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REVIEW ARTICLE

# Anticytokine Therapy: The Newer Horizons Revisited

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## ABSTRACT

In the recent era of molecular biology, the focus on the progression of periodontitis is mainly on inflammatory mediators, such as cytokines initiated due to microorganisms. Cytokines, such as interleukin (IL)-1, IL-6, tumor necrosis factor (TNF)-α, matrix metalloproteinases (MMPs), and prostaglandin $E_2$ ($PGE_2$) play a vital role in alveolar bone destruction and extracellular matrix degradation in the pathogenesis of periodontitis. Hence, the concept of inhibition of cytokine production or action through anticytokine therapy is implicated in various immune and inflammatory disorders and periodontitis. The concept of anticytokine therapy has grabbed quite an attention when compared with other existing treatment strategies for immune and inflammatory disorders. However, literature on anticytokine therapy in dentistry, particularly periodontology explaining the newer concepts, is not available till date. Therefore, the present study reviews the comprehensive appraisal of the newer aspects of anticytokine therapy and its applications in periodontology.

**Keywords:** Anticytokines, Cytokines, Host modulation, Periodontitis, Rheumatoid arthritis, Signaling pathways, Soluble receptors.

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## INTRODUCTION

Periodontitis is a chronic inflammatory disease caused primarily by bacteria in dental plaque, associating the supporting structures of the teeth.[1] Specific periodontal pathogens, such as the Gram-negative anaerobic bacteria inhabiting within the subgingival plaque, are associated with the progressive form of the disease. Although bacteria are the major etiological agents, the host immune response to these bacteria is of fundamental importance.[2,3]

Hence, it is evident that periodontitis is a multifactorial disease, associated with specific microorganisms, social and behavioral factors, genetic or epigenetic trait, all of which are modulated and controlled by the underlying immune and inflammatory responses of the host. Inflammation in periodontitis causes elevated T-lymphocytes, neutrophils, monocytes, and dendritic cells at the inflammatory site and becomes activated by virulence factors, antigens (lipopolysaccharides) or products from bacteria. These activated cells secrete proinflammatory cytokines and inflammatory mediators like IL-1β, IL-6, TNF-α, IL-7, IL-17, MMPs, and $PGE_2$, which accelerate osteoclastic development and activities through receptor activator of nuclear factor kappa B/receptor activator of nuclear factor kappa B ligand (RANK/RANKL) pathway, leading to alveolar bone destruction and extracellular matrix degradation.[4]

Host modulation therapy is one of the modalities to prevent and treat periodontal diseases by regulating the proinflammatory cytokines and inflammatory mediators.[5,6] The various approaches for host modulation are: (1) inhibition of MMPs through subantimicrobial dose of doxycycline and chemically modified tetracyclines, (2) inhibition of arachidonic acid metabolite through nonsteroidal anti-inflammatory drugs, (3) modulation of bone metabolism through bisphosphonates and hormone replacement therapy, (4) regulation of immune and inflammatory response through suppressing proinflammatory cytokines (anticytokine therapy) and oxidative stress, (5) proresolution of inflammation by endogenous lipid mediator through resolvins. Therefore, the use of anticytokine therapy along with conventional treatments, such as scaling and root planing has shown to be advantageous.[7]

### Cytokines

Cytokines are soluble proteins produced by nucleated cells throughout the body, especially from lymphocytes (majorly T cells), monocytes, macrophages, and granulocytes, and also by epithelial cells, endothelial cells,

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**Table 1:** Functional classification of cytokines[8,9]

| *Family* | *Members* |
|---|---|
| Hematopoietic | IL-3, G-CSF, GM-CSF, M-CSF, EPO, SCF |
| Proinflammatory | IL-1α, IL-1β, IL-6, IL-17, TNF-α, LT, LIF |
| Anti-inflammatory | IL-1Rα, 4, 10, 13 |
| Growth and differentiation | PDGF, TGF-β, VEGF, EGF, FGF, IGF |
| Immunoregulatory | TGF-β, IFN, IL-2, 4, 5, 7, 9–18 |
| Chemotactic | IL-8, MIP-1α, MIP-1β, MCP-1, RANTES |

G-CSF: Granulocyte colony-stimulating factor; GM-CSF: Granulocyte macrophage colony-stimulating factor; M-CSF: Macrophage colony-stimulating factor; EPO: Erythropoietin; SCF: Stem cell factor; LT: Lymphotoxin; LIF: Leukemia inhibitory factor; IL-1Ra: Interleukin-1 receptor antagonist; PDGF: Platelet-derived growth factor; TGF: Transforming growth factor; VEGF: Vascular endothelial growth factor. EGF: Epidermal growth factor; FGF: fibroblast growth factor; IGF: Insulin-like growth factor; IFN: Interferon; MIP: Macrophage inflammatory protein; MCP: Monocyte chemotactic protein; RANTES: Regulated upon activation, normal T-cell expressed and secreted; modified from Arend WP

