similarly, Treg-associated cytokines such as IL-10 are described to attenuate host response, acting over inflammatory (11) and Th1 (12) cytokines, and also directly inducing the expression of OPG and TIMPs (13), and to contribute to bone formation activity in the periodontal environment (14).

Thus, it is suggested that anti-inflammatory cytokines play a crucial role in preventing further tissue destruction and are needed to be reviewed in detail.

ANTI-INFLAMMATORY CYTOKINES

As opposed to the destructive pathway that involves primarily pro-inflammatory cytokines, regulatory pathways mediated by following anti-inflammatory cytokines can control or attenuate periodontal disease development.

INTERLEUKIN-4

IL-4 is an anti-inflammatory cytokine produced by CD_4 (T_H) cells, mast cells, eosinophils and basophils. Its receptor is the Interleukin-4 (IL-4) receptor.

History

It was co-discovered by Maureen Howard and William Paul and by Dr. Ellen Vitetta and her research group in 1982. The nucleotide sequence for human IL-4 was isolated four years later confirming its similarity to a mouse protein called B-cell stimulatory factor-1 (BCSF-1).

The IL-4 gene has been mapped to human chromosome 5, and the corresponding cDNA was cloned in 1986[22,23].

Structure

IL-4 has a compact, globular fold, stabilised by 3 disulphide bonds. One half of the structure is dominated by a 4 alpha-helix bundle with a left-handed twist. The helices are anti-parallel, with 2 overhand connections, which fall into a 2-stranded anti-parallel beta-sheet. It is expressed as a 15-19 kDa protein and exists as a dimer (Figure 2).

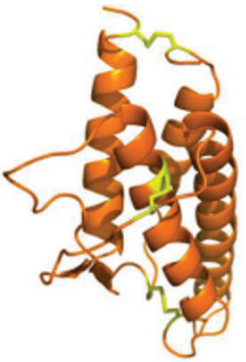


Figure 2. Structure of IL-4 (Courtesy- Plugis MN et al 2018)[24]

IL-4 receptor

Interleukin 4 receptor is a type I cytokine receptor. IL4R is its human gene.

The N-terminal (extracellular) portion of interleukin-4 receptor is related in overall topology to fibronectin type III modules and folds into a sandwich comprising seven antiparallel beta sheets arranged in a three-strand and a four-strand beta-pleated sheet (Figure 3). They are required for binding of interleukin-4 to the receptor alpha chain, which is a crucial event for the generation of a Th2-dominated early immune response.

IL-4 gene encodes the alpha chain of the interleukin-4 receptor, a type I transmembrane protein that can bind interleukin- 4 and interleukin- 13 to regulate IgE antibody production in B cells. Among T cells, the encoded protein also can bind interleukin 4 to promote differentiation of Th2 cells.

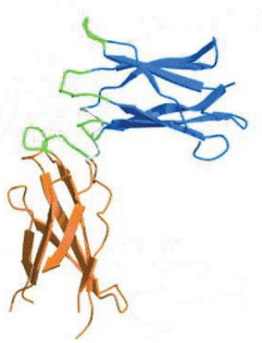


Figure 3. Structure of IL-4 receptor (Courtesy- Mueller TD et al 2002)[25]

Functions

IL-4 induces CD_4 T cells to differentiate into T_H2 cells while suppressing the development of T_H1 cells. It also acts as a B cell, T cell, and mast cell growth factor, it enhances class II MHC expression on B cells, and it provides immunoglobulin class switching to IgG_1 and IgE. In fact, IL-4 is necessary for IgE response induction, and its absence also leads to significantly lower levels of IgG_1 in T cell-dependent immune responses[26]. The stimulatory effects of IL-4 on IgG_1 and IgE production and on MHC class II induction are downregulated by IFN-γ, a cytokine whose functions are antagonized by IL-4 and vice versa. It is a key regulator in humoral and adaptive immunity. IL-4 also inhibits the production of pro-inflammatory cytokines IL-1β, TNF-α, thus, attenuating the disease progression.

INTERLEUKIN-5

Interleukin 5 or IL-5 is an interleukin produced by T helper-2 cells and mast cells. Through binding to the IL-5 receptor, IL-5 stimulates B cell growth and increases immunoglobulin secretion. It is also a key mediator in eosinophil activation.

History

Cloned in 1987, the IL-5 cDNA encodes a protein of 20-22 kDa which has an apparent molecular weight of 45 kDa upon dimerization. Like IL-4, the gene for IL-5 has also been mapped to chromosome 5 in humans[23].

Structure

IL-5 is a 115-amino acid (in human, 133 in the mouse) -long T_H2 cytokine that is part of the hematopoietic family. Also known as B cell growth factor II (BCGTFII) and T cell replacing factor (TRF), it exists as a dimer linked by disulfide bonds[27](Figure 4).

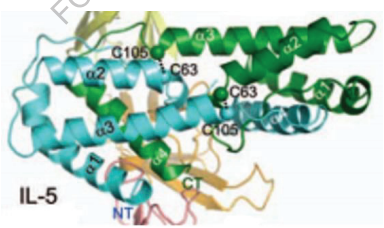


Figure 4. Structure of IL-5 (Courtesy- Kusano S et al 2012)[28]

IL-5 receptor

The Interleukin-5 receptor is a type I cytokine receptor. It is a heterodimer of the interleukin 5 receptor alpha subunit and CSF2RB.

The IL-5 receptor (IL-5R) belongs to the type I cytokine receptor family and is a heterodimer composed of two polypeptide chains, one α subunit, which binds IL-5 and confers upon the receptor cytokine specificity, and one β subunit, which contains the signal transduction domains.

α – subunit

The IL-5Rα chain is exclusively expressed by eosinophils, some basophils and murine B1 cells or B cell precursors. Like many other cytokine receptors, alternative splicing of the α-chain gene results in expression of either a membrane bound or soluble form of the bα-chain. The soluble form does not lead to signal transduction and therefore has an antagonistic effect on IL-5 signaling. Both monomeric forms of IL-5Rα are low affinity receptors, while dimerization with the β-chain produces a high affinity receptor. In either case, the α-chain exclusively binds IL-5 and the intra-cellular portion of IL-5Rα is associated with Janus kinase (JAK) 2, a protein tyrosine-kinase essential in IL-5 signal transduction (Figure 5).

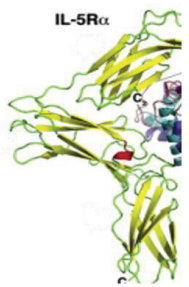


Figure 5. Structure of IL-5 receptor α- subunit (Courtesy- Patino E et al 2011)[29]

β – subunit

The β-subunit of the IL-5 receptor is responsible for signal transduction and contains several intracellular signaling domains. Unlike the α-chain, the β-chain does not bind IL-5, is not specific to this cytokine, and is expressed on practically all leukocytes. In fact, the β-subunit of the IL-5 receptor is also found in IL-3 and GM-CSF receptors where it is associated with IL-3Rα and GM-CSFRα subunits respectively. Therefore, it is known as the common β receptor or βc. As with the IL-5Rα subunit, the β subunit's cytoplasmic domain is constitutively associated with JAK2, as well as LYN, another tyrosine kinase, which are both essential for IL-5 signal transduction.

Functions

IL-5 is involved in eosinophil differentiation and activation and stimulation of immunoglobulin class switching to IgA. Other properties of IL-5 include increased activation of B cell proliferation, and enhancement of T cell cytotoxicity[23]. The combined production of IL-4 and IL-5 by CD_4 T_H2 cells therefore results in IgE and IgA production and mast cell and eosinophil stimulation.

INTERLEUKIN-6

IL-6 is a multifunctional cytokine that possesses a wide range of activities. In humans, it is encoded by the IL6 gene. It is produced by a variety of cell types, including monocytes/ macrophages, T and B cells, keratinocytes, endothelial cells, adipocytes, fibroblasts, and several tumor cells. The most important sources in periodontal diseases are macrophages and monocytes at the site of inflammation[30-32]. It is an interleukin that acts as both a pro-inflammatory and anti-inflammatory cytokine.

History

The IL-6 cDNA was cloned in 1986 and the gene encoding IL-6 was mapped to chromosome 7 in humans[33].

Structure

IL-6 is a glycoprotein ranging from 21 to 28 kDa depending on the degree of post-translational modification (Figure 6)

Figure 6. Structure of IL-6 (Courtesy- Metcalfe RD et al 2020)[34]

IL-6 receptor

IL-6 signals through a cell-surface type I cytokine receptor complex consisting of the ligand-binding IL-6Rα chain (CD126), and the signal-transducing component gp130 (also called CD130) (Figure 7).

CD130 is the common signal transducer for several cytokines including leukemia inhibitory factor (LIF), ciliary neurotropic factor, oncostatin M, IL-11 and cardiotrophin-1, and is almost ubiquitously expressed in most tissues. In contrast, the expression of CD126 is restricted to certain tissues. As IL-6 interacts with its receptor, it triggers the gp130 and IL-6R proteins to form a complex, thus activating the receptor. These complexes bring together the intracellular regions of gp130 to initiate a signal transduction cascade through certain transcription factors, Janus kinases (JAKs) and Signal Transducers and Activators of Transcription (STATs).

IL-6 is probably the best studied of the cytokines that use gp130, also known as IL-6 signal transducer (IL6ST), in their signalling complexes.

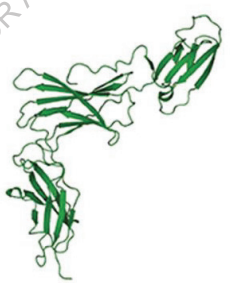


Figure 7. Structure of IL-6 receptor (Courtesy- Metcalfe RD et al 2020)[34]

Functions

According to the review by Irwing & Myrillas, IL-6 has been found to exert anti-inflammatory properties, e.g. enhancement of tissue inhibitor of metalloproteinase (TIMP) production and suppression of pro-inflammatory cytokines IL-1β and TNF-α. In addition, down-regulation of pro-inflammatory cytokines and up-regulation of anti-inflammatory molecules (e.g. IL-1 receptor antagonist, TNF soluble receptor) by IL-6 in acute inflammatory processes have been reported[35]. However, the balance between the protective and destructive activities of IL-6 in periodontitis is yet to be determined[36].

INTERLEUKIN-10

Interleukin 10 (IL-10) is an anti-inflammatory cytokine, also referred to as B cell-derived T cell growth factor and cytokine synthesis inhibitory factor (CSIF) because it inhibits IFN-γ production by activated T cells. IL-10 is produced by monocytes, macrophages and T cells and is capable of inhibiting several functions of dendritic cells, monocytes, and T cells including their cytokine production while stimulating B cell immunoglobulin (Ig) production and cytotoxic T lymphocyte generation[37].
In humans, IL-10 is encoded by the IL10 gene.

History

The cDNA for human IL-10 was cloned in 1990 and found to erode an 18 kDa protein.

Structure

IL-10 is an intercalated dimer of two subunits (39–41) consisting of six amphipathic helices. The polypeptide chains of each subunit contribute to both parts of the dimer consisting of 160 amino acids. The structure of human IL-10 was studied by X-ray

crystal structure analysis[38]. It is 18.5 kDa acid-sensitive protein that lacks detectable carbohydrate moieties (Figure 8).

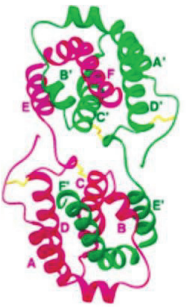


Figure 8. Structure of IL-10 (Courtesy- Zdanov A 2004)[37]

IL-10 receptor

Interleukin-10 receptor is a type II cytokine receptor. It consists of α and β subunits.

α - subunit

Interleukin 10 receptor, alpha subunit is a subunit for the interleukin-10 receptor. IL10RA, is its human gene (Figure 9).

IL10RA has also recently been designated CDW210A (cluster of differentiation W210A).

The protein encoded by this gene is a receptor for interleukin 10. IL-10 receptor is reported to promote survival of progenitor myeloid cells through the insulin receptor substrate-2/PI 3-kinase/AKT pathway. Activation of this receptor leads to tyrosine phosphorylation of JAK1 and TYK2 kinases.

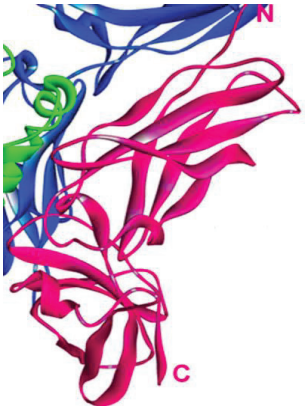


Figure 9. Structure of IL-10 receptor α-subunit (Courtesy- Acuner-Ozbabacan ES et al 2014)[39]

β -subunit

Interleukin 10 receptor, beta subunit is a subunit for the interleukin-10 receptor. IL10RB is its human gene.

IL10RB has also recently been designated CDW210B (cluster of differentiation W210B).

The protein encoded by this gene belongs to the cytokine receptor family. It is an accessory chain essential for the active interleukin 10 receptor complex. Co-expression of this and IL10RA proteins has been shown to be required for IL10-induced signal transduction. This gene and three other interferon receptor genes, IFAR2, IFNAR1, and IFNGR2, form a class II cytokine receptor gene cluster located in a small region on chromosome 21.

