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POTENCIJALNI UTICAJ IL-33/ST2 SIGNALNOG PUTA NA GUBITAK ALVEOLARNE KOSTI

POTENTIAL EFFECT OF IL-33/ST2 SIGNAL PATHWAY ON ALVEOLAR BONE LOSS

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Sažetak

Uvod*: ST2 je član IL-1R familije receptora, dok je interleukin-33 (IL-33) njegov prirodni ligand. Kako se u ekstracelularni prostor oslobađa iz nekrotičnih ćelija, IL-33 može da ima ulogu „alarmina" koji obaveštava imunski sistem o postojanju destrukcije tkiva. S obzirom da je ST2 receptor eksprimiran na gotovo svim ćelijama imunskog sistema, IL-33/ST2 signalni put ima važnu ulogu u patogenezi brojnih bolesti u kojima uglavnom aktivacija ovog puta podstiče razvoj Th2 imunskog odgovora. Protektivna ili proinflamatorna uloga IL-33/ST2 signalne osovine direktno je zavisna od dominantnog imunskog odgovora koji je u osnovi ovih oboljenja. Podaci iz literature o uticaju IL-33/ST2 signalnog puta na resorpciju alveolarne kosti su veoma oskudni. Mi smo ispitali uticaj delecije ST2 gena i administracije IL-33 na periapeksnu inflamatornu destrukciju kosti kod BALB/c miševa. Periapeksne lezije ST2-/- miševa sadržale su veći procenat CD4+ T limfocita, CD3+CCR6+ T limfocita, IFN-γ-, IL-17-, TNF-α- i IL-6-produkujućih ćelija u okviru gejtovanih CD4+ T limfocita u poređenju sa lezijama WT miševa. Nasuprot tome, administracija IL-33 kod WT miševa prouzrokovala je smanjenje procenta CD4+ T limfocita koji produkuju proinflamatorne citokine i povećanje procenta IL-4-produkujućih ćelija.*
Zaključak: *IL-33/ST2 signalni put negativno reguliše intenzitet periapeksne destrukcije alveolarne kosti prevencijom razvoja Th1/Th17 imunskog odgovora i ukazuju na moguću protektivnu ulogu IL-33 u terapiji gubitka alveolarne kosti. Buduća istraživanja implementiraće novu strategiju lečenja u stomatološku praksu.*

Ključne reči: *IL-33/ST2 signalni put, alveolarna kost, gubitak*

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Abstract

Introduction: *ST2 is a member of IL-1R receptor family and interleukin-33 (IL-33) is its natural ligand. IL-33 functions as an „alarmin" released upon necrotic cell death to alert the immune system to tissue damage. As ST2 receptor is expressed on many immune cells, IL-33/ST2 signal pathway has important proinflammatory or protective role in the pathogenesis of numerous diseases. IL-33/ST2 signaling promotes Th2 immune response in allergy, autoimmunity and chronic inflammatory disorders, but its role in the pathogenesis of alveolar bone loss is still unclear. We have investigated the effects of ST2 gene deletion and IL-33 administration on the periapical inflammatory bone destruction in BALB/c mice. We found that periapical lesions in ST2-/- mice are characterized by increased frequencies of CD4+ T cells, CD3+CCR6+ T cells and IFN-γ+, IL-17+, TNF-α+ and IL-6+ cells in gated CD4+ T cells compared with lesions in WT mice. A significant decrease in the percentage of CD4+ T cells producing proinflammatory cytokines and an increase in the percentage of IL-4 producing cells was observed in the periapical lesions in WT mice after IL-33 administration.*
Conclusion: *IL-33/ST2 signaling negatively regulates severity of periapical inflammatory bone destruction by preventing Th1/Th17 cell-mediated immune responses and indicate a possible protective role of IL-33 in the therapy of alveolar bone loss. Future research will implement new therapeuthic strategy in dental practice.*

Key words: *IL-33/ST2 signal pathway, alveolar bone, loss*

Uvod

Struktura i funkcija interleukina-33(IL-33)

Interleukin-33 (IL-33) je novi član IL-1 familije citokina. Prvi put je identifikovan 2005. godine kada su Schmitz i saradnici analizirajući članove IL-1 familije otkrili ligand za ST2 molekul koji interakcijom sa receptorskim kompleksom indukuje Th2 imunski odgovor[1]. Ovakvo delovanje IL-33 je u suprotnosti sa karakteristikama drugih citokina IL-1 familije, IL-1, IL-1Ra i IL-18, koji stimulišu Th1 imunski odgovor[2]. Kasnije je ustanovljeno da je IL-33 identičan nuklearnom faktoru venula sa visokim endotelom, koji je povezan sa hromatinom i reguliše transkripciju, tako da istovremeno deluje i kao citokin i kao nuklearni faktor[3]. Interleukin-33 konstitutivno je eksprimiran u mnogim tkivima (pluća, mozak, koža, kičmena moždina), pri čemu su njegovi glavni izvori fibroblasti, endotelne i epitelne ćelije[4,5]. Kod miševa je prisutan i u makrofagima, dendritskim i glatko-mišićnim ćelijama[1,6]. U odsustvu pro-inflamatornih citokina i inflamacije lokalizovan je u jedru endotelnih ćelija, gde je za hromatin vezan kratkim motivom koji je homologan protein virusa herpesa LANA (*latency associated nuclear antigen*)[3,7]. IL-33 se pasivno oslobađa iz ćelija nakon njihovog oštećenja ili nekroze i alarmira imunski sistem o postojanju destrukcije tkiva[8,9]. Oslobođeni IL-33 se zatim enzimski razgrađuje dejstvom kaspaze-7 i kaspaze-3, čime nastaju biološki inaktivni produkti IL-33[10,11]. Ranije se smatralo da IL-33 tek nakon enzimske obrade stiče optimalnu biološku aktivnost i da kaspaza-1 obrađuje pro-IL-33, nakon čega nastaje zrela forma IL-33. Međutim, nedavno je pokazano da ovaj enzim nije u stanju da obradi pro-IL-33[12].