**Table 2:** Proinflammatory cytokines and its membrane and soluble receptors

| *Cytokines* | *Membrane receptors* | *Soluble receptors* |
|---|---|---|
| IL-1β | IL-1RI | sIL-1RI |
| | IL-1RII | sIL-1RII |
| | IL-1RAcP | |
| TNF-α | TNF-RI | sTNF-RI |
| | TNF-RII | sTNF-RII |
| IL-6 | IL-6R | |
| | Glycoprotein-130 | sIL-6R |

IL-1R: Interleukin-1 receptor; IL-1RAcP: Interleukin-1 receptor associated protein; sIL-1RI: Soluble interleukin-1 receptor; TNF-R: Tumor necrosis factor receptor; sTNF-RI: Soluble tumor necrosis factor receptor; IL-6R: Interleukin-6 receptor; sIL-6R: Soluble interleukin-6 receptor

**Table 3:** Cytokines and its agonists and antagonists

| *Cytokines* | *Agonist* | *Antagonist* |
|---|---|---|
| IL-1β | IL-1RI | sIL-1R |
| | IL-1RII | |
| | IL-1RAcP | IL-1Ra |
| TNF-α | TNF-RI | sTNF-RI |
| | TNF-RII | sTNF-RII |
| | | Anti-TNF antibody |
| IL-6 | IL-6R | Anti-IL-6 antibody |
| | (gp-130) | Sgp-130 |
| | sIL-6R | |

IL-1R: interleukin-1 receptor; IL-1RAcP: interleukin-1 receptor associated protein; sIL-1R: soluble interleukin-1 receptor; TNF-R: tumor necrosis factor receptor; sTNF-RI: soluble tumor necrosis factor receptor; IL-6R: interleukin-6 receptor; sIL-6R: soluble interleukin-6 receptor; gp-130: Glycoprotein 130; sgp-130: Soluble form of glycoprotein 130

and fibroblasts.[4,8] They play a central role in the pathogenesis of various inflammatory diseases, including periodontal diseases. Adequate amount of proinflammatory cytokine production against microorganisms is necessary to protect host against the virulent effects of microorganisms, although inadequate or excessive production of these cytokines can damage the host via tissue destruction and leads to disease progression (Table 1). Cytokines have a complicated built-in biological redundancy, such that many cytokines have overlapping functions. Hence, the overall biological effect is the result of the balance between all cytokines, rather than their individual levels.[4,7]

Cytokines act through receptors, which are located on the cell surface. These receptors are membrane-bound receptors (signal transducers), and when shed from cell surface by proteolytic enzymes, they are called soluble receptors (Table 2). Soluble receptors of certain cytokines act as antagonists or agonists to respective membrane-bound receptors of cytokines by downregulating (prevents downstream signaling) or transactivating mechanisms (activates nonresponsive cells) respectively[2,7,10] (Table 3).

Cytokines bind to specific receptors on target cells and initiate intracellular signaling cascade. This causes alteration in gene regulation and activation, thereby releasing secondary mediators like MMPs and $PGE_2$. These mediators cause connective tissue breakdown and bone resorption.[3] Interleukin-1α, IL-1β, TNF-α, IL-6 are proinflammatory cytokines, produced for prolonged periods at local inflammatory sites, essential for the initiation of periodontitis and its progression.[11]

Interleukin-1 is a proinflammatory cytokine, subdivided into IL-1α, IL-1β; both are synthesized as 31 kDa precursors, and they have a large difference in posttranslational modifications. Interleukin-1α is biologically active, cleaved to a small extent. Interleukin-1β needs a proteolytic cleavage and intracellularly activated by specific enzymes like caspases, and it is a potent stimulator of MMPs and $PGE_2$.[11,12] Hence, IL-1β has a central role in mediating a variety of inflammatory responses and bind to membranous receptors on target cells. Tumor necrosis factor-α is also a proinflammatory cytokine, produced mainly by macrophages and has a capacity to induce bone resorption and upregulate $PGE_2$, MMPs, and adhesion molecules on leukocyte and stimulate the production of chemokines, finally resulting in severe inflammatory response. These effects are mediated through membranous-bound TNF-receptor (mTNF-R). There are type I TNF-R (55 kDa molecular weight) and type II TNF-R (75 kDa molecular weight) and differ with each other in their intracellular domain, thereby producing different cellular responses. Tumor necrosis factor-α binds to these receptors with high affinity and produces downstream signaling through mitogen-activated protein kinases (MAPKs) and nuclear factor kappa B (NF-κB) activation cascades. Moreover, TNF-R associated factor domain, death domain containing adapter proteins, and associated signaling enzymes are