Functions

IL-10 is produced by a variety of cell types, including CD_4 T cells, activated CD_8 T cells, and activated B cells[40]. It is known to affect several steps in antimicrobial immunity, and its main functions are to limit and ultimately terminate inflammatory responses and regulate the differentiation and proliferation of immune cells such as T cells, B cells, NK cells, and mast cells[8,41]. The immunoregulatory cytokine IL-10 suppresses pro-inflammatory cytokine production and the antigen-presenting capacity of monocytes/macrophages and dendritic cells, and therefore indirectly reduces cytokine production by T cells and natural killer (NK) cells[42,43].

Thus, IL-10 inhibits the production of pro-inflammatory cytokines by inhibiting MHC class II expression in monocytes and macrophages and directly inhibiting the proliferation of CD4+ T cells[8,44]. IL-10 favors the phagocytic activity of monocytes and macrophages[45] and regulates innate and adaptive Th1 and Th2 responses by limiting T cell activation and differentiation in the lymph nodes[8]. In addition, it prevents apoptosis of B cells and enhances their proliferation and differentiation towards plasma cells[46,47].

INTERLEUKIN-11

IL-11 is a multifunctional cytokine produced by bone marrow-derived stromal cells and some fibroblasts. In humans it is encoded by the IL11 gene.

History

The corresponding cDNA of IL-11 was cloned in 1990[48].

Structure

IL-11 is a cytokine of 24 kDa encoded by a gene located on the long arm of chromosome 19 (Figure 10).

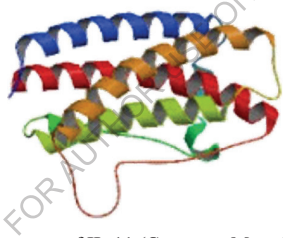


Figure 10. Structure of IL-11 (Courtesy- Metcalfe R et al 2020)[49]

IL-11 receptor

The interleukin 11 receptor is a type I cytokine receptor, binding interleukin 11. It is a heterodimer composed of an interleukin 11 receptor alpha subunit and an incompletely characterized beta subunit.

α – subunit

Interleukin 11 receptor, alpha subunit is a subunit of the interleukin 11 receptor. IL11RA is its human gene (Figure 11).

Interleukin 11 is a stromal cell-derived cytokine that belongs to a family of pleiotropic and redundant cytokines that use the gp130 transducing subunit in their high affinity receptors. This gene encodes the IL-11 receptor, which is a member of the hematopoietic cytokine receptor family. This particular receptor is very similar to ciliary neurotrophic factor, since both contain an extracellular region with a 2-domain structure composed of an immunoglobulin-like domain and a cytokine receptor-like domain. Alternative splicing has been observed at this locus and two variants, each encoding a distinct isoform, have been identified.

Figure 11. Structure of IL-11 receptor α-subunit (Courtesy- Metcalfe RD et al 2020)[49]

β- subunit

Interleukin 11 receptor, beta subunit is a subunit of the interleukin 11 receptor and is incompletely characterized.

Functions

IL-11 is a functional homologue of IL-6 and can replace IL-6 for the proliferation of certain plasmacytoma cell lines[50]. Additional IL-11 activities include stimulation of T

cell-dependent B cell immunoglobulin secretion, increased platelet production, and induction of IL-6 expression by CD_4 T cells.

INTERLEUKIN-13

IL-13 is cytokine secreted by many cell types, but especially T helper type 2 (Th2) cells, mast cells and NK cells. In humans it is encoded by the IL13 gene.

History

IL-13 was originally identified as a protein produced by activated murine T_H2 lymphocytes and referred to as P600[51]. The cDNA for IL-13 was recently cloned and the gene was mapped to human chromosome 5, closely linked to the gene encoding IL-4[52].

Structure

It is a 12-17 kDa protein, closely related to IL-4 protein (Figure 12).

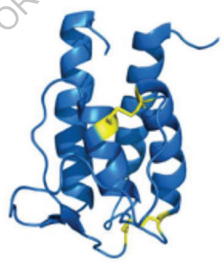


Figure 12. Structure of IL-13 (Courtesy- Plugis MN et al 2018)[24]

IL-13 receptor

The interleukin-13 receptor is a type I cytokine receptor, binding Interleukin-13. It consists of two subunits, encoded by IL13RA1 and IL4Rα, respectively. These two

genes encode the proteins IL-13Rα1 and IL-4Rα. These form a dimer with IL-13 binding to the IL-13Rα1 chain and IL-4Rα stabilises this interaction. This IL-13 receptor can also instigate IL-4 signalling. (Figure 13). In both cases this occurs via activation of the Janus kinase(JAK)/Signal Transducer and Activator of Transcription (STAT) pathway, resulting in phosphorylation of STAT6. Phosphorylated STAT6 dimerises and acts as a transcription factor activating many genes, such as eotaxin.

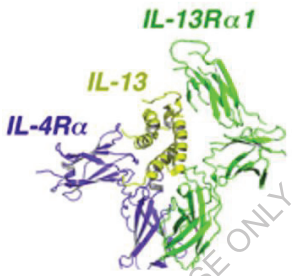


Figure 13. Structure of IL-13 receptor α_1 (Courtesy- Lupardus PJ et al 2010)[53]

There is also another receptor that can bind IL-13: IL-13Rα2 encoded by the IL13RA2 gene. This binds IL-13 with very high affinity (and can therefore sequester it) but does not allow IL-4 binding. It acts as a negative regulator of both IL-13 and IL-4, however the mechanism of this is still undetermined (Figure 14).

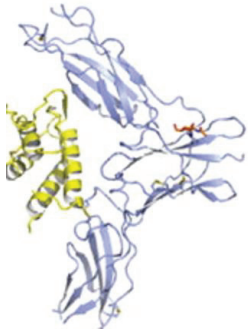


Figure 14. Structure of IL-13 receptor α_2 (Courtesy- Lupardus PJ et al 2010)[53]

Functions

IL-13 has effects on immune cells that are similar to those of the closely related cytokine IL-4. IL-13 exhibits anti-inflammatory activities by inhibiting the production of inflammatory cytokines, such as IL-1β, TNF-α, IL-8, and IL-6, by human peripheral blood monocytes induced with lipopolysaccharide[54]. In addition, it enhances monocyte and B lymphocyte differentiation and proliferation, increases CD23 expression, and induces IgG_4 and IgE class switching[55].

TRANSFORMING GROWTH FACTOR-β

The transforming growth factor-β (TGF-β) family of cytokines includes three isoforms, TGF-β_1, β_2, and β_3 which are encoded by separate genes yet bind to the same high affinity receptor. TGF-β is the most multi-functional cytokine. It affects a vast range of biological processes including regulation of cellular differentiation and growth, inflammation, wound healing, and bone formation.

History

TGF-β_1 was first identified in human platelets as a protein with a molecular mass of 25 kDa with a potential role in wound healing. It was later characterized as a large protein precursor (containing 390 amino acids) that was proteolytically processed to produce a mature peptide of 112 amino acids.

The human cDNA for TGF-β_1 was cloned in 1985[56].

Structure

TGF-β_1 contains 390 amino acids and TGF-β_2 and TGF-β_3 each contain 412 amino acids. They each have an N-terminal signal peptide of 20-30 amino acids that they

require for secretion from a cell, a pro-region (called latency associated peptide or LAP), and a 112-114 amino acid C-terminal region that becomes the mature TGF-β molecule following its release from the pro-region by proteolytic cleavage. The mature TGF-β protein dimerizes to produce a 25 kDa active molecule with many conserved structural motifs. TGF-β has nine cysteine residues that are conserved among its family; eight form disulfide bonds within the molecule to create a cysteine knot structure characteristic of the TGF-β superfamily while the ninth cysteine forms a bond with the ninth cysteine of another TGF-β molecule to produce the dimer. Many other conserved residues in TGF-β are thought to form secondary structure through hydrophobic interactions. The region between the fifth and sixth conserved cysteines houses the most divergent area of TGF-β molecules that is exposed at the surface of the molecule and is implicated in receptor binding and specificity of TGF-β (Figure 15).

Figure 15. Structure of TGF-β (Courtesy- Jay Groppe et al 2008)[57]

TGF-β receptor

TGF-β receptors are single pass serine/threonine kinase receptors. They exist in several different isoforms that can be homo- or heterodimeric. The number of characterized ligands in the TGF-β superfamily far exceeds the number of known receptors, suggesting the promiscuity that exists between the ligand and receptor interactions.

Three TGF-β receptor types can be distinguished by their structural and functional properties. Receptor types I and II have similar ligand-binding affinities and can be distinguished from each other only by peptide mapping. Both receptor types I and II

have a high affinity for TGF-β1 and low affinity for TGF-β2. TGF-β receptor type III has a high affinity for both TGF-β1 and -β2 and in addition TGF-β1.

Transforming growth factor, beta receptor I (activin A receptor type II-like kinase, 53kDa) is a TGF beta receptor. TGFBR1 is its human gene (Figure 16).

The protein encoded by this gene forms a heteromeric complex with type II TGF-beta receptors when bound to TGF-beta, transducing the TGF-beta signal from the cell surface to the cytoplasm (Figure 17). The encoded protein is a serine/threonine protein kinase.

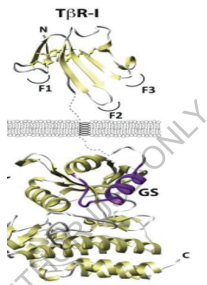


Figure 16. Structure of TGF-β1 receptor (Courtesy- Hinck AP 2012)[58]

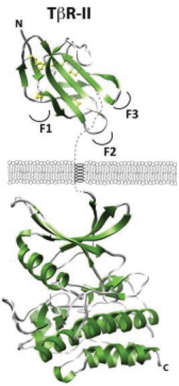


Figure 17. Structure of TGF-β2 receptor (Courtesy- Hinck AP 2012)[58]

Functions

TGF-β is produced by T cells, platelets, and monocytes and inhibits T cell and NK cell proliferation and activation and may play an important role in inflammation[55]. It stimulates the synthesis of connective tissue matrix components, such as collagen[56,59], fibronectin[60], proteoglycan[61], glycosaminoglycan[62], osteonectin[63], and osteopontin in many cell types, including PDL cells[64]. It also inhibits the degradation of matrix proteins by inhibiting the synthesis of metalloproteinases such as collagenase[65,66] and by increasing the synthesis of proteinase inhibitors[67,68].

TGF-β_1 can also inhibit the secretion and activity of many other cytokines including interferon-γ, tumor necrosis factor-alpha (TNF-α) and various interleukins. It can decrease the expression levels of cytokine receptors, such as the IL-2 receptor to down-regulate the activity of immune cells. However, TGF-β_1 can also increase the expression of certain cytokines in T cells and promote their proliferation, particularly if the cells are immature.

TGF-β further inhibits proliferation and stimulates apoptosis of B cells, and plays a role in controlling the expression of antibody, transferrin and MHC class II proteins on immature and mature B cells.

IL-1 RECEPTOR ANTAGONIST (IL-1Ra)

IL-1receptor antagonist is a member of the interleukin 1 cytokine family. It is a protein that in humans is encoded by the IL1RN gene.

History

IL-1Ra was initially called the IL-1 inhibitor and was discovered separately in 1984 by two independent laboratories.

Structure

A specific natural protein of 22-25 kDa molecular weight has been described, acting as an IL-1 receptor antagonist. IL-1Ra gene and five other closely related cytokine genes form a gene cluster spanning approximately 400 kb on chromosome 2 (Figure 18).

Human IL1RN gene (interleukin -1 receptor antagonist gene) maps to the long arm of chromosome 2. It is also a region for the IL-1β and IL-1 receptors I and II loci. Yet the meaning of sharing the same chromosomal localization by three distinct IL-1 forms as well as two receptor types in humans is not clear.

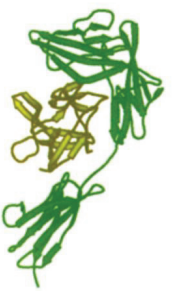


Figure 18. Structure of IL-1Ra (Courtesy- Schreuder H et al 1997)[69]

Functions

IL-1Ra, is an agent that binds non-productively to the cell surface interleukin-1 receptor (IL-1R). IL-1R is the same receptor that binds interleukin 1 (IL-1). Hence IL-1Ra prevents IL-1 from sending a signal to that cell.

IL1Ra is secreted by various types of cells including immune cells, epithelial cells, and adipocytes, and is a natural inhibitor of the pro-inflammatory effect of IL1β. This protein inhibits the activities of interleukin 1, alpha (IL-1α) and interleukin 1, beta (IL-1β), and modulates a variety of interleukin 1 related immune and inflammatory responses.

Lipopolysaccharide (LPS), some cytokines [IL-4, IL-10, granulocyte-macrophage colony stimulating factor (GM-CSF) and transforming growth factor-β (TGF-β)], as well as various immune complexes can stimulate expression of IL-1 receptor antagonist, capable of binding to both IL-1 receptor types. Dinarello claims the most frequent stimulus promoting the secretion of this specific protein are the bacterial endotoxins[70]. Stimulated cells, such as polymorphonuclear leukocytes (PMN), can activate IL1RN gene expression and protein translation[71]. Interleukin-1 receptor antagonist blocks IL-1 activity, both in vivo and in vitro. Recombinant IL-1Ra inhibits IL-1-promoted thymocyte and chondrocyte proliferation, as well as collagenase, GMCSF and IL-6 synthesis[72]. High IL-1Ra activity has been reported in the reduction of leukaemic cells proliferation[73].