Struktura i funkcija ST2 molekula

ST2 gen (poznat još i kao T1, DER4 ili Fit 1 gen) je član IL-1R familije receptora koji je prvobitno identifikovan u mišjim fibroblastima[13,14]. Transkripciju ST2 gena kontrolišu dva posebna promotera. „Gornji“ upravlja transkripcijom u hematopoetskim ćelijama, dok „donji“ promoter reguliše ST2 ekspresiju u fibroblastima[15]. Alternativnim splajsingom informacione RNK nastaju dva različita produkta ST2 gena: solubilna sekretovana forma (ST2) i transmembranska

Introduction

Structure and function of interleukin-33 (IL-33)

Interleukin-33 (IL-33) is a new member of IL-1 cytokine family. It was identified in 2005 by Schmitz et al. as an interleukin-1-like cytokine that signals via the IL-1 receptor-related protein ST2 and induces Th2-associated cytokines[1]. Unlike IL-33, the other IL-1 family members, IL-1, IL-1Ra and IL-18, primarily induce Th1 immune response[2]. IL-33 is chromatin-associated nuclear factor in high endothelial venules with transcriptional properties. IL-33 acts intracellularly as a nuclear factor and extracellularly as a cytokine[3]. This cytokine is constitutively expressed in many tissues (lung, brain, skin, spinal cord). Fibroblasts, endothelial and epithelial cells are the main cellular source of IL-33[4,5]. In mice, IL-33 was also found in macrophages, dendritic and muscle cells[1,6]. In the absence of proinflammatory cytokines and inflammation, IL-33 is chromatin-associated factor in the nucleus of endothelial cells. IL-33 chromatin-binding peptide shares striking similarities with a motif found in herpesvirus LANA (*latency associated nuclear antigen*)[3,7]. IL-33 function as an „alarmin” released following cell necrosis to alert the immune system to tissue damage or stress[8,9]. Processing of released IL-33 by caspase-7 and caspase-3 dramatically attenuate IL-33 bioactivity[10,11]. It was previously believed that IL-33 requires maturation by caspase-1 for optimal biological activity. Recently, it was reported that processing by caspase-1 results in IL-33 inactivation, rather than activation[12].

Structure and function of ST2 molecule

ST2 gene (also known as T1, DER4 or Fit 1 gene) is classified as a member of IL-1R receptor family. It was primarily identified in murine fibroblasts[13,14]. Two promoters control the transcription of ST2 gene. Proximal promoter used for the expression of ST2 gene in hematopoetic cells is distinct from distal promoter which regulates ST2 expression in fibroblasts[15]. Two distinct types of ST2 gene products, a soluble form (ST2) and a transmembrane form (ST2L) are produced by alternative splicing of pre-mRNA. The structure of ST2L is similar to that

forma (ST2L). ST2L je strukturno sličan receptoru za IL-1 tipa I (IL-1RI), i sadrži tri ekstracelularna imunoglobulinska domena i jedan intracelularni Toll-interleukin-1 receptorski domen. Iako je solubilni ST2 molekul strukturno homolog sa ekstracelularnim regionom ST2L, nedostaju mu transmembranski i intracelularni Toll-interleukin-1 receptorski domen[16]. ST2L molekul je membranski receptor za IL-33. Iako je ST2L član IL-1R familije receptora, on ne vezuje ostale članove IL-1 familije citokina (IL-1, IL-1Ra i IL-18)[17,18]. ST2 molekul funkcioniše kao tzv. „mamac" receptor, koji vezivanjem IL-33 blokira njegovo vezivanje za ST2 receptor[6]. Solubilnu formu ST2 molekula stvaraju mnoge ćelije u organizmu: Th2 limfociti, makrofagi, fibroblasti, ćelije karcinoma dojke, mnoge embrionalne ćelije, dok je membranska forma eksprimirana na Th2 limfocitima, makrofagima, dendritskim ćelijama, NK i NKT ćelijama, mastocitima, bazofilnim i eozinofilnim granulocitima[19-21].