responsible for initiating signaling by above-mentioned cascades and these are specific for TNF-α signaling.[7] By action of proteolytic enzymes on the cell membrane, TNF-R shed from the cell surface. These shed receptors are soluble (sTNF-R) in various biological fluids and act as antagonist to TNF-α by preventing the binding of TNF to mTNF-R. There exists a vast evidence in literature suggesting the higher levels of sTNF-R that are detected in inflammatory conditions like periodontitis.[2,5,7,13]

Interleukin-6 is another important proinflammatory cytokine responsible for various biological activities in most of the cells. It acts via ligand binding receptor (IL-6R) and signal transducer glycoprotein-130 (gp-130), located on the cell membrane. The cytosolic signaling pathways involved in IL-6 initiation include Janus associated kinases (JAKs), signal transduce and activator of transcription 3 (STAT3), and MAPK pathways. In contrast to other cytokine-soluble receptors, soluble forms of IL-6 receptors (IL-6Rs) are agonist to ligand binding IL-6R. However, soluble form of gp-130 has antagonistic effect to IL-6R (Table 3) and it contains only single domain. Therefore, it can release in large amounts, can act as endocrine cytokine, activate liver to produce acute phase proteins, and also activate hypothalamus for thermoregulation.[4,7,14-16]

## Anti-inflammatory Cytokines

Inflammatory mediators that lead to bone resorption depend on the expression of proinflammatory cytokines, and to the contrary, anti-inflammatory cytokines, such as IL-4, IL-10, IL-12, IL-13, and IL-18, serve to inhibit bone resorption.[12] Interleukin-1 receptor antagonists (IL-1Ra) competitively block the IL-1 binding without activating signaling pathways through binding, specifically to cell surface receptors, such as IL-1RI with high affinity and not to the IL-1R-associated proteins.[13] Interleukin-4 potent anti-inflammatory cytokine decreases osteoclastogenic activity of osteoblasts and directly targets osteoclast progenitor cells, thereby decreasing bone resorption. Interleukin-10 decreases RANKL and increases osteoprotegerin, thereby inhibits bone resorption.[4,14-16] Interleukin-11 decreases tissue destruction by stimulation of a tissue inhibitor of MMP-1 (TIMP-1) and also inhibits TNF-α, IL-1β, IL-12p40, and nitric oxide.[17]

## Anticytokine Therapies in Various Immune and Inflammatory Diseases

Regulation of the effects of cytokines has been suggested for therapeutics used in tissue destructive inflammatory diseases, such as rheumatoid arthritis (RA), Crohn's disease, and various other immune and inflammatory diseases. Hence, inhibitors of cytokine production or action are widely investigated as potential therapeutic modalities in a variety of immune and inflammatory diseases, including periodontitis (Tables 4 and 5).[8,15,18]

## Strategies to Inhibit Cytokine Activity[5,10,19]

- Antibodies to specific cytokines
- Immunoadhesins (recombinant soluble receptors)
- Soluble cytokine receptors
- Blockade of cytokine receptors
- Disruption of cell signaling pathways or activation of anti-inflammatory pathway.

Antibodies to specific cytokines are a leading approach to neutralize cytokines in the use of specific antibodies directed against the cytokine or its corresponding receptor. The advantages of this are excellent solubility, high specificity, and long half-life in serum. It has certain limitations due to rapid metabolism of antibodies and necessitates repeated administration. Administration of cytokine receptor antagonists can induce an antibody and neutralize cytokine and it eliminates the need for repeated administration of anticytokine antibodies,[4,18-20] e.g., IL-6R antagonist.

Immunoadhesins are another approach used to develop biological inhibitors of cytokine activity to engineer a fusion protein that combines the constant domain of an antibody molecule with the ligand recognition domain of a cytokine receptor. Its advantages are that it eliminates the need to immunize an animal, circumvent screening for cytokine-specific antibodies, antigen recognition, and extended half-life in serum.[14-16,19]

Soluble cytokine receptors are other means for regulating cytokine-induced pathways. Cytokines produced during inflammation are strongly regulated at transcriptional and translational levels. Production of soluble cytokine binding receptors blocks cytokine action (except for IL-6) at the inflammatory site by downregulation mechanisms. These are found in blood and extracellular fluid and are derived from the proteolytic cleavage of the extracellular domains of cell membrane-bound cytokine receptors. An antagonist of cytokines downregulates the respective cytokines by blocking the signaling pathways.[19]

## Blockade of Cytokine Receptors[13-15,19]

Natural cytokine receptor antagonists bind to the membrane receptors present on target cell and prevent respective cytokine binding to the target cell, thereby preventing activation of the target cells. For example, IL-1Ra bind to IL-1RI but not to the IL-1R associated proteins; it can bind to the IL-1RI with high affinity without activating signaling pathways and competitively blocks the IL-1 binding.