Decreased concentration of IL-1Ra is observed in crevicular fluid of patients suffering from periodontal disease suggesting protective role of IL-1Ra[74,75]. It should be noted that the intracellular form of IL-1Ra can only act when released from dead or necrotizing tissues together with IL-1β (in case of macrophages) and IL-1α (in case of keratinocytes)[75]. Multiannual own studies have proved the presence of IL-1Ra in saliva in patients with clinically healthy periodontal tissue[76]. In some cases this glycoprotein may not act as a cytokine inhibitor in course of inflammatory process within periodontal

tissue. The periodontal pathogens can influence the cytokine network within periodontal tissue and gingival crevicular fluid, blocking the activity of the inhibitors.

On the other hand, the course of pathogenic process in periodontium depends on the proportion of pro-inflammatory and anti-inflammatory cytokines[77]. Only extremely high IL-1Ra concentration, significantly exceeding the IL-1β concentration, could inhibit the biological activity of this cytokine.

It was observed (in the in vitro studies) that to decrease by 50% prostaglandins and collagenase synthesis, which is induced both by IL-1α and IL-1β, the IL-1Ra concentration should overwhelm the pro-inflammatory cytokine by over 100 times. This observation has been explained by the "spatial receptor effect". Target cells can present a couple thousands of receptors, and yet even much smaller amount of the IL-1 receptors would be enough to induce regular biological response[78].

The functions of anti-inflammatory cytokines thus, can be summarized as below-

Cytokine	Functions
IL-4	- Inhibits the production of pro-inflammatory cytokines IL-1β, TNF-α - Induces CD_4 T cells to differentiate into T_H2 cells - Necessary for IgE response induction
IL-5	- Eosinophil differentiation and activation - Increased activation of B cell proliferation - Enhancement of T cell cytotoxicity
IL-6	- Enhancement of Tissue inhibitor of metalloproteinase (TIMP) - Suppression of proinflammatory cytokines IL-1β, TNF-α - Upregulation of anti-inflammatory molecules IL-1ra
IL-10	- Suppresses pro-inflammatory cytokine production - Favors the phagocytic activity of monocytes and macrophages - Prevents apoptosis of B cells - Regulates the differentiation and proliferation of immune cells
IL-11	- Stimulation of T cell-dependent B cell immunoglobulin secretion - Increased platelet production - Induction of IL-6 expression by CD_4 T cells

IL-13	- Inhibits the production of inflammatory cytokines IL-1β, TNF-α, IL-8 - Enhances monocyte and B lymphocyte differentiation and proliferation - Induces IgG_4 and IgE class switching
TGF-β	- Stimulates the synthesis of connective tissue components, such as collagen, proteoglycan, glycosaminoglycan, osteonectin and osteopontin - Inhibits the degradation of matrix proteins by inhibiting the synthesis of metalloproteinases such as collagenase - Inhibit the secretion and activity of other cytokines IFN-γ, TNF-α - Decrease the expression levels of cytokine receptors
IL-1ra	- Blocks IL-1 activity, both in vivo and in vitro - Natural inhibitor of the pro-inflammatory effect of IL-1β

APPLICATIONS IN PERIODONTAL DISEASE ACTIVITY

When inflammatory responses are generated in any given tissue, the expression of a variety of cytokines is generally increased at the site and then down-regulated to control the local inflammatory response. Thus, it is possible to suggest that the host inflammatory immune responses present a dual role because, while it has been determined that cytokines are required to control periodontal infection[11], these same mediators may trigger inflammatory responses and hence tissue destruction.

This raises the possibility of objectively diagnosing the severity of inflammation by monitoring the cytokine level or profile at the inflamed site. In order to diagnose the disease activity of periodontal tissues, researchers have investigated the possible relationships between levels of various inflammatory cytokines in GCF and the periodontal status or conditions.

Cytokines can be accurately measured in biological fluids via two methodologies - Serum cytokine analysis and whole blood stimulated cellular analysis.

Serum cytokine analysis:

Sample type-Serum

Assay principle- Direct cytokine measurement

Indication- Cost-effective method for suspected acute inflammation, differentiates among bacterial, viral, allergic and asthmatic immune responses

Whole blood stimulated cellular analysis:

Sample type- Whole blood

Assay principle- Cytokine measurement from stimulated immune cells

Indication- Preferred method for chronic disease and inflammation management

Thus, monitoring the expression of multiple cytokines in inflamed periodontal tissues might be an objective way of evaluating disease activity in periodontitis. This information may be useful for objectively establishing the presence of an activated immune response as well as guide targeted therapeutic regimens designed to reduce inflammation and its secondary effects.

REVIEW OF LITERATURE

INTERLEUKIN-4

A study was done to evaluate the potential anti-inflammatory effects of IL-4 in suppressing human monocyte TNF-α, IL-1 and prostaglandin E_2 (PGE_2). Mononuclear cells were selected by centrifugation (170 x g for 30 min) of leukocyte-rich fractions and suspended in Hanks' balanced salt solution. Monocytes were isolated by countercurrent centrifugal elutriation with a constant rotor speed (2000 rpm) but increasing pump rates from 8 to 22 ml/min. For each elutriation process, monocyte fractions were collected at a rate between 14.5 and 22.0 ml/min; monocyte enrichment> 90% was confirmed by cell morphology on Giemsa-stained cytocentrifuged smears and by nonspecific esterase staining. Lymphocytes were the main contaminating cell type; polymorphonuclear cells always were 3% or less. TNF-α activity was measured with actinomycin D-treated L929 target cells. IL-1 was assayed by the murine thymocyte comitogenesis assay. Levels of PGE_2 in monocyte culture supernatants (-0.03 ng/ml) were determined by immunoassay using competitive adsorption to dextran-coated charcoal. Also, total cellular RNA from 4-hr monocyte cultures was prepared and fractionated. It was found out that cotreatment of stimulated cells with the purified human lymphokine, interleukin 4 (IL-4 2 0.1-0.5 unit/ml; 12-60 pM) dramatically blocked the increased levels of these three mediators; for TNF-α and IL-1, the inhibition was manifest at the level of mRNA. Thus, IL-4 can suppress some parameters of monocyte activation and, as for B cells, have opposite effects to IFN-γ[79].

A study was done to examine whether the productions of IL-1 and a specific receptor antagonist of IL-1 (IL-1Ra) in human blood monocytes were regulated differently. Highly purified blood monocytes, isolated by centrifugal elutriation from healthy donors, were stimulated with LPS in the presence or absence of IL-4, and their

productions of IL-1 and IL-1Ra were measured by Northern blot and immunoblot analyses. IL-1 and IL-1Ra were produced by monocytes stimulated with LPS, but not with IL-4 alone. Marked up-regulation by IL-4 of IL-1Ra production in LPS-stimulated monocytes was observed at both the mRNA and protein levels. Maximal expressions of IL-1β and IL-1Ra mRNA in LPS-stimulated monocytes were observed 2 h and 8 h, respectively, after stimulation. The enhancement of IL-1Ra production by IL-4 was concluded to be due to enhanced gene transcription, because there was no difference in the half-lives of IL-1Ra mRNA in monocytes cultured with and without IL-4. Up-regulation of IL-1Ra production by IL-4 was also observed in monocytes stimulated with adherent IgG at both the mRNA and protein levels. Thus, it was suggested that this unique property of IL-4 may be important in down-regulation of the IL-1-initiated immune and/or inflammatory response, not only directly through inhibition of IL-1 production, but also indirectly through up-regulation of IL-1Ra production[80].

A study was done to investigate whether a relationship exists between IL-4 receptor (IL-4R) expression and macrophages (MO) persistence in the absence of exogenous IL-4. Gingival MOs, when compared with monocyte MOs from peripheral blood mononuclear cells, expressed high levels of IL-4R mRNA. Furthermore, in vitro cultures of gingival MOs remained viable whereas identically treated peripheral blood MOs rapidly lost viability. However, when gingival MOs were incubated with recombinant IL-4 (rIL-4), the cell viability was dramatically reduced. When the frequency of apoptotic cells was assessed in rIL-4-treated gingival MO cultures, higher numbers of apoptotic cells were noted in rIL-4-treated versus control cultures. Furthermore, rIL-4-treated MOs from inflamed gingiva showed DNA fragmentation as assessed by electrophoresis. These findings clearly show that addition of exogenous rIL-4 to gingival MO cultures leads to cell death by apoptosis. Thus, the results of this study suggested that topical application of rIL-4 may inhibit the persistence of MOs in adult periodontitis, which could then lead to decreased inflammation[81].

A study was done to determine the levels of interleukin IL-1β, IL-4, IL-6 and IL-8 in gingival crevicular fluid (GCF) of periodontally healthy and diseased individuals and to study their association to smoking, stress and clinical periodontal parameters. A total of 80 patients including 20 patients with early onset or aggressive periodontitis (EOP), 20 with chronic adult periodontitis (AP), 20 with gingivitis (G) and 20 patients with healthy periodontium (H) were enrolled in the study. GCF was collected by means of Durapore strips, from four sites per patient, randomly selected in each quadrant. The contents of IL- 1β, IL-4, IL-6 and IL-8 were measured in 320 samples by use of commercially available sandwich ELISA assays. In periodontally diseased subjects the total amounts of IL-1β, IL-6 and IL-8 were significantly elevated as compared to healthy subjects, whereas IL-4 showed an inverse relationship to periodontal status and higher amounts were found in the healthy group. The amounts of all four cytokines were positively correlated with probing depths. IL-4, IL-6 and IL-8 were significantly correlated to smoking while stress was associated with IL-1β, IL-6 and IL-8 levels. Thus, the results of study suggest that crevicular IL-1β, IL-6 and IL-8 reflect the activity of periodontal destruction, whereas IL-4 shows an inverse correlation to it[82].

A study was done to determine the effects of IL-4 on the IL-1-induced expression of MMP-3 in human gingival fibroblasts isolated from patients with periodontitis. Northern blot analysis was performed to determine the effects of IL-4 on the IL-1 induction of MMP-3 mRNA. MMP-3 protein levels were determined by enzyme-linked immunosorbent assay (ELISA), and prostaglandin E_2 (PGE_2) levels were measured by enzyme immunoassay (EIA). DNA binding of activator protein (AP)-1 and NF-κβ was assessed by electrophoretic mobility shift assay (EMSA). Northern blot analysis revealed that co-incubation of gingival fibroblasts with IL-1 and IL-4 resulted in a significant decrease in MMP-3 mRNA levels compared to IL-1 alone, with a concomitant decrease in protein levels. This inhibition is dose-dependent, and is apparent as early as 3 hours after stimulation. IL-1-induced production of PGE_2 was not

affected in four of six cultures isolated from different individuals. Addition of exogenous PGE_2 had no effect on the suppressive effects of IL-4. DNA binding of transcription factors AP-1 and NF-κβ was not affected by IL-4. Thus, it was suggested that IL-4 inhibits the IL-1 induction of MMP-3 in human gingival fibroblasts isolated from patients with periodontitis. This effect is independent of PGE_2 and is not due to inhibition of the DNA binding activity of known transcription factors binding to the MMP-3 promoter[83].

A study was done to examine the expression of CD13/ APN on human gingival fibroblasts (hGF) and to examine how T cell-derived cytokines may regulate the expression of CD13/ APN on hGF. hGF cells were prepared from the explants of clinically non-inflamed gingival from 8-25 years old patients with informed consent. Also total cellular RNA was extracted from hGF cultured in a six-well multiplate for the RT-PCR assay. CD13/ APN was expressed on hGF at the mRNA and protein levels as determined by RT-PCR and flow cytometry, respectively. Enzymatic activities accompanying the expression were assessed by colorimetric analysis using the synthetic substrate Leu-p-nitroanilide. On examining the possible regulation of CD13/APN expression on hGF in response to T cell-derived cytokines, it was found out that T helper (Th 2) cell type cytokines such as IL-4 and IL-13, preferentially increased the expression of proteins as well as the enzymatic activities of CD13/ APN in a dose-dependent manner. Thus, these findings suggested that CD13/ APN expressed by hGF could contribute to the anti-inflammatory response in periodontal tissue, and may be involved in disease processes mediated by Th2 cells[84].

A study was done to investigate the levels of IL-4 and IL-10 in gingival crevicular fluid (GCF). Seventeen patients with Chronic periodontitis (CP), 17 patients with Rheumatoid arthritis (RA) and 17 healthy controls (HC) were included. The RA group was divided into two groups according to gingival sulcus depths (RA-a: PD < or =3mm, (n=12), RA-b: PD>3mm, (n=5)). For each patient, clinical parameters were recorded. The GCF samples were evaluated by ELISA for IL-4 and IL-10 levels. It was found out

that IL-4 levels in the RA-a, RA-b and CP subjects were significantly lower compared to the HC subjects ($p<0.05$). The mean level of IL-4 in RA-b group was significantly higher than that in CP group ($p<0.05$). IL-10 mean level in the HC group was higher than those in the other groups ($p<0.05$). In the RA-a group, higher IL-10 level was found compared to the CP patients ($p<0.05$). Thus, it can be suggested that the initiation and progression of periodontal inflammation may be due to a lack or inappropriate response of the anti-inflammatory cytokines in both Chronic periodontitis and Rheumatoid arthritis[85].