Il-33/ST2 signalni put

IL-33 ostvaruje svoje dejstvo vezujući se na membrani ćelije za heterodimerni receptorski kompleks, koga čine ST2 receptor i IL-1R pomoćni protein (*IL-1R accessory protein*, IL-1RacP). Signal se posredstvom myD88 molekula (*myeloid differentiation primary-response protein 88*) prenosi unutar ćelije i aktiviraju se NF-κB (*nuclear factor-kappa B*) i/ili MAP kinaze (*mitogen-activated protein kinases*), i na taj način indukuju produkciju različitih citokina i hemokina. Aktivacija NF-κB indukuje produkciju IL-1β, IL-3, IL-6, TNF, CXCL2, CCL2, CCL3, PGD2 i LTB4, a aktivacijom MAP kinaza (p38, JNK i ERK) produkuju se IL-5, IL-13, CCL5, CCL17 i CCL24[14].

Aktivaciju IL-33/ST2 signalnog puta reguliše ST2 molekul mehanizmom negativne povratne sprege. On se u ćelijama masovno stvara i oslobađa u prisustvu citokina i hemokina produkovanih nakon aktivacije IL-33/ST2 signalnog puta. Solubilni ST2 molekul vezuje slobodni IL-33, i na taj način sprečava njegovo vezivanje za membranski ST2 recepor[22]. IL-33 može da modulira funkciju svih ćelija koje eksprimiraju ST2 receptor. Kao što je već pomenuto, ST2 molekul se specifično eksprimira na Th2 limfocitima i predstavlja važan efektorski molekul Th2 imunskog odgovora. Ukoliko se

for IL-1 receptor type I (IL-1RI), consisting of three extracellular immunoglobulin domains and an intracellular Toll-interleukin-1 receptor domain. Although the extracellular domain is common to soluble ST2 and ST2L, the soluble ST2 lacks the transmembrane and intracellular Toll-interleukin-1 receptor domains and is secreted from the cells[16]. ST2L molecule is a membrane receptor for IL-33. ST2L belongs to IL-1R receptor family, but it does not bind IL-1, IL-1Ra or IL-18[17,18]. Soluble ST2 may act as a „decoy"receptor by binding IL-33 and preventing its binding to ST2L[6]. ST2 is expressed in Th2 lymphocytes, macrophages, fibroblasts, mammary tumors and embryonic tissues, while ST2L expression was originally detected in Th2 lymphocytes, macrophages, dendritic cells, NK and NKT cells, mastocytes, basophil and eosinophil granulocytes[19-21].

IL-33/ST2 signal pathway

IL-33 binds a heterodimer receptor complex consisting of ST2 receptor and IL-1R accessory protein (IL-1RacP), which leads to recruitment of myD88 molecule (*myeloid differentiation primary-response protein 88*) and activation of nuclear factor-kappa B (NF-κB) and/or mitogen-activated protein kinases (*MAP kinases*). The activation of these molecules induces the production of different chemokines and cytokines. The activation of NF-κB induces the production of IL-1β, IL-3, IL-6, TNF, CXCL2, CCL2, CCL3, PGD2 and LTB4, whereas MAP kinases activation result in increased production of IL-5, IL-13, CCL5, CCL17 and CCL24[14].

Soluble ST2 molecule regulates the activation of IL-33/ST2 signal pathway by the negative feedback mechanism. Cells produce large amounts of ST2 in the presence of cytokines and chemokines generated by IL-33/ST2 pathway activation. Soluble ST2 molecule may actually be a decoy to bind circulating IL-33 and prevent its binding to ST2L[22]. IL-33 may modulate the function of ST2 positive cells. As previously described, ST2 molecule is preferentially expressed on Th2 cells and is important for Th2 effector function. In the presence of IL-33 naïve T cells differentiate into the Th2 lineage, even in the absence of IL-4, the key driver of Th2 immune response[23]. IL-33-dependent stimulation of Th2 cytokine producing cells induce the

IL-33 deluje na naivne T limfocite, oni diferenciraju u Th2 limfocite, čak i u odsustvu IL-4, ključnog citokina koji usmerava imunski odgovor u Th2 smeru[23]. Stimulacija efektorskih Th2 limfocita IL-33 indukuje produkciju IL-4, IL-5 i IL-13 i hemotaksu Th2 limfocita[24,25].

Nedavno je pokazano da NK i NKT ćelije eksprimiraju ST2 receptor. Iako je očekivano da izazove Th2 efekat, tretman IL-33 indukovao je produkciju i Th1 i Th2 citokina u ovim ćelijama[24].

IL-33 je snažan aktivator mastocita, bazofilnih i eozinofilnih granulocita. Tretiranje IL-33 u mastocitima indukuje produkciju IL-5, IL-13, granulocitno-monocitnog faktora stimulacije kolonija (granulocyte-macrophage colony-stimulating factor, GM-CSF) i TNF-α, i podstiče sazrevanje i preživljavanje ovih ćelija u inflamiranim tkivima[26]. Bazofilni granulociti nakon stimulacije IL-33 sekretuju brojne citokine, uključujući IL-4, IL-5, IL-6, IL-8, IL-13 i GM-CSF. Uz to, IL-33 funkcioniše i kao hemoatraktant bazofila[24,27]. Eozinofilni granulociti tretirani IL-33 pojačano stvaraju IL-8 i imaju produžen životni vek[28].