**Table 4:** Various commercially available anticytokine drugs, strategies to inhibit cytokine and targeted cytokine molecules[19,21]

| *Type of strategy to inhibit cytokine activity* | *Cytokine antagonists* | *Target cytokine* | *Biological type* | *Clinical status* | *Company name* |
|---|---|---|---|---|---|
| Antibodies to specific cytokines | Infliximab™—Remicade® [5,6] | TNF-α | Chimeric monoclonal antibody mAb | Approved | Johnson & Johnson/ Centocor |
| | Golimumab™ (CNTO 148)[22] | TNF-α | Human mAb | Approved | Simpani® |
| | Certolizumab pegol™ (CDP870)[23] | TNF-α | Human mAb | Phase III | Cimzia® |
| | Canakinumab™ (ACZ885)[24] | IL-1β | Humanized mAb | Approved | Ilaris® |
| | Tocilizumab (Atlizumab)[25] | IL-6R | Humanized mAb | Approved | Actemra and RoActemra® |
| | Bevacizumab—Avastin™ [26] | VEGF-A | Humanized mAb | Approved | Genentech |
| | HuMax-IL-15/AMG714[27] | IL-15 | Human mAb | Phase I/II | Genmab A/S, immunex |
| | Basiliximab—Simulect®[28] | IL-2Rα | Chimeric IgG1 mAb | Approved | Novartis |
| | Daclizimab—Zenapax®[29] | IL-2Rα | Humanized mAb | Approved | Protein design labs/ Hoffman LaRoche |
| | Secukinumab—AIN457[30] | IL-17A | Human IgG1k mAb | Phase IIIb | Cosentyx |
| | Denosumab[31] | RANKL | Human mAb | Approved | XGEVA® |
| | Ustekinumab (CNTO 1275)[32] | IL-12 and IL-23 | Human mAb | Approved | Stelara® |
| Immunoadhesins | Etanercept—Enbrel®[33] | TNF-α, LT-α | Receptor/IgG fusion protein | Approved | Immunex |
| | Altrakincept—Nuvance® | IL-4 | Receptor/IgG fusion protein | Phase II | Immunex |
| | Rilonacept (IL-1 Trap)—Arcalyst®[34] | IL-1 | Dimeric fusion protein | Approved | Regeneron pharmaceuticals, Inc. |
| Cytokine antagonists | Kineret–Anakinra[13] | IL-1R | Receptor antagonist | Approved | Amgen |
| | D2E7-Adalimumab[35] | TNF-α | Human IgG1 mAb | Phase III | Abbott |
| Disruption of cell signaling pathways | RWJ 67657[36] VX-745[37] | TNF-α, IL-6, and IL-8 | p38 mitogen activated protein kinase inhibitors | | Vertex Pharmaceuticals |
| | SP600125[38] | TNF-α, interferon-gamma, IL-6, COX-2, and MMPs | c-Jun N-terminal kinase pathway inhibitors | | SelleckChem |

Phase I: Testing of drug on healthy volunteers for dose-ranging, with subtherapeutic, but with ascending doses; Phases II and III: testing of drug on patients to assess efficacy and safety at therapeutic doses, differs in terms of number of participants, 100 to 300, 1000 to 2000 respectively

**Table 5:** Various anticytokine drugs and their indications

| *Drug* | *Indications* | *Company name* |
|---|---|---|
| Avastin® (bevacizumab) | Advanced breast and colorectal cancer | Genentech |
| Remicade® (infliximab) | Ankylosing spondylitis<br>Crohn's disease<br>Psoriatic arthritis<br>Rheumatoid arthritis<br>Psoriasis | Johnson & Johnson/ Centocor |
| Enbrel® (etanercept) | Ankylosing spondylitis<br>Juvenile rheumatoid arthritis<br>Psoriatic arthritis<br>Rheumatoid arthritis<br>Psoriasis | Immunex |
| D2E7 and HuMax-IL-15® (adalimumab) | Rheumatoid arthritis | Abbott |
| Kineret® (Anakinra) | Rheumatoid arthritis | Amgen |
| Simulect® and Zenapax® (Basiliximab) | Acute kidney transplantation rejection | Novartis |
| Nuvance® (altrakincept) | Asthma | Immunex |

## Disruption of Cell Signaling Pathways or Activation of Anti-inflammatory Pathway[20]

Pharmacological inhibitors of MAPK, NF-κB, and JAK/STAT pathways are being developed to treat RA, periodontal diseases, and other inflammatory diseases.