A study was done to investigate the changes of gingival crevicular fluid (GCF) IL-4 and IFN-γ in patients with chronic periodontitis before and after initial nonsurgical periodontal therapy (NSPT). GCF was collected from 17 patients by means of Periopaper at baseline and 1 month after NSPT. IL-4 and IFN-γ were measured by enzyme-linked immunoabsorbent (ELISA) assay. NSPT resulted in decreased total amount of IFN-γ, increased concentration of IL-4, and increased ratio of IL-4 to IFN-γ levels. Thus, it was suggested that a low ratio of IL-4 to IFN-γ levels might be involved in the destruction (diseased sites) of periodontal tissue, whereas an increased ratio of IL-4 to IFN-γ levels could be related to the improvement of clinical periodontal health[86].

A study was done to investigate the role of IL-4 in periodontal disease by assessing its gingival crevicular fluid (GCF) levels in subjects in an Indian population with healthy periodontium, gingivitis or periodontitis and to further determine the influence of non-surgical periodontal treatment on the IL-4 levels. A total of 60 subjects were divided into three groups based on gingival index (GI), pocket probing depth and clinical attachment loss (CAL): healthy (group 1), gingivitis (group 2) and chronic periodontitis (group 3). A fourth group (group 4) consisted of 20 subjects from group 3, 6-8 weeks after treatment. Only one site per subject was selected as a sampling site. GCF samples collected from each patient were quantified for IL-4 using a Human IL-4 ELISA KIT. The highest mean concentration of IL-4 was obtained for group 1 and the

lowest mean concentration of IL-4 was obtained for group 3. The mean IL-4 concentrations for group 2 and group 4 were intermediate between the levels in healthy subjects and periodontitis subjects. Thus, the mean concentration of IL-4 decreased from periodontal health to disease, suggesting that type 2 helper T cell cytokine, as represented by IL-4, was associated with the remission or improvement of periodontal disease[87].

A study was done to evaluate TNF-α and IL-4 levels in healthy sites and sites exhibiting signs of moderate and advanced generalized aggressive periodontitis (GAgP) in the same subject. Healthy sites (HS), Moderate sites (MS) [probing depth between 4 and 6 mm], and Advanced sites (AS) [probing depth >or= 7 mm] were selected for crevicular fluid sampling in the same AgP subject. One site from periodontally healthy subjects was sampled for use as a control. TNF-α and IL-4 levels were measured using ELISA. It was found out that the total amount of TNF-α was lower for control sites, while there were no differences among healthy and diseased sites from GAgP subjects ($P < 0.05$). The concentration of TNF-α was higher in HS, in relation to the other sites ($P < 0.05$). There were no significant differences among the groups regarding total amounts of IL-4 ($P > 0.05$), while IL-4 concentration was significantly higher in control sites, when compared with sites from GAgP subjects ($P < 0.05$). Thus, in this study, it was observed that high levels of TNF-α and low levels of IL-4 were observed in both healthy and diseased sites within the same generalized AgP individuals[88].

A study was done to assess and compare the levels of IL-4, IL-6 and IL-12 in serum samples of patients with generalized aggressive periodontitis patients (GAgP) and control individuals. A total of 50 subjects including 25 patients with GAgP and 25 healthy patients undergoing extraction and surgical crown lengthening (control group) were enrolled. Local blood samples of patients were collected from surgical sites of pocket reduction and from healthy individuals before tooth extraction or crown lengthening from non inflamed sites. The levels of IL-4, IL-6 and IL-12 were

determined by an ELISA assay using serum samples separated from the whole blood of both groups. It was found out that the level of IL-4 decreased significantly in test group in comparison with the control group ($p= 0.002$). The amount of IL-6 in GAgP patients increased strongly compared with control group ($p < 0.0001$). There was no significant difference between the two groups concerning the level of IL-12. Thus, the results of this study indicate that the concentration of IL-4 in sera of GAgP subjects is lower compared with healthy subjects, highlighting the protective role of IL-4 in the disease. Lack of IL-4 may result in a breakdown of the regulation of immune function and enhanced macrophage survival in the inflammatory lesion[89].

A study was done to compare genotypes and soluble protein of pro and anti-inflammatory cytokines (IL-1α, IL-1β, IL-6, IFN-γ, IL-10, TNF-α and IL-4) in subjects with or free of chronic periodontitis. A total of 1,290 Chinese subjects were recruited to this clinical trial: 850 periodontally healthy controls and 440 periodontal patients. All subjects were free of systemic diseases. Oral examinations were performed, and the following parameters were recorded for each subject: Supragingival/ subgingival calculus, gingival recession, bleeding on probing (BOP), probing depth (PD), clinical attachment loss (CAL) and tooth mobility. The peripheral blood samples were collected for genetic and ELISA analysis. Restriction enzymes were used for digestion of amplified fragments of IL-1α, IL-1β, IL-6, IFN-γ, IL-10, TNF-α and IL-4. It was observed that the protein expressions of patient and control samples for IL-1α, IL-1β, IL-6, IFN-γ, IL-10, TNF-α and IL-4 measured by ELISA confirmed a statistically significant difference ($p < 0.001$). The digestion of fragments of various genes showed that the pro-inflammatory cytokines IL-1α and TNF-α, and the anti-inflammatory cytokines IL-4 and IL-10 demonstrated a correlation with chronic inflammation in patients. The remaining genes investigated in patients and healthy subjects did not show any significant difference. Thus, it is suggested that the cytokine gene polymorphisms may be used as a marker for periodontitis susceptibility, clinical behavior and severity[90].

INTERLEUKIN-5

A study was done to show that the RgpA-Kgp complexes hydrolyze and inactivate IL-4 and IL-5 under physiologically relevant conditions. Using the IL-4/IL-5-dependent TF1.8 T-cell line, it was found that at equimolar ratios of cytokine to RgpA-Kgp complexes, IL-4 and IL-5 were inactivated in the culture medium. The inactivation of IL-4 and IL-5 was RgpA-Kgp concentration dependent, as at an enzyme-to-cytokine molar ratio of 1:8, the bioactivity of the cytokines was greater than at the higher concentration of RgpA-Kgp of 1:1. Furthermore, inactivation of the cytokines by the RgpA-Kgp complexes was time dependent, as longer preincubation times resulted in lower cytokine activity. IL-5 was found to be slightly more resistant to inactivation than IL-4. Mass spectrometric analyses of IL-4 and IL-5 showed that hydrolysis by RgpA-Kgp complexes was C terminal to Arg and Lys residues of the cytokines. The peptides released indicated that the regions of IL-4 and IL-5 important for bioactivity were being hydrolyzed in the first 15 min of incubation. Thus, it was suggested that the ability of the RgpA-Kgp complexes to degrade Th2 cytokines may contribute to immune dysregulation and may play a role in the pathology of chronic periodontitis[91].

INTERLEUKIN-6

A study was done to investigate the role of IL-6 during virulent Escherichia coli (E.coli) infection and in lipopolysaccharide (LPS)-induced mortality. In this study, an increased susceptibility of IL-6-deficient mice to E. coli infection in terms of mortality and accumulation of viable bacteria in tissues was described, indicating a protective role for IL-6 during the immune response against E. coli. In contrast, mortality rates of IL-6-deficient mice and control animals undergoing LPS-induced shock did not differ, indicating that IL-6 was inconsequential for survival in this model. Furthermore, it was shown that neutrophils were crucial for resistance to E. coli in normal mice. IL-6-

deficient mice were unable to efficiently induce neutrophilia in the bloodstream immediately following challenge with E. coli, in contrast to a characteristic neutrophilia induced in control animals. Prophylactic treatment of the mutant animals with recombinant IL-6 protein reverted both the deficit of neutrophilia and the accumulation of bacteria in tissues. Thus, the results of this study clarify the role of IL-6 as protective in virulent E. coli infection and suggest that the protective effect may be at least partially mediated through neutrophils[92].

A study was done to investigate whether endogenous IL-6 plays pro- or antiinflammatory roles in local or systemic responses. In this study, the role of IL-6 in acute inflammatory responses was investigated in animal models of endotoxic lung or endotoxemia by using IL-6+/+ and IL-6-/- mice. Aerosol exposure of endotoxin induced increased IL-6 and proinflammatory cytokines TNF-α and MIP-2 and a neutrophilic response in the lung of IL-6+/+ mice. It was observed that the levels of TNF-α and MIP-2 and neutrophilia were significantly higher in the lung of IL-6-/- mice. The rate of neutrophil apoptosis in these mice was similar to that in IL-6+/+ mice. A low constitutive level of antiinflammatory cytokine IL-10 was not enhanced by endotoxin and remained similar in the lung in both IL-6+/+ and IL-6-/- mice. Systemically, intraperitoneal delivery of endotoxin resulted in much more pronounced circulating levels of TNF-α, MIP-2, GM-CSF, and IFN-γ in IL-6-/- mice than in IL-6+/+ mice, and administration of recombinant IL-6 to IL-6-/- mice abolished these differences. In contrast, circulating IL-10 levels were induced to a similar degree in both IL-6+/+ and IL-6-/- mice. Thus, results of this study reveal that endogenous IL-6 plays a crucial antiinflammatory role in both local and systemic acute inflammatory responses by controlling the level of proinflammatory, but not antiinflammatory, cytokines, and that these antiinflammatory activities by IL-6 cannot be compensated for by IL-10 or other IL-6 family members[35].

A study was done to determine the role of IL-6 in regulating IL-1 expression and bone resorption. The first molars of IL-6 knockouts (IL-6-/-) and wild-type mice were subjected to surgical pulp exposure and infection with a mixture of four common pulpal pathogens, including *Prevotella intermedia*, *Fusobacterium nucleatum*, *Peptostreptococcus micros*, and *Streptococcus intermedius*. Mice were killed after 21 days, and bone destruction and cytokine expression were determined. Surprisingly, bone destruction was significantly increased in IL-6−/− mice versus that in wild-type mice (by 30%; $P < 0.001$). In a second experiment, the effects of chronic (IL-6−/−) IL-6 deficiency and short-term IL-6 deficiency induced by in vivo antibody neutralization were determined. Both IL-6−/− (30%;P< 0.001) and anti-IL-6 antibody-treated mice (40%;P < 0.05) exhibited increased periapical bone resorption, compared to wild-type controls. The increased bone resorption in IL-6-deficient animals correlated with increases in osteoclast numbers, as well as with elevated expression of bone-resorptive cytokines IL-1α and IL-1β, in periapical lesions and with decreased expression of the anti-inflammatory cytokine IL-10. Thus, the results of this study suggest that endogenous IL-6 expression has significant anti-inflammatory effects in modulating infection-stimulated bone destruction in vivo[93].

A study was done to investigate the expression of IL-6 as well as the incidence of IL-6 (−174) gene polymorphism and their correlation to the severity of periodontitis in Brazilians. Peripheral blood mononuclear cells were collected from 12 non-smoker individuals with periodontitis for evaluation of IL-6 expression using flow cytometry. A positive correlation was observed between the mean clinical attachment loss and intensity of expression of IL-6, in which the greater the attachment loss, the higher the expression of IL-6 (P= 0·007, R^2 = 0·52). Also, patients with severe periodontitis displayed a higher intensity of IL-6 expression compared to moderate periodontitis (P= 0·04). To determine the occurrence of IL-6 gene polymorphism, DNA was obtained from oral swabs of 209 Brazilian individuals with and without periodontitis. Polymerase

chain reaction, restriction endonuclease digestion and electrophoresis were performed, allowing for detection of the IL-6(−174) polymorphism. It was observed that non-smokers with moderate periodontitis (P= 0·05) and control (P= 0·04) groups displayed a higher incidence of the G^{-} genotype when compared to severe periodontitis. This suggests that the G^{-} genotype may represent a protective role in severity of periodontitis. Thus, the increased expression of IL-6 and IL-6 (−174) polymorphism are associated with periodontal disease severity in Brazilian individuals[94].

A study was done to examine the effects of IL-6 and its soluble receptor (sIL-6R) on osteoblastic differentiation of periodontal ligament cells. Osteoblastic differentiation was induced by ascorbic acid. Osteoblast markers, including alkaline phosphatase activity and Runx2 gene expression, were examined. The mechanism of action of IL-6 on osteoblastic differentiation was evaluated by insulin-like growth factor (IGF)-I production and specific inhibitors for the IL-6-signaling molecule. It was observed that IL-6/sIL-6R enhanced alkaline phosphatase activity and Runx2. Alkaline phosphatase activity was reduced by anti-IGF-I antibody. Mitogen-activated protein kinase and Janus protein tyrosine kinase inhibitors diminished alkaline phosphatase induced by IL-6/sIL-6R. Thus, it was suggested that IL-6/sIL-6R increases ascorbic-acid-induced alkaline phosphatase activity through IGF-I production, implying that IL-6 acts not only as an osteolytic factor, but also as a mediator of osteoblastic differentiation in periodontal ligament cells[95].

A study was done to investigate the role of IL-6 promoter polymorphism (-174 G/C) in Indian patients with chronic periodontitis. Prevalence of single nucleotide polymorphism in IL-6 at position -174 G>C in a population of 30 South Indians was investigated. Blood samples were collected from 15 chronic periodontitis patients and 15 healthy controls. The results showed that the G/G genotype was significantly more frequent in the chronic periodontitis group and that the C/C genotype was significantly more frequent in the control group (P= 0.0069 for both). The G allele was more frequent

in chronic periodontitis patients (76.67%), whereas the C allele was more frequent in the control group (73.33%). Among chronic periodontitis patients, the odds ratio for having the G allele, as compared with the controls, was 9.04. In this population, the presence of the G/G genotype of IL-6 (-174) might increase susceptibility to chronic periodontitis, whereas the C/C genotype may have a protective effect[96].