IL-33 aktivira i antigen-prezentujuće ćelije. Primena IL-33 amplifikuje polarizaciju alternativno aktiviranih makrofaga i stimuliše produkciju CCL17 i CCL[24] hemokina[29]. Mayuzumi i saradnici pokazali su da IL-33 podstiče razvoj dendritskih ćelija[30]. Dendritske ćelije dobijene nakon stimulacije IL-33 su fenotipski i funkcionalno nezrele, sa smanjenim kapacitetom aktivacije naivnih T limfocita, ali mogućnošću polarizacije imunskog odgovora u Th2 smeru[31].

Il-33/ST2 signalni put u patološkim stanjima

S obzirom da je ST2 receptor eksprimiran na gotovo svim ćelijama imunskog sistema, kao i u mnogim tkivima i organima, IL-33/ST2 signalni put ima važnu protektivnu ili proinflamatornu ulogu u patogenezi brojnih bolesti u kojima uglavnom aktivacija ovog puta podstiče razvoj Th2, i istovremeno suprimira Th1/Th17 imunski odgovor. Administracija IL-33 ublažava eksperimentalni autoimunski encefalomijelitis supresijom Th1/Th17 imunskog odgovora i indukcijom alternativno aktiviranih makrofaga[32]. Blokiranje ST2 receptora primenom anti-ST2 antitela redukuje inflamaciju u plućima uzrokovanu respiratornim sincicijalnim virusom i indukuje rezistenciju na bakteriju

production of IL-4, IL-5 and IL-13 and Th2 cells chemotaxis[24,25].

More recent evidence suggests that NK and NKT cells express ST2 receptor. Although it was expected that IL-33 application induces the Th2 immune response, both Th1 and Th2 cytokines were produced in these cells[24].

IL-33 is a potent activator of mastocytes, basophil and eosinophil granulocytes. The administration of IL-33 stimulate mastocytes to produce IL-5, IL-13, granulocyte-macrophage colony-stimulating factor (GM-CSF) and TNF-α, and drives maturation and survival of these cells in inflamed tissues[26]. After stimulation by IL-33, basophil granulocytes produce many cytokines, including IL-4, IL-5, IL-6, IL-8, IL-13 and GM-CSF. In addition, IL-33 acts as a chemoattractant of basophils[24,27]. Eosinophil granulocytes stimulated with IL-33 produce high levels of IL-8 and have longer survival[28].

IL-33 activates antigen-presenting cells. The administration of IL-33 amplifies the polarization of alternatively activated macrophages and stimulate the production of CCL17 and CCL24 chemokines[29]. Mayuzumi et al. have shown that IL-33 promotes dendritic cell development[30]. IL-33-activated dendritic cells are functionally and phenotipically immature with low capacity to activate naïve T cells, but possibility to drive polarization of the immune response toward Th2 subset[31].

Il-33/ST2 signal pathway in pathological conditions

As ST2 receptor is expressed on many immune cells, tissues and organs, IL-33/ST2 signal pathway has an important proinflammatory or protective role in the pathogenesis of numerous diseases. The activation of IL-33/ST2 signal axis stimulates Th2 and suppresses Th1/Th17 immune response. The administration of IL-33 attenuates experimental autoimmune encephalomyelitis by suppressing Th1/Th17 immune response and inducing polarization of alternatively activated macrophages[32]. Blocking ST2 receptor by using anti-ST antibodies reduces inflammation in the lung during respiratory syncytial virus infection and induces the resistance to *Leishmania major* in susceptible BALB/c mice by shifting of harmful Th2 into protective

Leishmania major kod BALB/c miševa preusmeravanjem štetnog Th2 u zaštitni Th1 imunski odgovor[33,34]. Korišćenje rekombinantnog ST2 fuzionog proteina smanjuje eozinofilnu inflamaciju disajnih puteva i suprimira produkciju Th2 citokina u mišjem modelu astme, pre svega kroz ometanje interakcije IL-33 sa ST2 receptorom na Th2 limfocitima[35].

Uloga IL-33/ST2 signalnog puta u patogenezi periapeksne inflamacije i gubitka alveolarne kosti nije poznata. Hipoteza našeg istraživanja bila je da je IL-33/ST2 signalni put uključen u modulaciju imunskih odgovora u toku razvoja i progresije periapeksne inflamatorne destrukcije kosti. Kako bismo testirali ovu hipotezu, ispitali smo uticaj delecije ST2 gena i administracije IL-33 na formiranje eksperimentalno indukovanih lezija kod BALB/c miševa.

Naša sopstvena istraživanja su sprovedena na sledeći način:

Eksperimentalne životinje

BALB/c (WT) i ST2-deficijentni (ST2-/-) miševi, muškog pola, 6 do 8 nedelja starosti, korišćeni su za indukciju periapeksnih lezija. Eksperimente je odobrio Etički komitet Fakulteta medicinskih nauka Univerziteta u Kragujevcu, Srbija.