## Implications in Periodontal Diseases

Rheumatoid arthritis is one of the best disease models, while describing the implications of anticytokine therapy. It has been noticed that RA resembles periodontitis with respect to pathogenesis, progression of disease, and cytokine levels except IL-1β. Therefore, anticytokine therapy has been implicated in the treatment of experimental periodontitis.[11-15,39]

Anticytokine therapy for periodontal diseases primarily targets production or actions of IL-1β, IL-6, TNF-α, because they are necessary for the initiation and progression of periodontal diseases and persistent production at the inflammatory site. There exists

convincing evidence that inflammation associated with gingivitis is actively protective, since blocking further upregulation of the host response with IL-1/TNF antagonists inhibits the inflammatory response and bone loss in periodontitis.[14] It has been shown that this utilization of soluble receptors, specific to inflammatory cytokines, which potentially stimulate fibroblasts to regulate biological events is involved in the pathogenesis of periodontal diseases.[7] By targeting the specific proinflammatory cytokines like IL-1 and TNF-α via soluble antagonist, we can be able to arrest the periodontal disease progression.[16]

Treatment strategies currently available for controlling inflammation/bone resorption in periodontal diseases are (Table 6):

- Natural cytokine antagonists [4]
- Neutralizing antibody to TNF-α: Infliximab®[5,6]
- Recombinant soluble receptor to TNF-α: Etanercept®[32]
- Soluble human rhIL-1R type I[14]
- Antagonist to IL-1R: Anakinra®[13]
- Recombinant human IL-11[17]
- Cytokine suppressive anti-inflammatory drugs[36-38,40]
- Gene therapeutics[7]

### Natural Cytokine Antagonists

It binds to the membrane receptors present on the target cell and prevent respective cytokine binding to the target cell, thereby preventing activation of target cells. For example, IL-1Ra binds to IL-1RI but not to the IL-1R associated proteins; it can bind to the IL-1RI with high affinity without activating signaling pathways and competitively blocks the IL-1 binding.[4]

### Neutralizing Antibody to TNF-α: Infliximab™—Remicade®[5,6,41]

It is a chimeric immunoglobulin G (IgG) monoclonal antibody, which neutralizes proinflammatory cytokine, TNF-α. It has been shown in various studies that periodontitis presents heaps similarities with RA with respect to TNF-α-induced bone resorption. The benefits of TNF-a blockade in RA prompted to determine infliximab efficacy in treating coexisting periodontitis. Patients with RA receiving infliximab had lower periodontal indices and gingival crevicular fluid TNF-alpha levels.[5,6] Pers et al[6] compared the efficacy of infliximab in group of RA patients with chronic periodontitis group and periodontally healthy group and concluded that infliximab can decrease clinical attachment loss in chronic periodontitis group.

Gonçalves et al[41] evaluated the efficacy of infliximab on periodontitis in Wistar rats with different dosages and concluded that 5 mg/kg infliximab might reduce the proinflammatory cytokines like IL-1β and TNF-α, thereby preventing further progression of periodontitis and proved that infliximab had significant anti-inflammatory and bone-protective effects.

### Recombinant Soluble Receptor to TNF-α: Etanercept (Enbrel)®

Etanercept (75 kDa) is a dimeric, recombinant soluble form of the TNF-R consisting of extracellular domain of TNF-RII linked to the Fc portion of a human $IgG_1$. The anti-inflammatory effects of etanercept are due to its ability to bind to TNF-α, preventing it from interacting with cell membrane-bound receptors and making it biologically inactive. Etanercept can inhibit TNF, thereby modifying its biological actions like the adhesion molecules expression for leukocyte migration, cytokine levels in serum, and MMP-3.[32] Di Paola et al[33] postulated that treatment with etanercept significantly reduced the signs of periodontitis (degree of periodontitis inflammation and tissue injury), infiltration of neutrophils, the expression cytokines (e.g., TNF-α), and apoptosis genes (Bax and Bcl-2 expression). Hence, periodontitis (tissue destruction and clinical attachment loss) can be reduced/retarded with etanercept treatment.