A study was done to analyse the levels of IL-6 and IL-8 in GCF of patients with chronic periodontitis prior to and following surgical and/or nonsurgical periodontal therapy for a period of 32 weeks. GCF samples were obtained from 24 nondiseased and 72 diseased sites of 12 periodontal patients prior to as well as at 6, 16, and 32 weeks following non-surgical and surgical periodontal therapy. IL-6 and IL-8 levels were determined by enzyme-linked immunosorbent assay (ELISA). It was found out that periodontal treatment improved all clinical parameters. Both treatment modalities resulted in similar IL-6 as well as IL-8 levels. Mean IL-6 and IL-8 concentrations were significantly higher in non-diseased compared to diseased sites and increased significantly following treatment in diseased sites. Mean total amounts of IL-6 and IL-8 (TAIL-6, TAIL-8) did not differ significantly between diseased and nondiseased sites, while following therapy TAIL-8 levels decreased significantly. Thus, the results of this study suggest that periodontal therapy reduced the levels of IL-8 in GCF. However, a strong relationship between IL-6, IL-8 amounts in GCF and periodontal destruction and inflammation was not found[97].

INTERLEUKIN-10

A study was done to demonstrate that human monocytes activated by lipopolysaccharides (LPS) were able to produce high levels of interleukin 10 (IL-10), previously designated cytokine synthesis inhibitory factor (CSIF), in a dose dependent

fashion. IL-10 was detectable 7 hours after activation of the monocytes and maximal levels of IL-10 production were observed after 24-48 hours. These kinetics indicated that the production of IL-10 by human monocytes was relatively late as compared to the production of IL-1α, IL-1β, IL-6, IL-8, TNF-α, and granulocyte colony-stimulating factor (G-CSF), which were all secreted at high levels 4-8 hours after activation. The production of IL-10 by LPS activated monocytes was, similar to that of IL-1α, IL-1β, IL-6, IL-8, TNF-α, granulocyte-macrophage colony-stimulating factor (GM-CSF), and G-CSF, inhibited by IL-4. Furthermore it was observed that IL-10, added to monocytes, activated by IFN-γ, LPS, or combinations of LPS and IFN-γ at the onset of the cultures, strongly inhibited the production of IL-1α, IL-1β, IL-6, IL-8, TNF-α, GM-CSF, and G-CSF at the transcriptional level. Viral-IL-10, which has similar biological activities on human cells, also inhibited the production of TNF-α and GM-CSF by monocytes following LPS activation. Activation of monocytes by LPS in the presence of neutralizing anti-IL-10 monoclonal antibodies resulted in the production of higher amounts of cytokines relative to LPS treatment alone, indicating that endogenously produced IL-10 inhibited the production of IL-1α, IL-1β, IL-6, IL-8, TNF-α, GM-CSF, and G-CSF. In addition, IL-10 had autoregulatory effects since it strongly inhibited IL-10 mRNA synthesis in LPS activated monocytes. Furthermore, endogenously produced IL-10 was found to be responsible for the reduction in class II major histocompatibility complex (MHC) expression following activation of monocytes with LPS. Thus, the results of this study indicate that IL-10 has important regulatory effects on immunological and inflammatory responses because of its capacity to downregulate class II MHC expression and to inhibit the production of proinflammatory cytokines by monocytes[43].

A study was done to demonstrate that IL-10 upregulates IL-1 receptor antagonist production from lipopolysaccharide-stimulated human polymorphonuclear leukocytes by delaying mRNA degradation. Highly purified (>99.5%) PMN were isolated under endotoxin-free conditions from buffy coats of healthy donors, and were immediately P

suspended in RPMI 1640 medium supplemented with 10% low endotoxin FCS in the presence or absence of purified recombinant human IL-10. Cells were then stimulated with 1µg/ml LPS (from E. coli)and cultured at 37˚C either in 24-well tissue culture plates at 10^6 cells/300 µ1, or in polystyrene flasks at 5 x 10^6 cells/ml. Extraction and Northern blot analysis of total RNA from PMN were performed. Extracellular IL-lra was determined using a specific ELISA kit (50 pg/ml detection limit). Data were assessed for statistical significance using Student's t test for paired data, and expressed as means + SEM. It was observed that LPS is also a potent stimulus for the mRNA expression and release of the IL-1 receptor antagonist (IL-1ra). Also, the release of IL-1ra from LPS-stimulated PMN is markedly potentiated in the presence of IL-10 (from two to threefold after 18 hours of stimulation). Moreover, it was observed that this upregulation of IL-1ra production by IL-10 in LPS- stimulated PMN took place through IL-1ra mRNA stabilization. Indeed, the half-life of IL-1ra mRNA was prolonged in PMN stimulated in the presence of IL-10 and LPS, as compared with cells stimulated with LPS alone. IL-10 selectively upregulates IL-1ra production in LPS- activated PMN, while it inhibits the production of IL-1β, TNF, and IL-8 under the same conditions, thus, suggesting that IL-10 may be an important physiologic regulator of cytokine production from PMN, and emphasizes the potential role of IL-10 in inflammatory responses[98].

A study was done to investigate the autoregulatory effect of IL-10 on proinflammatory cytokine production by *Porphyromonas gingivalis* Lipopolysaccharide-tolerant Human monocytes. It was observed that pretreatment of human peripheral blood monocytes with a very low concentration (0.1 ng/ml) of *P. gingivalis* lipopolysaccharides (LPS) resulted in a significant decrease of IL-6 production, but not IL-8 production, by restimulation of a high concentration (1 µg/ml) of the same LPS. In contrast, the same pretreatment with Escherichia coli (E.coli) LPS resulted in the enhanced production of both IL-6 and IL-8 after restimulation. The selective induction by *P.gingivalis* LPS tolerance of IL-6 production developed in a time-dependent manner during the primary culture. *P. gingivalis* LPS-pretreated cells

were also refractory to a high-dose E.coli LPS restimulation in terms of IL-6 production. The expression of IL-6 mRNA decreased 10 hours after restimulation of *P. gingivalis* LPS-pretreated monocytes. Furthermore, an upregulation of anti-inflammatory cytokine IL-10 upon a second high-dose LPS rechallenge occurred at the same time point in the pretreated cells. The role of IL-10 in the process of IL-6 down-regulation was studied and it was found out that neutralization by an anti-IL-10 polyclonal antibody prevented IL-6 down-regulation in *P.gingivalis* LPS-pretreated monocytes, whereas IL-8 production was not affected. Addition of exogenous IL-10 during the high-dose LPS stimulation of untreated cells substituted for the LPS pretreatment and resulted in the inhibition of IL-6 production in a dose-dependent manner. A higher dose of IL-10 was required to suppress IL-8 synthesis from monocytes. Thus, it was suggested that IL-10 mediates IL-6 down-regulation in *P.gingivalis* LPS-tolerant monocytes in an autocrine manner[99].

A study was done to investigate the involvement of IL-1β, IL-8, and IL-10 and RANTES (regulated on activation, normally T cell expressed and secreted) and the cell populations associated with the immune response in destructive periodontitis, as well as the effect of periodontal therapy on cytokine levels in gingival crevicular fluid (GCF). Data were obtained from 12 patients with moderate to advanced periodontitis and 6 healthy controls. Patients presenting at least 2 sites with ≥ 2 mm clinical attachment loss were included in the destructive periodontitis group. After monitoring for 4 months, only 6 patients showed destructive periodontitis and GCF samples and soft tissue biopsies were collected from these patients. GCF samples and biopsies were collected both from active (12 GCF samples and 6 biopsies) and inactive (12 GCF samples and 6 biopsies) sites. The comparison with healthy controls was carried out by collecting GCF samples from 6 healthy volunteers (12 samples) and biopsies during the surgical removal of wisdom teeth. In periodontal patients, clinical data and GCF samples were obtained prior to periodontal treatment (72 samples) and 2 months after periodontal therapy (72 samples). GCF was collected using a paper strip; eluted and ELISA were performed to

determine cytokine levels. The inflammatory infiltrate was analyzed by immunohistochemistry of gingival biopsy samples with monoclonal antibodies against CD3, CD8, CD4, CD11c, and CD 19 antigens. It was observed that cellular components of the inflammatory infiltrate include B and T lymphocytes and monocyte/ macrophages. Active sites contained a higher number of B lymphocytes and macrophages. IL-8 and IL-1β and RANTES in GCF were detected in the majority of sites from periodontal patients (100%, 94% and 87%, respectively); IL-10 was found in only 43%. IL-8 was the only cytokine detected in the GCF (75%) of the control group. Moreover, IL-1β levels were significantly higher in active sites versus inactive sites ($P < 0.05$). IL-8 and IL-10 and RANTES were increased in active sites; however, differences were not significant ($P > 0.05$). A positive correlation between the IL-8 and RANTES ($r = 0.677$, $P < 0.05$) was observed in periodontitis patients. Periodontal therapy reduced the total amount of IL-1β, IL-8, and IL-10 and RANTES. Data showed a weak correlation between the clinical parameters and the total amount of cytokines in periodontitis. Thus, it was suggested that the amount of crevicular IL-1β, IL-8, and IL-10 and RANTES is associated with periodontal status[100].

A study was done to investigate the role of anti-inflammatory cytokine IL-10 and T cell cytokine profile in periodontitis granulation tissue. Biopsies were obtained from 10 patients with early onset periodontitis (EOP) and 10 patients with adult periodontitis (AP). Gingival tissue was obtained and sectioned from all of the patients in the AP group, to serve as controls. Polyclonal monospecific antibodies were used to detect cells expressing IL-2, IL-4, IL-6, IL-10, and IL-15, TNF-α and IFN-γ in formalin-fixed, paraffin-embedded sections of granulation tissue from periodontitis lesions. Also a series of oligonucleotide probes were employed to detect cells expressing the cytokine transcripts in the same tissue biopsies. It was observed that cells that expressed IL-4 or IL-6 were more numerous than cells expressing either IL-2 or IFN-γ. Th2 cells were more numerous in EOP and AP tissues. IL-15 substitutes for IL-2 in a number of biological activities related to the Th1 immune response, and interestingly, in

periodontal lesions the IL-15- expressing cells outnumbered IL-2- expressing cells, suggesting that this is the pattern of immune regulation by T cells in the periodontium. Thus, the functional balance in the T cell subsets detected by their cytokine profiles underlies the importance of the anti-inflammatory mechanisms taking place in the diseased tissue. The numbers of inflammatory leukocytes that express the anti-inflammatory cytokine IL-10 are much more widely distributed than those that express the proinflammatory cytokines IL-6 and TNF-α suggesting that large numbers of infiltrating inflammatory cells as well as accessory cells are involved in the down-regulation of the inflammatory and immune response in periodontitis[17].

A study was done to investigate whether the IL-10 gene promoter polymorphisms in Japanese patients are associated with adult periodontitis (AP) and generalized early-onset periodontitis (G-EOP). Genomic DNA was obtained from 34 AP patients, 18 G-EOP patients and 52 controls. The promoter region between -506 and -1140 was amplified by PCR, and polymorphisms were detected by nucleotide sequencing. It was found out that the haplotype frequencies in Japanese were quite different from those of Caucasian and were even slightly different from those of southern Chinese with systemic lupus erythematosus. No significant difference in allele or haplotype frequencies was found between patients and controls. Thus, it is suggested that IL-10 production may be regulated within the complex cytokine network in chronic inflammatory periodontal disease, rather than the gene polymorphisms[101].

A study was done to analyse the levels of the proinflammatory cytokine IL-1β and the anti-inflammatory cytokine IL-10 in GCF of patients with chronic periodontitis prior to, and following, periodontal therapy for a period of 32 weeks. GCF samples were obtained from 24 non-diseased and 72 diseased sites of 12 periodontal patients prior to as well as at 6, 16 and 32 weeks post-periodontal therapy. All sites received conventional periodontal treatment and IL-1β and IL-10 levels (concentration and total amount) were determined by ELISA. Additionally, probing pocket depth (PD), clinical attachment loss (CAL), gingival (GI) and plaque (PII) indices were evaluated pre- and

post-therapy. It was observed that IL-1β was detected in 382 out of 384 samples, while IL-10 was detected in 337 out of 384 samples. The total amount of IL-1β was significantly higher at diseased compared to non-diseased sites ($p < 0.01$). Following therapy, IL-1β total amounts were reduced, while IL-1β concentration gradually increased. IL-10 total amounts (per 30 s sample) were similar in diseased and non-diseased sites, and following therapy they remained almost unchanged. By contrast, IL-10 concentration was significantly higher in non-diseased sites ($p < 0.01$) and displayed a significant increase post-therapy. Moreover, IL-1β concentration and total amount were significantly greater in smokers following therapy, while IL-10 total amount was significantly higher in non-smokers both prior to and following therapy. Total IL-1β amounts were positively correlated with GI and PII. A weak negative correlation between IL-1β and IL-10 levels was noted ($p > 0.05$). Thus, it was suggested that the total amount rather than the concentration of IL-1β in GCF seemed to be closely associated with periodontal disease severity. Moreover, smoking status influenced IL-1β and IL-10 levels and an inverse relationship was evident between IL-1β and IL-10[102].