Indukcija periapeksnih lezija

Nakon uvođenja u anesteziju intraperitonealnim ubrizgavanjem ketaminhidrohlorida i ksilazina, WT i ST2 -/- miševima otvorene su pulpe mandibularnih molara i izložene mikroflori usne duplje. Miševi sa zdravim, intaktnim zubima služili su kao kontrola.

Administracija IL-33

Grupi WT miševa sa eksperimentalno indukovanim periapeksnim lezijama (n=6) intraperitonealno je ubrizgavan mišji rekombinantni IL-33 (R&D Systems, Minneapolis, MN, USA; 1μg/injekciji) dva puta nedeljno u toku dve nedelje.

Protočna citometrija

Ćelijske suspenzije periapeksnog tkiva inkubirane su sa antitelima specifičnim za CD3, CD4, CD8, CXCR3, CCR6, CD11c,

Th1 immune response[33,34]. The administration of recombinant fusion proteins reduces eosinophil inflammation and suppresses the production of Th2 cytokines in murine model of asthma by preventing binding of IL-33 to ST2 receptor on Th2 lymphocytes[35].

The data on the role of IL-33/ST2 signaling in the pathogenesis of periapical inflammation and alveolar bone loss are lacking. We hypothesized that IL-33/ST2 signaling pathway is involved in the modulation of immune responses during the development and progression of periapical inflammatory bone destruction. To test this hypothesis, we investigated the effects of ST2 gene deletion and IL-33 administration on the formation of experimentally-induced periapical lesions in BALB/c mice.

Our own research was conducted in the following way:

Experimental animals

Male BALB/c (WT) and ST2 knockout (ST2-/-) mice on BALB/c background, 6 to 8 weeks old, were used for the induction of periapical lesions. The experiments were approved by the Ethics Board of the Faculty of Medical Sciences, University of Kragujevac, Serbia.

Induction of periapical lesions in mice

WT and ST2-/- mice were anesthetized with i.p. injection of ketamine-hydrochloride and xylazinen, and mandibular molar pulps were exposed and left open to the oral environment. Mice that did not undergo pulp exposure were used as controls.

IL-33 administration in mice

The WT mice with experimentally induced periapical lesions (n=6/group) were injected intraperitoneally with murine recombinant IL-33, twice per week for 2 weeks (R&D Systems, Minneapolis, MN, USA; 1μg/injection).

Flow cytometry

Periapical tissue cell suspensions were incubated with antibodies specific for CD3, CD4, CD8, CXCR3, CCR6, CD11c, CD11b, F4/80 and CD49b (BD Pharm-

USA). Nakon površinskog bojenja, urađena je permeabilizacija i intracelularno bojenje antitelima specifičnim za TNF-α, IL-6, IFN-γ, IL-17, IL-4 i IL-5 (BD Pharmingen). Ćelije su analizirane pomoću FACSCalibur protočnog citometra (BD Biosciences) i programa Flowing Software verzija 2.5 (Informer Technologies).

Statistička analiza

Podaci su analizirani korišćenjem SPSS programa.Verzije 13. Rezultati su prikazani sa statističkom značajnošću $p<0,05$.

Rezultati

Delecija ST2 gena povećava influx mononuklearnih ćelija i produkciju proinflamatonih citokina u periapeksnim lezijama

Broj mononuklearnih ćelija bio je statistički veći kod ST2-deficijentnih miševa (ST2-/-) u poređenju sa WT miševima ($p<0,05$). Periapeksne lezije ST2-/- miševa sadržale su veći procenat $CD4^+$ T limfocita i CCR6-pozitivnih ćelija u okviru gejtovanih $CD3^+$ T limfocita ($p<0,05$), dok su procenti drugih analiziranih ćelijskih populacija bili slični između ova dva. Procenti TNF-α-, IL-6-, IFN-γ- i IL-17-produkujućih ćelija u okviru gejtovanih $CD4^+$ T limfocita bili su značajno veći kod ST2-/- miševa u poređenju sa WT miševima ($p<0,05$; Slika 1). Nije pronađena razlika u procentima IL-4- i IL-5-produkujućih $CD4^+$ T limfocita.

Administracija IL-33 negativno reguliše Th1/Th17 imunski odgovor u periapeksnim lezijama

Nije bilo razlike u ukupnom broju mononuklearnih ćelija u periapeksnim lezijama miševa tretiranih IL-33 u poređenju sa kontrolama. Egzogeno aplikovani IL-33 značajno je smanjio influks $CD4^+$ T limfocita, CXCR3- i CCR6-pozitivnih ćelija u okviru gejtovanih $CD3^+$ T limfocita ($p<0.05$), dok druge ćelijske populacije nisu bile pogođene (rezultati nisu prikazani).

Procenti TNF-α-, IL-6-, IFN-γ- i IL-17-produkujućih ćelija u okviru gejtovanih CD 4 + T limfocita bili su značajno niži u periapeksnim lezijama miševa tretiranih IL-33 u poređenju sa kontrolama. Nasuprot tome,

ingen, USA). Following the surface staining, cells were permeabilized and intracellular staining was done with Abs specific for TNF-α, IL-6, IFN-γ, IL-17, IL-4 and IL-5 (BD Pharmingen). Cells were analyzed with FACSCalibur Flow Cytometer (BD Biosciences) and Flowing Software Version 2.5 (Informer Technologies).