### Soluble Human rhIL-1R

Soluble human rhIL-1R type I consists of the extracellular portion of the type I receptor. It has been shown in various studies that function blocking of soluble receptors to IL-1 was applied by local injection to sites (6.6 μg/injection three times each week for 6 weeks) in experimental animals with induced periodontitis that inhibits approximately 80% of the recruitment of inflammatory cells in close proximity to bone. The formation of osteoclasts was reduced by 67% at the experimental sites compared with that at the control sites (sites injected with vehicle alone), and the amount of bone loss was reduced by 60%.[14]

Delima et al[16] statistically proved that IL-1 and TNF antagonists can reduce the clinical attachment loss by approximately 51% and alveolar bone resorption by almost 91%. Gravas et al[15] studied the effects of soluble receptors and receptor antagonists of IL-1 and TNF-α and showed that IL-1 and TNF-α antagonists block the progression of the inflammatory cell infiltrate toward the alveolar bone crest, the recruitment of osteoclasts, and periodontal attachment and bone loss.

Compared with control animals, intrapapillary injection of soluble receptor antagonists of IL-1 and TNF-α reduced the pattern of bone loss by approximately 50% as assessed by Computer-Assisted Densitometric Image Analysis.[15,16]

**Table 6:** Detailed description of various anticytokine therapies used in periodontal disease

| *Anticytokine therapy on periodontal disease* | *Drug used* | *Study by* | *Number of subjects or animals* | *Periodontal disease with or without RA* | *Dosage* | *Duration of the study* |
|---|---|---|---|---|---|---|
| Neutralizing antibody to TNF-α | Infliximab™—Remicade® | Pers et al[6] | 40 subjects (Groups I and II) | With RA | 3 mg/kg | >22 months |
| | | Periodontal outcome: Attachment loss and alveolar bone resorption was reduced after infliximab treatment | | | | |
| | | Mayer et al[5] | 30 patients (RA + RA + and control) | With RA | 200 mg | Every 8 weeks patient had received treatment |
| | | Patients with RA receiving infliximab had lower periodontal indices and TNF-α gingival crevicular fluid levels | | | | |
| | | Gonçalves et al[41] | Wistar rats | Experimental periodontitis | 1, 5, 7, and 10 mg/kg | 11 days |
| | | Infliximab (5 mg/kg) reduced granulocyte blood counts, gingival IL-1β, TNF-α, and MPO levels, diminished MMP-1/-8, RANK, and RANK-L bone immunolabeling with better periodontal histopathological scores and collagen network in comparison with the challenged saline group | | | | |
| Recombinant soluble receptor to TNF-α | Etanercept (Enbrel)® | Di Paola et al[33] | Sprague-Dawley rats | Experimental periodontitis | 5 mg/kg sc | |
| | | Significantly reduced the degree of (1) periodontitis inflammation and tissue injury (histological score), (2) infiltration of neutrophils (MPO evaluation), (3) iNOS [the expression of nitrotyrosine and cytokines (e.g., TNF-a)], and (4) apoptosis (Bax and Bcl-2 expression) | | | | |
| Soluble receptors/ antagonists | Soluble receptors to IL-1 plus soluble receptors to TNF | Assuma et al[14] | *Macaca fascicularis* | Experimental periodontitis | 6.6 µg/ injection | 6 weeks |
| | | Inhibits approximately 80% of the recruitment of inflammatory cells in close proximity to bone. The formation of osteoclasts was reduced by 67% at the experimental sites compared with that at the control sites (sites injected with vehicle alone), and the amount of bone loss was reduced by 60% | | | | |
| | Soluble antagonists to IL-1 plus soluble receptors to TNF | Delima et al[16] | *Macaca fascicularis* | Experimental periodontitis | 6.6 mg/100 mL | 4 weeks |
| | | Reduces the clinical attachment loss by approximately 51% and alveolar bone resorption by almost 91% | | | | |
| | Interleukin-1 and tumor necrosis factor antagonists | Gravas et al[15] | *Macaca fascicularis* | Experimental periodontitis | 6.6 mg/ 100 mL | 4 weeks |
| | | 67% reduction in the number of osteoclasts and a 60% reduction in the amount of bone loss in animals with ligature-induced alveolar bone loss that have been treated with IL-1/ TNF blockers compared with controls | | | | |
| Recombinant human IL-11 (rhIL-11) | | van den Berg[18] | Beagle dog | Experimental periodontitis | | 8 weeks |
| | | Minimizes inflammation and tissue destruction | | | | |
| Cytokine suppressive anti-inflammatory drugs | SD-282 | Kirkwood et al[40] | Wistar rat | Experimental periodontitis | 15 or 45 mg/kg | 8 weeks |
| | | Reduced LPS induced periodontal disease, inflammatory cytokine expression, osteoclastogenesis, and alveolar bone loss by inhibiting p38α MAPK inhibitors | | | | |