A study was done to examine the timing of the manifestation of accelerated alveolar bone loss in IL-10 (-/-) mice. Twenty four IL-10 (-/-) and twenty one IL-10 (+/+) age-matched male 129/ SvEv mice were used in the study. Sacrifice times occurred at 1, 3 and 9.5 months of age. Alveolar bone loss was determined morphometrically on defleshed jaws. ELISA was used for determination of serum concentration of type I collagen C-telopeptide, a systemic marker of bone resorption. It was observed that alveolar bone loss for the entire IL-10 (-/-) group was significantly different than for the IL-10 (+/+) group ($p= 0.025$). There was no significant difference in alveolar bone loss between IL-10 (-/-) and IL-10 (+/+) mice at 1 and 3 months of age. At 9.5 months of age, IL-10 (-/-) mice exhibited 39 % greater alveolar bone loss than IL-10 (+/+) mice ($p =0.018$). For IL-10 (-/-) mice, alveolar bone loss significantly increased with age. Serum C-telopeptide levels significantly decreased with age in both groups. IL-10 (-/-) had consistently higher C-telopeptide levels than IL-10 (+/+) mice and the

difference between the two groups reached statistical significance (p= 0.011) for the 9.5 month old mice. Thus, it was suggested that the accelerated alveolar bone loss observed in IL-10 (-/-) mice is a late-onset condition and that lack of IL-10 may have an effect on bone homeostasis[103].

A study was done to investigate the role of IL-10 gene transfer in attenuating *P. gingivalis*-induced inflammation. The effect of IL-10 plasmid injection on the local cytokine response was examined. Two weeks after the implantation of chambers, either IL-10 plasmid or vector was injected into the mice. Four days later, they were challenged with an intra-chamber injection of *P. gingivalis*. The intra-chamber levels of IL-10, IFN-γ, TNF-α, and IL-1β were evaluated after 2 and 24 hours. It was observed that local IL-10 gene delivery elevated the levels of IL-10 at both time periods. It attenuated the levels of IFN-γ (656 ± 154 to 218 ± 144 pg/mL) and TNF-α (23 ± 2.0 to 12.5 ± 2.9 ng/mL) at 2 hours, and of IL-1β (21.5 ± 5.7 to 12.4 ± 3.0 ng/mL) at 24 hours. Thus, it is suggested that there is a possibility of modulating the local inflammatory response to *P. gingivalis* by direct IL-10 gene transfer[104].

A study was done to investigate the role of IL-10 in inhibiting osteoclastogenesis by reducing NFATc1 expression and preventing its translocation to the nucleus. It is known that several major pathways regulate osteoclastogenesis, with key signaling genes such as p38, TRAF6, NF-Kb and NFATc1 well established as playing vital roles. So the gene expression was looked upon in 11 of these genes using real-time quantitative PCR on RNA extracted from RANKL-treated RAW264.7 monocytes. It was observed that there was no downregulation by IL-10 of DAP12, FcγRIIB, c-jun, RANK, TRAF6, p38, NF-κB, Gab2, Pim-1, or c-Fos at the mRNA level. However, IL-10 significantly reduces RANKL-induced NFATc1 expression. NFATc1 is transcribed from two alternative promoters in Mus musculus and, interestingly, only the variant transcribed from promoter P1 and beginning with exon 1 was downregulated by IL-10 (isoform 1). In addition, immunofluoroscence studies showed that IL-10 reduces NFATc1 levels in RANKL-treated precursors and suppresses nuclear translocation. The

inhibitory effect of IL-10 on tartarate-resistant acid phosphatase-positive cell number and NFATc1 mRNA expression was reversed by the protein kinase C agonist phorbol myristate acetate, providing evidence that IL-10 disrupts NFATc1 activity through its effect on calcium mobilization. Thus, it was suggested that IL-10 acts directly on mononuclear precursors to inhibit NFATc1 expression and nuclear translocation. And it proves that IL-10 directly inhibits osteoclastogenesis by suppressing NFATc1 activity[105].

A study was done to evaluate the broad effects of the functional IL-10 promoter-592 polymorphism in modulation of IL-10, TIMP-3, and OPG expression and their association with periodontal disease outcome. In this study, the IL-10 promoter -592 C/A single nucleotide polymorphism (SNP), which is associated with a decrease in IL-10 production, was analyzed by RFLP in 116 chronic periodontitis (CP) patients and 173 control (C) subjects, and the IL-10, TIMPs, and OPG mRNA expression levels in diseased gingival tissues were determined by real-time-PCR. It was observed that the IL-10 -592 SNP CA (P= 0.0012/ OR=2.4/ CI:1.4-4.1), AA (P= 0.0458/ OR=2.3/ CI:1.1-4.9), and CA+AA (P= 0.0006/ OR=2.4/ CI:1.4-3.4) genotypes and the allele A (P= 0.0036/ OR=1.7/ CI:1.2-2.4) were found to be significantly more prevalent in the CP group when compared with control subjects. Both CA and AA genotypes were associated with lower levels of IL-10, TIMP-3, and OPG mRNA expression in diseased periodontal tissues and were also associated with disease severity as mean pocket depth. Thus, it can be suggested that IL-10-592 SNP is functional in CP, being associated with lower levels of IL-10 mRNA expression, which is supposed to consequently decrease the expression of the downstream genes TIMP-3 and OPG, and influence periodontal disease outcome[106].

A study was done to evaluate three polymorphisms at positions-1082G> A, -819C> T and -590C> A in patients with generalized chronic periodontitis (n = 27) and generalized aggressive periodontitis (n = 32) in comparison with periodontitis-free controls (n =34). IL-10 promoter polymorphisms were analyzed by PCR with sequence-

specific primers (PCR-SSP). Distributions of single alleles, genotypes and haplotypes were calculated by the chi-square test. Risk-factor analyses were carried out by logistic regression. Subgingival bacteria were subjected to molecular biological analyses using the micro-Ident test. It was found out that the combination ATA/ ATA was found only in patients with aggressive periodontitis (15.6 vs. 0.0 %, p = 0.023). Considering into account age, gender, smoking and plaque level, an increased odds ratio (3.7, p = 0.04) for aggressive periodontitis was shown for subjects with the haplotype ATA. *Prevotella intermedia* was found to be decreased in ACC-positive (41.3 vs. 66.7 %, p = 0.022), ATA-positive (33.3 vs. 57.1 %, p = 0.032) and ACC/ ATA-positive (20.0 vs. 55.9 %, p = 0.002) individuals. In GCC/ GCC-positive subjects, *P. intermedia* occurred more frequently (86.7 vs. 42.3 %, p = 0.002). Thus, it was suggested that the haplotype ATA, which is known as a 'low interleukin-10 producer' is a putative risk indicator for generalized aggressive periodontitis[107].

A study was done to examine relationships between subgingival biofilm composition and levels of gingival crevicular fluid (GCF) cytokines in periodontal health and generalized aggressive periodontitis (GAP). Periodontal parameters were measured in 25 periodontally healthy and 31 GAP subjects. Subgingival plaque and GCF samples were obtained from 14 sites from each subject. 40 subgingival taxa were quantified using checkerboard DNA-DNA hybridization and the concentrations of 8 GCF cytokines measured using Luminex. Cluster analysis was used to define sites with similar subgingival microbiotas in each clinical group. Significance of differences in clinical, microbiological and immunological parameters among clusters was determined using the Kruskal-Wallis test. It was observed that GAP subjects had statistically significantly higher GCF levels of IL-1β ($p<0.001$), granulocyte-macrophage colony-stimulating factor (GM-CSF) ($p<0.01$), and IL-1β/IL-10 ratio ($p<0.001$) and higher proportions of Red and Orange complex species than periodontally healthy subjects. There were no statistically significant differences in the mean proportion of cytokines among clusters in the periodontally healthy subjects, while the ratio IL-1β/IL-10

($p<0.05$) differed significantly among clusters in the aggressive periodontitis group. Thus, it was suggested that different subgingival biofilm profiles are associated with distinct patterns of GCF cytokine expression. Aggressive periodontitis subjects were characterized by a higher IL-1β/IL-10 ratio than periodontally healthy subjects, suggesting an imbalance between pro- and anti-inflammatory cytokines in aggressive periodontitis[108].

A study was done to detect the serum levels of pro-and anti-inflammatory cytokines in chronic periodontitis (CP) patients and determine its correlation with different clinical parameters of the periodontal status, as well as study the correlation among these cytokines and to evaluate the ratio between pro- and anti-inflammatory cytokines. A total of 50 patients with chronic periodontitis were studied, their ages range from 23-60 years with a mean age of 40.1±7.6 years. Apparently healthy volunteers consisted of 25 individuals who were their age range (21-50) years with a mean age of 33.4±9.1 years considered as control. Periodontal parameters used in this study were plaque index, gingival index, probing pocket depth, clinical attachment level and bleeding on probing. Blood samples were collected from CP patients and healthy control groups to assess serum concentrations of IL-1β, IL-2, IL-6, TNF-α and IL-10 by means of enzyme-linked immune-sorbent assay. It was observed that median serum levels of pro-inflammatory cytokines (IL-1β and TNF-α) were significantly higher in CP patients than in healthy control groups ($p<0.001$), whereas the serum levels of IL-2and IL-6 were not observed any significant differences between two groups ($p>0.05$). In contrast serum levels of antiinflammatory cytokines (IL-10) was significantly low in patients when compared to control ($p<0.001$). On the other hand, the ratios of IL-1β\IL-10 and TNF-α\IL-10 were significantly higher in patients when compared with the ratios in control group. Regarding correlation between serum cytokines and clinical periodontal parameters, serum IL-1β level was showed significant positive correlation with each of plaque index, gingival index, probing pocket depth and clinical attachment level. On the

other hand no association between serum IL-2 levels and clinical parameters of chronic periodontitis were found. Moreover, IL-6 showed significant positive correlation with probing pocket depth, while TNF-α revealed significant positive association with each of gingival index, probing pocket depth and clinical attachment level. Conversely, serum IL-10 levels had negative significant correlation with plaque index, probing pocket depth and bleeding on probing. Interestingly strong linear positive correlation was found among each of (IL-1β, IL-6 and TNF-α,).While strong negative correlation was noticed between IL-10 and each of (IL-1β and TNF-α).Thus, the results of this study may provide direct evidence for the systemic activation of immune cells in periodontitis, and suggests that cytokines may play an important role in pro-inflammatory response in serum of patients with chronic periodontitis. Moreover imbalance between pro and anti-inflammatory cytokines could be involved in the initiation and progression of chronic periodontitis and is indicative of a stronger systemic proinflammatory state in disease[109].

INTERLEUKIN-11

A study was done to elucidate the molecular mechanisms regulating the anti-inflammatory activities of recombinant human (rh)IL-11, the ability of rhIL-11 to reduce serum levels of inflammatory mediators such as TNF-α, IL-1β, IL-12, and IFN-γ in LPS-treated mice and to down-regulate macrophage function in culture. In a mouse model of endotoxemia, pretreatment with rhIL-11 blocked LPS-induced elevation of TNF-α, IL-1β, and IFN-γ serum levels, but had no effect on IL-12 p40, IL-6, or IL-10 serum levels. It was observed that the effects of rhIL-11 on the production of inflammatory mediators in vivo may occur in part through direct interactions with macrophages. rhIL-11 pretreatment of thioglycollate-elicited peritoneal macrophages resulted in greater than 60% inhibition of LPS-induced production of TNF-α, IL-1β, IL-12 p40, and nitric oxide. The activity of rhIL-11 was not mediated through induction of

IL-10, IL-6, or TGF-β1. Thus, it was suggested that the ability of rhIL-11 to modulate the inflammatory response is not dependent on known anti-inflammatory cytokines and substantiate a role for this cytokine in the attenuation of inflammatory conditions[110].

A study was done to determine if recombinant human interleukin-11 (rhIL-11), known to downregulate several inflammatory modulators, has the ability in subcutaneous administration to reduce the rate and/or extent of periodontal attachment loss and radiographic bone loss in a ligature-induced periodontal disease beagle dog model. Twenty 18-month-old female beagle dogs were brought to optimal periodontal health over a 2-week period. Periodontal disease was induced by placing 2.0 silk ligatures around the mandibular first molar and premolar teeth. The dogs were divided into 3 treatment groups and one control group. The 3 treatment groups received subcutaneous injections of either 15, 30, or 80 µg/kg of rhIL-11 in saline buffer twice a week. The placebo group received buffer only subcutaneously twice a week. The gingival health of each animal was measured by recording the presence or absence of gingival inflammation, plaque, and bleeding upon probing. Attachment levels and bone height were also measured. Treatment administration and clinical and radiographic evaluations were performed in a masked fashion. It was observed that at week 8, the placebo group had 3.89 mm of attachment loss and 73.8% radiographic bone remaining. The 15 µg/kg group had 1.99 mm attachment loss and 89.5% bone remaining; the 30 µg/kg group had 0.84 mm attachment loss and 92.5% bone remaining; and the 80 µg/kg group had 1.05 mm attachment loss and 85.5% bone remaining. All 3 treatment groups lost significantly less attachment and retained significantly more bone than did the placebo group. Thus, it was suggested that subcutaneous injections of rhIL-11 were able to slow the progression of attachment and radiographic alveolar bone loss in a ligature-induced beagle dog model[111].