Statistical Analysis

Data were analyzed using statistical package SPSS, version 13. The results were considered significantly different when $p<0.05$.

Results

ST2 deletion enhances the influx of mononuclear cells and proinflammatory cytokines production in periapical lesions

The number of MNCs in the periapical lesions was significantly higher in ST2-/- mice compared with WT mice ($p<0.05$). Periapical lesions of ST2-/- mice had increased percentages of $CD4^+$ T cells and $CCR6^+$ cells among gated $CD3^+$ T cells ($p < 0.05$), while the frequencies of other cell populations studied were similar between the two genotypes (data not shown). The percentages TNF-α^+, IL-6^+, IFN-γ^+ and IL-17^+ cells in gated $CD4^+$ T cells were significantly higher in ST2-/- mice compared with WT mice ($p<0.05$; Fig. 1). No difference in the percentages of IL-4- and IL-5-producing $CD4^+$ T cells was found (data not shown).

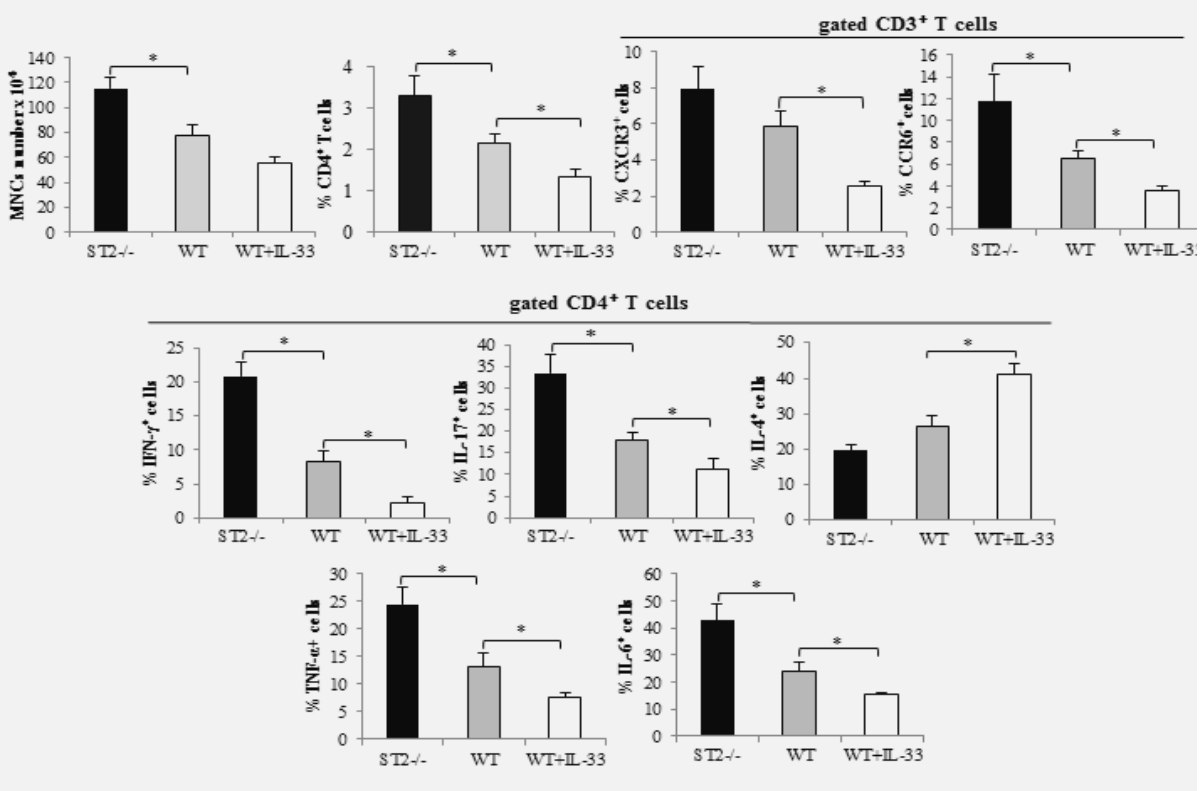
IL-33 administration negatively regulates Th1/Th17 immune responses in periapical lesions

There was no difference in the total number of MNCs in the periapical lesions of IL-33-treated mice compared to untreated controls. Exogenous IL-33 significantly downregulated the influx of CD4+ T cells, CXCR3+ and CCR6+ cells in gated CD3+ T cells ($p<0.05$), while other cell populations studied were not affected (data not shown). The percentages of TNF-α^+, IL-6^+, IFN-γ^+ and IL-17^+ cells in gated $CD4^+$ T cells were significantly lower in the periapical lesions of IL-33-treated mice compared with untreated mice ($p<0.05$). In contrast, exogenous IL-33 significantly increased the percentages of IL-4^+ cells in gated $CD4^+$ T cells(Fig.1).

egzogeni IL-33 značajno je smanjio procenat IL-4- produkujućih ćelija u okviru gejtovanih CD4+ T limfocita (Slika 1).