## Antagonist to IL-1R: Anakinra (Kineret®)

It is an IL-1Ra and blocks the biological activity of IL-1 by competitively inhibiting the binding of IL-1 to the cell membrane-bound IL-1R in both *in vivo* and *in vitro* and prevents cell signaling pathways and thereby renders inflammation and reduces tissue destruction in periodontal diseases.[13]

## Recombinant Human IL-11 (rhIL-11)

It inhibits TNF-α and other proinflammatory cytokines and stimulates TIMP-1 and minimizes inflammation and tissue destruction respectively. Subcutaneous administration of rhIL-11 twice a week had the ability to reduce the rate or extent of periodontal attachment loss and

radiographic bone loss in a ligature-induced beagle dog model after 8 weeks.[18]

## Cytokine Suppressive Anti-inflammatory Drugs

Plaque accumulation at gingival margin can cause inflammatory cascade through series of signaling pathways that help in recognizing external antigen. These signals pass to nucleus from cell membrane via cytoplasm and alter the gene expression by transcriptional and posttranscriptional mechanisms. Cytokines, proteases, and bacterial virulence factors like lipopolysaccharide (LPS) can affect the multiple signal transduction pathways, which in turn affect acquired and innate immunity.[36-38,40]

Gene expression of cytokines regulated at the level of transcriptional and posttranscriptional, translational and posttranslational modifications. Various conditions like chronic diseases (periodontitis), autoimmune diseases, precancerous and cancer lesions show exaggerated and uncontrolled response on gene expression, cause excessive production which further activates PGs and proteases, leading to tissue damage.

Hence, inhibition of these signaling pathways prevents tissue destruction and inflammation. But the main drawbacks are lack of specificity and development of side effects. After improving this therapy as target specific and minimal side effects, it can be used as adjunctive host modulating strategy for periodontal treatment (Table 7).

**Table 7:** Cytokine suppressive anti-inflammatory drugs targeted signaling pathways

| *Targeted cell signaling pathways* | *Drug* | *Target cytokine* |
|---|---|---|
| p38 MAPKs inhibitors | RWJ 67657 | TNF-α, IL-6, and IL-8 |
| | VX-745 (Vertex pharmaceuticals) | |
| c-Jun N-terminal kinase pathway inhibitors | SP600125 | TNF-α, IL-6, and MMPs |
| p38α MAPK | SD-282 | |

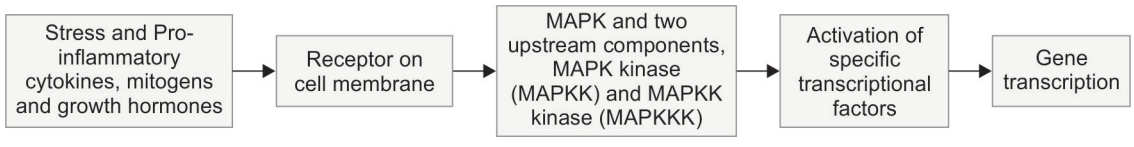
## Major Signaling Pathways Seen in Periodontitis

- Mitogen activated protein kinases play an important role in many aspects of host-mediated inflammatory response and responsible for signal transduction of cytokines and growth factors (Flow Charts 1 and 2).