A study was done to evaluate the regulatory effects of TGF-β on IL-11 production by human periodontal ligament cells (PDL) and human gingival fibroblasts (HGF). The

expression of TGF-β receptor in PDL and HGF were observed using flow cytometry. PDL and HGF were stimulated with TGF-β with or without protein kinase C (PKC) inhibitors and activator. IL-11, bone morphogenetic protein-2 (BMP-2) and TGF-β mRNA expression was quantified by real-time polymerase chain reaction (PCR). IL-11 production was measured using ELISA assay. It was observed that PDL and HGF expressed both TGF-β receptor I and TGF-β receptor II on the cell surfaces. IL-11 mRNA expression and IL-11 production were augmented by TGF-β in both PDL and HGF, with higher values in PDL. PKC inhibitors partially suppressed TGF-β-induced IL-11 production in PDL and HGF, whereas activator enhanced it. TGF-β mRNA and BMP-2 mRNA expression were up-regulated by TGF-β in PDL. Thus, the results of this study suggested that PDL produce IL-11 in response to TGF-β[112].

A study was done examine the effect of IL-11 on the osteoblastic differentiation of periodontal ligament cells. Cultured periodontal ligament cells were stimulated with IL-11 and/or ascorbic acid, with or without inhibitors for type 1 collagen, janus kinase/signal transducers and activator of transcription, and mitogen-activated protein kinase (MAPK). Osteoblastic differentiation was investigated by examining the alkaline phosphatase activity and gene expression of Runx2, osteocalcin and bone sialoprotein using reverse transcription-polymerase chain reaction. Type 1 collagen and tissue inhibitor of metalloproteinase-1 production were measured using ELISA assays. It was found out that IL-11 enhanced alkaline phosphatase activity and Runx2, osteocalcin and bone sialoprotein gene expression in the presence of ascorbic acid. IL-11 induced type 1 collagen and tissue inhibitor of metalloproteinase-1 production in periodontal ligament cells. Type 1 collagen inhibitor completely inhibited the alkaline phosphatase activity enhanced by IL-11 and ascorbic acid. Furthermore, janus kinase/signal transducers and activator of transcription and MAPK signaling inhibitors reduced IL-11/ascorbic acid-induced alkaline phosphatase activity in periodontal ligament cells. Thus, it was suggested that IL-11/ascorbic acid induced the osteoblastic differentiation of periodontal ligament cells through type 1 collagen production and janus kinase/signal transducers

and activator of transcription, and MAPK signaling pathways were involved in this process. Also, IL-11 may function as an osteopromotive cytokine, stimulating the osteoblastic differentiation of periodontal ligament cells mainly through the synthesis of type 1 collagen and possibly by the induction of tissue inhibitor of metalloproteinase-1[113].

A study was done to analyse IL-1β, IL-11 and IL-12 levels in GCF of patients with gingivitis (G) and chronic periodontitis (CP). 40 subjects including 12 CP, 14 G and 14 controls (C) were enrolled in the study. GCF samples were collected from 6 maxillary sites per patient and analysed for IL-1β, IL-11 and IL-12 by an ELISA assay. It was observed that significantly lower concentrations of IL-11 were detected in CP compared with both G and C groups ($p < 0.05$). The CP group had a significantly higher total amount of IL-12 and IL-1β compared with the C group ($p < 0.05$). The IL-11/ IL-1β cytokine ratio was higher in both G and C groups compared with the CP group. The IL-11/ IL-1β ratio became progressively lower with increasing probing depth ($p < 0.01$). Thus, it was suggested that IL-11 levels are significantly decreased in GCF from sites with periodontitis compared with G and healthy sites. And because of the possible preventive effect of IL-11 on inflammation, IL-11 may be an important factor in the therapeutic modulation of periodontal disease[114].

A study was done to investigate the impact of the IL-11/ IL-17 ratio on the pathogenesis of chronic periodontitis. Forty chronic periodontitis (CP) patients and 20 healthy controls (C) were included. The CP group was divided into two subgroups in line with the probing depth (PD) in GCF-sampling sites (CPa: PD >or= 5 mm, CPb: PD <or= 4 mm). For each patient, gingival index, plaque index, gingival bleeding time index, PD, and clinical attachment level values were recorded. IL-11 and IL-17 in GCF were evaluated by ELISA assays. It was found out that the total amount and concentration of IL-11 and IL-17 were significantly lower in the CPa group ($P < 0.05$). The C group has the highest IL-11: IL-17 ratio, followed by CPb and CPa groups

respectively. The ratio was significantly lower in CPa group than the CPb and C groups ($P < 0.01$). Thus, the results of this study suggested that the decreased ratio of IL-11:IL-17 may be a factor, which has shown this imbalance between the cytokines' activities in deeper pockets in this study[115].

A study was done to evaluate IL-17 and IL-11 in GCF of generalized chronic periodontitis (GCP) and generalized aggressive periodontitis (GAgP) patients in relation to periodontopathic bacteria. GCF samples were collected from 65 subjects including 25 CP, 25 GAgP, and 15 controls (C) and analyzed for IL-17 and IL-11 by an ELISA assay. Molecular detection of bacteria in the dental plaque was determined by PCR. It was observed that the total amount of IL-17 was significantly higher in GAgP group than in GCP and C groups ($P < 0.001$). The IL-11 concentration was significantly higher in C and GCP groups than GAgP group ($P < 0.001$). The IL-11/ IL-17 ratio was significantly higher in the C group than in GCP and GAgP groups ($P < 0.05$). Morevoer, GAgP group showed lower ratios of IL-11/ IL-17 when compared to the GCP group. The high positivity of P. gingivalis in the dental plaque was associated with significantly increased GCF levels of IL-17 in GCP and GAgP patients. Thus, it was suggested that the increased IL-17 level in GCF of GAgP points to a potential role in the aetiopathogenesis. Meanwhile, the decreased ratio of IL-11/ IL-17 might reflect an imbalance between the proinflammatory and anti-inflammatory cytokines in different periodontal disease[116].

A study was done to investigate the levels of IL-11 and IL-17 and their ratio in GCF in patients with Aggressive periodontitis (AgP). 20 patients with generalized AgP (GAgP) and 18 healthy controls (HC) were enrolled in the study. For each patient, the values of clinical parameters, such as gingival index, plaque index, probing depth, and clinical attachment level, were recorded. Levels of IL-11 and IL-17 in GCF samples were evaluated using ELISA assay. The values of clinical parameters, cytokine levels, and the ratios of cytokines were evaluated. It was observed that the values of all the

clinical parameters were significantly higher in the GAgP group than in the HC group (P <0.001). The total amount and concentration of IL-11 and the concentration of the IL-17 and IL-11/IL-17 ratio were significantly lower in the GAgP group than in the HC group (P <0.001). The total amount of IL-17 was not significantly different between the groups (P = 0.317). Thus, it was suggested that the IL-11/IL-17 ratio was decreased in the GAgP group because of the decreased IL-11 levels. The IL-11/IL-17 axis and the link between IL-17 and neutrophil function disorders in AgP should be investigated to clarify the role of the IL-11/IL-17 axis and its balance and imbalance in the pathogenesis of AgP[117].

INTERLEUKIN-13

A study was done to investigate the effects of IL-13 alone or in combination with IL-4, IFN-γ, or IL-10 on human monocytes. IL-13 induced significant changes in the phenotype of monocytes. Like IL-4, it enhanced the expression of CD11b, CD11c, CD18, CD29, CD49e (VLA-5), class II MHC, CD13, and CD23, whereas it decreased the expression of CD64, CD32, CD16, and CD14 in a dose-dependent manner. IL-13 induced up-regulation of class II MHC Ag and its down-regulatory effects on CD64, CD32, and CD16 expression were prevented by IL-10. IFN-γ could also partially prevent the IL-13-induced down-regulation of CD64, but not that of CD32 and CD16. However, IL-13 strongly inhibited spontaneous and IL-10 or IFN-γ-induced ADCC activity of human monocytes toward anti-D coated Rh+ erythrocytes, indicating that the cytotoxic activity of monocytes was inhibited. Furthermore, IL-13 inhibited production of IL-1α, IL-1β, IL-6, IL-8, IL-10, IL-12 p35, IL-12 p40, macrophage inflammatory protein-1α, granulocyte/macrophage-CSF, granulocyte-CSF, IFN-α, and TNF-α by monocytes activated with LPS. In contrast, IL-13 enhanced the production of IL-1 ra by these cells. Similar results on cytokine production were observed or have been obtained

with IL-4. Thus IL-13 shares most of its activities on human monocytes with IL-4, but no additive or synergistic effects of IL-4 and IL-13 on human monocytes were observed, suggesting that these cytokines may share common receptor components. Thus, it was suggested that IL-13 has anti-inflammatory and important immunoregulatory activities[118].

A study was conducted to demonstrate that Interleukin 13 induces interleukin 4-independent IgG4 and IgE synthesis and CD23 expression by human B cells. IL-13-induced IgG4 and IgE synthesis by unfractionated peripheral blood mononuclear cells and highly purified B cells cultured in the presence of activated CD4+ T cells or their membranes. IL-13-induced IgG4 and IgE synthesis is IL-4-independent, since it was not affected by neutralizing anti-IL-4 monoclonal antibody. Highly purified, surface IgD+ B cells could also be induced to produce IgG4 and IgE by IL-13, indicating that the production of these isotypes reflected IgG4 and IgE switching and not a selective outgrowth of committed B cells. IL-4 and IL-13 added together at optimal concentrations had no additive or synergistic effect, suggesting that common signaling pathways may be involved. This notion is supported by the observation that IL-13, like IL-4, induced CD23 expression on B cells and enhanced CD72, surface IgM, and class II major histocompatibility complex antigen expression. In addition, like IL-4, IL-13 induced germ-line IgE heavy-chain gene transcription in highly purified B cells. Thus, the results of this study suggest that IL-13 is another T-cell-derived cytokine that, in addition to IL-4, efficiently directs naive human B cells to switch to IgG4 and IgE production[119].

A study was done to investigate the T lymphocytic cytokine production representing Th1 and Th2 subpopulations in smokers and non-smokers. Venous blood was collected from 30 treated periodontitis patients (12 smokers) and 24 healthy subjects (12 smokers). Whole blood cell cultures were stimulated and IFN-γ and IL-13 were measured in the culture supernatants, representing types 1 and 2 Th subpopulations,

respectively. Unadjusted data showed that smokers had more lymphocytes, and higher levels of IFN-γ and IL-13, irrespective of being periodontal patient. However in a multivariate analysis, increased IFN-γ production was not significantly explained by smoking, while higher IL-13 was strongly explained by smoking (21%, $p<0.001$).Thus, it was suggested that the increased Th activity and specifically an elevated Th2 profile in smokers may constitute a risk for smoking patients which may induce conversion of periodontal stability into progressive disease[120].

A study was done to investigate the effects of IL-4 and IL-13 on the expression of osteotropic cytokines in the IL-6 family expressed in human gingival fibroblasts. IL-4Rα and IL-13Rα1 mRNA were constitutively expressed in human gingival fibroblasts. The inflammatory cytokines IL-1β and TNF-α increased expression of IL-6, LIF, and IL-11 mRNA and protein in the gingival fibroblasts. Addition of IL-4 or IL-13 had no effect on IL-6 expression, but significantly inhibited LIF and IL-11 mRNA and protein stimulated by IL-1β and TNF-α. No involvement of NF-κB or STAT1 was observed in the inhibition. STAT6 was phosphorylated at Y641 by treatment with IL-4 and knockdown of STAT6 with siRNA decreased the inhibition of IL-11 and LIF expression by IL-4 in IL-1β and TNF-α stimulated cells. Thus, the results of this study suggested that a negative modulatory role of IL-4 and IL-13 in osteotropic cytokine production could be a mechanism playing an important inhibitory role in inflammation induced periodontitis[121].

TRANSFORMING GROWTH FACTOR- β

A study was done to evaluate changes in TGF-β1 levels in gingival crevicular fluid (GCF) and serum associated with periodontal inflammation in humans and dogs. TGF-β was quantified in gingival fluid and serum of beagles with experimentally-

induced periodontitis. Disease was monitored by several clinical parameters including Plaque Index, Gingival Index, probing depth, and epithelial attachment loss. Gingival tissues were obtained from 9 patients at the time of periodontal surgery, and gingival fluid samples were collected from an additional population of 10 periodontal patients. In 14 beagles, experimental periodontitis was induced and gingival fluids collected 6 months later. Fluid was collected by paper strips and volume measured by Periotron. Additionally, sera was collected before and 9 months after the ligature-induced periodontitis in 7 beagles. The levels of TGF-β1 were measured by ELISA. A significantly higher concentration of TGF-β1 was observed both in the gingival tissues and fluid samples obtained from the sites with deeper periodontal pockets than in the less involved sites. In beagles, TGF-β1 levels measured in gingival fluid were elevated in moderate disease, declining in fluid samples obtained from the pockets during more advanced experimental periodontitis. Furthermore, with the progression of experimental periodontitis, a decrease in TGF-β1 occurred in the sera of the beagle dogs. Thus, results of this study suggest that TGF-β1 may play a role in the pathogenesis and diagnosis of periodontal disease[122].