Ukupan broj mononuklearnih ćelija bio je značajno veći u periapeksnim lezijama ST2-/- miševa u poređenju sa WT miševima. Procenat CD4+ T limfocita i CCR6-pozitivnih ćelija u okviru gejtovanih CD3+ T limfocita, kao i TNF-α-, IL-6-, IFN-γ- i IL-17-pozitivnih ćelija u okviru gejtovanih CD4+ T limfocita bio je značajno veći kod ST2-/- miševa u poređenju sa WT miševima. Broj CD4+ T limfocita, CXCR3- i CCR6-pozitivnih ćelija u okviru gejtovanih CD3+ T limfocita bio je značajno niži nakon aplikacije IL-33 kod WT miševa. Miševi tretirani IL-33 imali su značajno niži procenat TNF-α-, IL-6-, IFN-γ- i IL-17-pozitivnih ćelija i značajno veći procenat IL-4-pozitivnih ćelija u okviru gejtovanih CD4+ T limfocita (*p<0,05).

The total MNCs number was significantly higher in periapical lesions of $ST2^{-/-}$ mice compared to WT mice. Percentages of $CD4^+$ T cells and $CCR6^+$ cells in gated $CD3^+$ T cells, as well as TNF-α^+, IL-6^+, IFN-γ^+ and IL-17^+ cells among gated $CD4^+$ T cells were significantly higher in ST2-/- mice compared to WT mice. The number of $CD4^+$ cells, $CXCR3^+$ and $CCR6^+$ cells in gated $CD3^+$ cells in periapical lesions were significantly lower in IL-33 treated mice compared to untreated WT mice. IL-33 treated mice had significantly lower percentages of TNFα^+, IL-6^+, IFN-γ^+ and IL-17^+ cells and significantly higher percentages of IL-4^+ cells in gated $CD4^+$ T cells (*p<0.05).



Slika 1. Protočna citofluorometrijska analiza mononuklearnih ćelija (MNCs) izolovanih iz periapeksnih lezija 14. dana nakon indukcije

Figure 1. Flow cytometric analysis of periapical lesion mononuclear cells (MNCs) was done at day 14 after the induction of periapical lesion in mice

Značaj dobijenih rezultata u okviru dostupnih naučnih činjenica

Prvi smo pokazali da IL-33/ST2 signalni put ima značajnu ulogu u patogenezi gubitka alveolarne kosti u periapeksnom regionu. Ciljana delecija ST2 gena kod BALB/c miševa izazvala je masivnu infiltraciju efektorskih ćelija i produkciju proinflamatornih citokina, dok je tretman WT miševa IL-33 izazvao suprotne efekte (Slika 1).

Zaiss i saradnici pokazali su da primena IL-33 inhibira destrukciju hrskavice i sistemski gubitak kosti i ukazali na potencijalnu terapijsku primenu ovog citokina u tretmanu resorpcije kosti. IL-33 preko ST2 receptora stimuliše produkciju GM-CSF koji deluje na prekursore osteoklasta i usmerava ih u pravcu sazrevanja alternativno aktiviranih makrofaga[36]. Ovi rezultati saglasni su sa rezultatima Schulzea i saradnika, koji su pokazali da IL-33 u potpunosti blokira stvaranje TRAP-pozitivnih (aktiviranih) osteoklasta[37]. Isti autori pokazali su da IL-33 suprimira ekspresiju nuklearnog faktora aktiviranih T limfocita (*nuclear factor of activated T-cells, cytoplasmic 1, Nfatc1),* ključnog transkripcionog faktora osteo-klastogeneze. Podaci iz literature o uticaju IL-33/ST2 signalnog puta na resorpciju alveolarne kosti su veoma oskudni i kontraverzni. U modelu parodontopatije indukovanom ligaturom pokazano je da ekspresija IL-33 korelira sa ekspresijom RANK-L-a i stepenom alveolarnog koštanog oštećenja[38]. Malcolm i saradnici su takođe pokazali da IL-33 indukcijom ekspresije RANK-L-a dovodi do egzacerbacije gubitka alveolarne kosti[39]. Nasuprot tome, mi smo nedavno pokazali da delecija ST2 gena povećava inflamatornu destrukciju alveolarne kosti u eksperimentalno indukovanim periapeksnim lezijama kod miševa. Povećani gubitak koštanog tkiva kod ST2 deficijentnih miševa povezan je sa povećanjem RANKL / OPG odnosa, ključnog pokazatelja resorpcije kosti, kao i sa povećanjem broja TRAP-pozitivnih osteoklasta[40]. Pored toga, u humanom modelu prvi smo pokazali ekspresiju IL-33 i ST2 receptora u peri-apeksnim granulomima i radiksnim cistama. Ovi rezultati ukazali su na aktivaciju IL-33/ST2 signalnog puta u inflamiranom periapeksnom regionu. Kako bismo bolje razumeli ulogu IL-33 u destrukciji alveola-rne kosti, ispitali smo ekspresiju IL-33 i ST2 molekula kako u periapeksnim granulomima

Significance of the obtained results in relation to the available data

We were first to provide the evidence that IL-33/ST2 signal pathway plays an important role in the pathogenesis of alveolar bone loss in the periapical region. Targeted disruption of ST2 gene in BALB/c mice led to massive infiltration of effector cells and production of proinflammatory cytokines, whereas the treatment of WT mice with IL-33 had the opposite effects (Fig. 1).