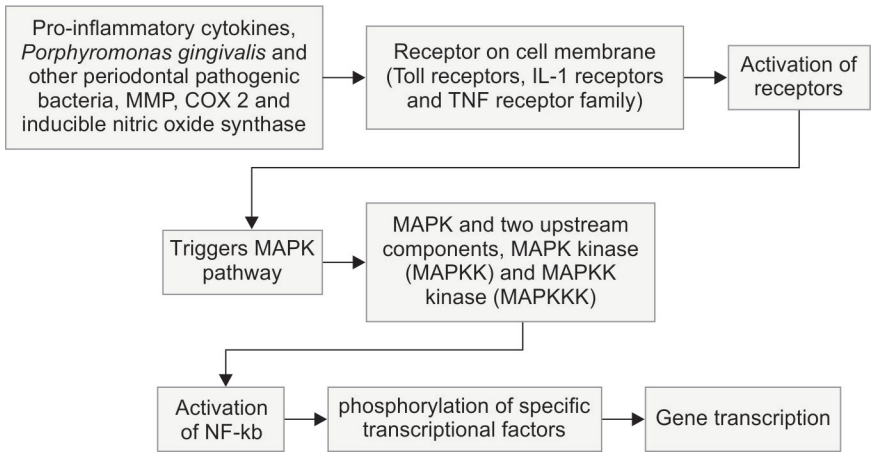
### *Subfamilies of MAPK*

- Extracellular-regulated kinases (ERK-1/-2): primarily activated by mitogens and growth factors
- C-Jun N-terminal activated kinases (JNK): activated by stress and proinflammatory cytokines
- p38: activated by stress and proinflammatory cytokines
- Nuclear factor kappa B: five members
  - REL-a (p65)
  - NF-kB1 (p50, p105)
  - NF-kB2 (p52, p100)
  - c-REL
  - REL-b
- Janus tyrosine kinase-signal transducer and activator of transcription (JAK/STAT) (Flow Chart 3: JAK-STAT pathway)
  - JAK1 (Interferon-γ and IL-6)
  - JAK2 (Interferon-γ)

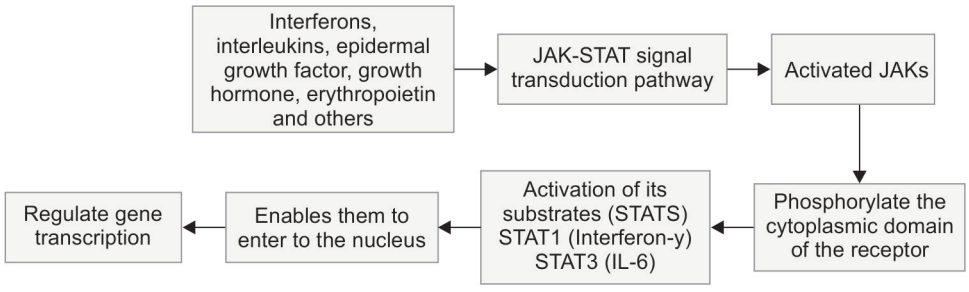
**Flow Chart 1:** Gene regulation via MAPK pathways by various stimulators




**Flow Chart 2:** Gene regulation in periodontitis via MAPK pathways by cytokines



**Flow Chart 3:** JAK-STAT pathway




– JAK3 and
– Tyk2

It has been stated that administration of SD-282 (15 or 45 mg/kg) reduced LPS-induced periodontal disease, inflammatory cytokine expression, osteoclastogenesis, and alveolar bone loss in rat model by inhibiting p38α MAPK inhibitors.[40]

### *Gene Therapeutics*

Human gingival fibroblasts (HGFs) constitute the major cell population in periodontal tissues. If we could modify HGF activities, it can serve as secreting anticytokine and antimicrobial molecules. Human gingival fibroblasts deport as anti-TNF-α system in periodontal tissue by secreting sTNF-RII antagonists for mTNF-Rs. Modified TNF-RII gene is introduced to gingival fibroblasts to overexpress sTNF-RII and this soluble form blocks binding of TNF-α to mTNF-Rs by binding TNF-α around gingival fibroblasts. It is seen that it has been suitable in the treatment of chronic infections and inflammations.[7]

Therapeutic strategies promising major breakthrough in medical and dental fields might also have certain limitations. There is evidence of infections without inflammatory symptoms. To prevent this event, antimicrobial therapy can be considered for chemical plaque control in addition to scaling and root planing and it also downregulates the immune system, so increases the risk of microbial infection. Hence, the screening of latent infectious diseases, such as tuberculosis, should be performed, and also with antimicrobial agents, caution must be taken to prevent apparent infection, without inflammatory symptoms when anticytokine therapy is performed.

### *Demerits*

Although in periodontitis the destruction is progressed by host-derived molecules, initiation of periodontal diseases needs pathogenic microorganism interaction with host. Hence by inhibiting only these host-derived molecules cannot arrest the disease progression. Major treatment approach should be focused on controlling the initiating factors of chronic periodontitis, such as local factors (plaque and calculus) by scaling on root planning, which is based on nonplaque hypothesis.

## CONCLUSION

Cytokines are known to play a key role in the pathogenesis of various inflammatory disorders like periodontitis by mediating the expressions of both innate and acquired immunity. Hence, it is evident that targeting these cytokines by anticytokine therapy can control the inflammatory signs of periodontal diseases, open newer horizons on molecular level targeted therapies in the treatment of periodontitis, and act as additional host modulating therapeutic approach in controlling periodontitis. Further, studies are anticipated toward the use of anticytokine therapy in the near future for better understanding and targeting the cellular and molecular pathways of periodontal disease pathogenesis.

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