A study was done to evaluate TGF-β1 expression in the peri-implant soft tissues of healthy and failing dental implants. Twenty patients participated in this study. Ten biopsies from healthy keratinized mucosa and 10 biopsies from peri-implant soft tissues surrounding failing implants were obtained (one biopsy per patient). The biopsies were obtained from different patients. It was observed that in 5 cases of healthy mucosa, the stromal cells were positive between 1 to 5. In 7 cases, the epithelial layers were positive, between 1 and 18 cells. The superficial epithelial layer was negative in all cases. In 9 cases, there was a positivity of the vascular component, between 2 and 16 vessels. In failing implants, the stromal cells were positive in 6 cases, between 1 and 4. In all cases, cells of the epithelial layers were positive, between 15 and 40. The vascular component was positive in all cases, between 12 and 30 vessels. The differences between TGF-β1

expression in the epithelium around healthy and failing implants were statistically significant ($P < 0.0001$). The differences between TGF-β1 expression in the blood vessels in the soft tissues around healthy and failing implants were also statistically significant ($P < 0.0001$). No statistically significant difference was observed between the 2 groups in the TGF-β1 expression in the stromal cells ($P = 0.88$). Thus, it was suggested that TGF-β1 may be one of the most important factors in the regulation of the infiltrate, and in the production of tissue repair with a stimulation of fibroblasts and endothelial cells[123].

A study was done to measure TGF-β1 in GCF collected from sites that have undergone guided tissue regeneration (GTR) and conventional flap (CF) surgery and to compare these with GCF collected from unaffected healthy sites. GCF samples were collected, using filter paper strips, at baseline (pre-surgical) and then at intervals up to 26 weeks from 16 patients undergoing GTR and from 11 patients undergoing CF surgery. After elution and acid treatment, TGF-β1 levels were measured by ELISA. It was found out that treatment of periodontal defect sites significantly reduced the mean probing pocket depth (PPD) and improved the mean lifetime cumulative attachment loss (LCAL). Average GCF volumes also significantly increased at all sites at 2 weeks post-surgery and thereafter declined to baseline levels, except at the GTR test sites that were still elevated at 7 weeks. TGF-β1 could be detected in almost all GCF samples, and 2 weeks after surgery, the average levels increased two-fold at the surgically treated but not at the control sites, which remained unchanged. Thus, it was suggested that changes in the levels of this growth factor in GCF might be useful for monitoring the progress of periodontal repair and regeneration[124].

INTERLEUKIN-1 RECEPTOR ANTAGONIST (IL-1Ra)

A study was done to investigate the correlation of gingival crevicular IL-1 molecules and the clinical status of patients with different severities of periodontitis. IL-lα, IL-1β, IL-1ra and the total IL-1/IL-1ra ratio (IL-1 activity index; IL-1AI) were measured in 75 GCF samples from non-inflamed gingiva sites in 2 healthy subjects and diseased sites in 7 patients with several types of periodontitis. IL-lα, IL-1β and IL-1ra were measured by specific non-cross-reactive ELISA assay. The probing depth, gingival index and alveolar bone loss of each site was recorded at the time of GCF sampling. The total amount of IL-lα, IL-1β and the IL-1AI, but not total IL-1ra, were found to be correlated with alveolar bone loss score. Three IL-1 molecules were also measured in the gingival tissue of patients with periodontitis. A similar progressive decrease of the IL-1AI was detected in gingival tissue with periodontitis. Thus, it was suggested that the amounts of both crevicular IL-1 and IL-1AI are closely associated with periodontal disease severity. And IL-1ra does not seem to play any role in alveolar bone loss due to periodontal disease[74].

A study was done to elucidate the role of cytokines in chronic adult periodontitis and to investigate whether the prevalence of mRNA for inflammatory cytokines generally associated with mononuclear phagocytes was higher in diseased than in healthy gingival tissue. Gingival mononuclear cells or whole gingival biopsies from 32 adult periodontitis patients and five healthy individuals used as controls were evaluated for inflammatory cytokine mRNA expression by reverse-transcription polymerase chain-reaction (RT-PCR) procedures. The cytokines assessed included IL-1α, IL-1β, IL-lra, IL-6, IL-8, IL-12, IL-13, TNF-α, TGF-β, and IFN-γ. The monocyte/macrophage lipopolysaccharide (LPS) receptor CD14 was also assessed. It was found out that TNF-α mRNA was present significantly more frequently in diseased than in healthy biopsies, whereas IL-la, IL-1β, and IL-lra mRNA were found in most (from 80 to 100%) healthy

tissues. Message for CD14 was present in both healthy and diseased tissue samples (100%). Thus, the results of this study suggest that IL-1ra is not prevalent in chronic adult periodontitis. Also suggesting that the mononuclear cells derived from periodontal tissues have the capacity to respond to components of periodontal pathogens and express both pro- and anti-inflammatory cytokines in these tissues[125].

A study was done to investigate the association between IL-1 and IL-1ra in the GCF and gingival tissue of periodontitis patients. 65 GCF samples were collected from 10 patients with different severities of periodontitis and probing depth (PD), gingival index (GI) and alveolar bone loss (ABL) were measured at each time of sampling. Based on the results of the ABL, GCF samples were divided into 3 groups, slight sites (slight alveolar bone loss: slight ABL), moderate sites (moderate alveolar bone loss: moderate ABL) and severe sites (severe alveolar bone loss: severe ABL). The total amounts of IL-1α, IL-1β and IL-1ra were measured by ELISA assay. The IL-1 activity index (IL-1AI) was calculated by the ratio of IL-1/ IL-1ra. The amount of IL-1α, IL-1β and IL-1ra from gingival tissue homogenated was also determined by ELISA. It was observed that before initial treatment, the mean total amount of gingival cervicular IL-1α was 77.02 ± 16.05 pg/site (0 ± 0-325 ± 17.4 pg/site), the mean total amount of gingival cervicular IL-1β was 35.68 ± 6.37 pg/site (0 ± 0-177 ± 3.7 pg/site), the mean total amount of gingival crevicular IL-1ra was 16.63 ± 9.29 ng/site (0 ± 0-209 ± 22.1 ng/site). The mean of the IL-1AI was 6.11 ± 0.86. The total amounts of IL-1α and IL-1β increased depending on the severity of ABL. However, the total amount of IL-1ra in the GCF was statistically lower than other two groups, suggesting the possible anti-inflammatory role of IL-1ra in periodontitis[126].

A study was done to investigate the cytokine IL-1β and its receptor antagonist IL-1ra in gingival crevicular fluid (GCF), in patients with adult periodontitis. A total of 40 GCF samples were harvested from 10 subjects with moderate to severe adult periodontitis and 10 healthy controls. Subjects were selected from both genders, with all

the upper anterior teeth present, and with no relevant systemic illness, pregnancy or recent medication. All subjects were non-smokers and had not received any periodontal therapy within the preceding 3 months. Deep bleeding sites, deep non-bleeding sites and healthy sites were investigated in relation to upper anterior teeth. Clinical measurements were recorded for each site, after obtaining a GCF sample. IL-1β and IL-1ra were quantified using new commercially available ELISA kits. It was found out that the mean concentration for IL-1β was 0.11 (SD 0.14) pg/µl for bleeding periodontitis sites, 0.04 (0.05) pg/µl for non-bleeding periodontitis sites, and 0.01 (0.03) pg/µl for healthy sites ($p<0.001$). In contrast, the mean concentration for IL-1ra was 6.99 (9.78) pg/µl for healthy sites, 0.59 (0.44) pg/µl for non-bleeding periodontitis sites, and 0.44 (0.36) pg/µl for bleeding periodontitis sites ($p<0.001$, except for comparisons between bleeding and non-bleeding periodontitis sites, $p>0.05$). For healthy sites, a strong inverse relationship was found between IL-1beta and IL-1ra levels in GCE. Thus, the results of this study suggest a strong relationship between the severity of adult periodontitis and the decreasing levels of IL-1ra[75].

A study was done to investigate IL-1β and IL-1ra concentrations in GCF of periodontally healthy teeth and to periodontitis teeth and to reveal their relationships to periodontal clinical indices. Thirty GCF samples were harvested from periodontally healthy sites in which 15 samples were from periodontally healthy subjects and 15 GCF samples were from patients with chronic periodontitis, and 43 GCF samples (including 28 samples from BOP positive sites and 15 samples from BOP negative sites) were harvested from periodontitis sites of 33 patients with chronic periodontitis. Clinical indices (GI, PPD and CAL) were recorded for each tooth. The concentrations of IL-1β and IL-1ra in GCF were quantified by ELISA method. It was observed that the mean concentrations of IL-1β and IL-1ra and the average value of IL-1β/IL-1ra for periodontally healthy teeth were (61.891 +/- 20.719) pg/ml, (739.410 +/- 249.121) ng/ml and 0.857 +/- 0.375, respectively. The mean concentration of IL-1β (224.402 +/- 87.416)

pg/ml and the average value of IL-1β /IL-1ra for periodontitis teeth 6.813 +/- 0.375 were greater than those for healthy teeth, respectively (P<0.0001), and in contrast, the mean concentration of IL-1ra for periodontitis teeth (366.722 +/- 104.188) ng/ml was lower than that for healthy sites(P<0.0001). For all sites, a strong inverse relationship was found between IL-1β and IL-1ra levels in GCF (P<0.01). Both of IL-1β and IL-1β /IL-1ra values had positive correlations with GI, PPD and CAL (P<0.01). And the negative correlation was found between GI and IL-1ra (P<0.05). Also, the mean concentration of IL-1β and the average value of IL-1β/IL-1ra at BOP positive teeth were greater than those at BOP negative teeth (P<0.0001), and the mean concentration of IL-1ra at BOP positive teeth was lower than that at BOP negative teeth (P<0.01). Thus, it was suggested that the increasing GCF level of IL-1β and decreasing level of IL-1ra may be an important factor for the pathogenesis and development of chronic periodontitis and IL-1ra has an inhibitory effect on IL-1β activity. The GCF level of IL-1β and the value of IL-1β /IL-1ra, especially the latter one, are closely associated with the clinical indices of chronic periodontitis[127].

A study was done to examine the effect of phase I periodontal treatment on the levels of IL-1β, IL-1ra, and IL-10 in GCF in patients with generalized aggressive periodontitis (G-AgP). Data were obtained from 15 patients with aggressive periodontitis and 15 healthy controls. GCF was collected from at least four pre-selected sites (one shallow, at least two moderate, or at least one deep pockets) in patients with G-AgP. In the healthy group, GCF samples were collected from one site. The cytokine levels were determined by an ELISA assay. Probing depth, clinical attachment level (CAL), gingival and plaque indices, and bleeding on probing were measured. The GCF sampling and clinical measurements were recorded at baseline and 6 weeks later after periodontal treatment. It was observed that IL-1β levels were significantly higher at the moderate and deep pocket sites compared with the shallow sites (p<0.05). After periodontal therapy, IL-1β levels were significantly reduced in the moderate and deep

pocket sites ($p<0.05$). IL-1ra levels at baseline of the moderate and deep pocket sites were significantly lower than the control sites ($p<0.05$). IL-10 levels were similar in all pockets and did not change after periodontal therapy. Thus, it was suggested that the periodontal treatment improves the clinical parameters in G-AgP, and this improvement is evident in deep pocket sites for pocket depth and CAL values. And IL-1ra plays an important role in evaluating the progress of inflammation[128].

FUTURE PERSPECTIVES

Understanding the role of cytokines and immune response in the initiation and progression of periodontal disease has led to the development of cytokine-targeted therapies. These therapies have a great role in the treatment of periodontal diseases by inducing protective anti-inflammatory agents such as anti-inflammatory cytokines and receptor antagonists[129]. However, use of cytokine-targeted therapies have been utilized in inflammatory diseases like Rheumatoid arthritis and in limited experimental periodontitis studies using primates, dogs, mice and rats[130-132]. Thus, more studies need to be conducted in humans to assess the role of cytokine targeted therapies in the treatment of periodontitis. Further, deepened understanding of the relation between host response and cytokine network will help in the development of more tissue-specific targeted therapies for the treatment of periodontal diseases.

CONCLUSION

Periodontitis is one of the main chronic inflammatory diseases presenting the most prevalent form of bone pathology in humans[133]. This disease is characterized by an infectious condition leading to the occurrence of supporting tissue destruction, which is host-mediated by local production of immune-inflammatory markers in response to pathogens and their products[134]. By using inhibitors, it was shown that cytokines play an important role in this process. Innumerous pro- and anti-inflammatory cytokines have been identified in the gingival tissue or in crevicular fluid as a result of cellular responses to periodontopathogens[135-137].

The effects of cytokines that promote osteoclast formation and bone resorption seem to be counteracted by other cytokines that are anti-inflammatory. It is probable that the balance between stimulatory and inhibitory cytokines, together with the regulation of their receptors and signaling cascades, determines the level of periodontal tissue loss. Thus, a dual nature for host inflammatory immune responses exists in periodontal diseases, where the control of periodontal infection and the destruction of periodontal tissues seem to share similar cellular and molecular pathways.

Given the pleiotropic and redundant nature of cytokines, a successful approach for treatment of periodontal diseases might not involve inhibition of one particular cytokine but rather aim to shift the balance between pro- and anti-inflammatory cytokines.

In periodontal diseases, marked inflammatory responses cause cytokines to spill into general circulation, resulting in detectable levels in biological fluids, such as serum and plasma. Changes in the circulating levels of these proteins have been linked to many disease states, making them valuable functional biomarkers, providing information regarding an individual's inflammatory status. Such knowledge might allow the development of diagnostic, preventive and therapeutic strategies in order to improve the clinical management of periodontal diseases.

Thus, clarification of how various cytokines contribute to the problem and/or solution of pathological situations such as periodontal diseases may enable us to diagnose and treat the disease at the molecular and cellular levels to an extent previously thought to be impossible. And, great excitement awaits further studies in this field to diagnose and treat the disease at the earliest.

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