Zaiss et al. have demonstrated that IL-33 administration inhibits cartilage destruction and systemic bone loss. These results suggest the potential use of IL-33 in bone loss therapy. IL-33 acts directly on ST2-positive murine osteoclast precursors, shifting their differentiation toward alternatively activated macrophages via GM-CSF in an autocrine fashion[36]. These results are in agreement with the results of Schulze et al. who showed that IL-33 absolutely blocks the formation of TRAP-positive (active) osteoclasts[37]. The same authors have demonstrated that IL-33 suppresses the expression of nuclear factor of activated T-cells (cytoplasmic 1, Nfatc1), the key transcription factor of osteo-clastogenesis. The data on IL-33/ST2 signaling in the pathogenesis of alveolar bone loss are scarce and controversial. In the murine ligature-induced periodontitis model, the expression of IL-33 was correlated with RA bone destruction[38]. Malcolm et al. have also shown that IL-33 exacerbates the alveolar bone loss in periodontal disease through induction of RANKL[39]. On the contrary, we have recently shown that ST2 deletion increases inflammatory bone destruction in experimentally induced periapical lesions in mice. Increased bone loss in ST2-deficient mice was related to increased RANKL/OPG NKL expression and the extent of alveolar ratio, the key indicator of bone resorption, as well as increased number of TRAP-positive osteo-clasts [40]. In addition, in the human model we were the first to demonstrate the expression of IL-33 and ST2 receptor in periapical granulomas and radicular cysts. These findings suggested that IL-33/ST2 pathway is activated in inflamed periapical region. To better understand the role of IL-33/ST2 pathway in periapical bone destruction, we investigated the expression patterns of IL-33 and ST2 in both periapical granu-

i cistama, tako i u zdravom periapeksnom tkivu. Povećan broj IL-33 i ST2-pozitivnih ćelija u periapeksnim lezijama, u poređenju sa zdravim periapeksnim tkivom, sugerisao je na moguću umešanost IL-33/ST2 signalnog puta u periapeksnoj destrukciji alveolarne kosti[41]. Zatim smo ispitali uticaj delecije ST2 gena ili administracije IL-33 na formiranje eksperimentalno indukovanih periapeksnih lezija kod BALB/c miševa. Periapeksne lezije ST2-deficijentnih miševa sadržale su veći procenat $CD4^+$ T limfocita i $CCR6^+$ Th17 limfocita. Procenat TNF-α-, IL-6-, IFN-γ- i IL-17-produkujućih $CD4^+$ T limfocita bio je takođe značajno veći u periapeksnim lezijama ST2-deficijentnih u poređenju sa WT miševima. Egzogeno aplikovan IL-33 značajno je smanjio influks $CD4^+$ T limfocita, $CXCR3^+$ Th1 i $CCR6^+$ Th17 limfocita u periapeksne lezije. Procenat TNF-α-, IL-6-, IFN-γ- i IL-17-produkujućih $CD4^+$ T limfocita u periapeksnim lezijama BALB/c miševa bio je značajno manji nakon aplikacije IL-33. Nasuprot tome, egzogeni IL-33 značajno je smanjio procenat IL-4-produkujućih $CD4^+$ T limfocita. Ovi rezultati pokazuju da IL-33/ST2 signalni put negativno reguliše periapeksnu destrukciju alveolarne kosti prevencijom razvoja Th1/Th17 imunskog odgovora.

Zaključak

Interleukin-33 bi mogao da predstavlja novu metu terapijskih intervencija u brojnim bolestima. Vezujući se za ST2 receptor IL-33 indukuje prevashodno Th2 imunski odgovor. Manipulisanje IL-33/ST2 signalnim putem, bilo monoklonskim anti-ST2 antitelima, bilo ST2 fuzionim proteinima ili monoklonskim anti-IL-33 antitelima može da predstavlja nov terapijski pristup u lečenju resorpcije alveolarne kosti posredovane Th1/Th17 imunskim odgovorom. Buduća istraživanja rasvetliće uticaj IL-33/ST2 signalnog puta na gubitak alveolarne kosti i implementiraće novu strategiju lečenja u stomatološku praksu.

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lomas and radicular cysts, as well as in healthy periapical tissues. Increased numbers of IL-33 and ST2-positive cells in periapical lesions, when compared to healthy periapical tissues, suggested that IL-33/ST2 signaling may be involved in periapical inflammatory bone destruction[41]. Further, we investigated the effects of ST2 gene deletion or IL-33 administration on the formation of experimentally-induced periapical lesions in BALB/c mice. Periapical lesions of ST2-/- mice had increased percentages of CD4+ T cells and CCR6+ cells among gated CD3+ T cells. The percentages TNF-α+, IL-6+, IFN-γ+ and IL-17+ cells in gated CD4+ T cells in periapical lesions were significantly higher in ST2-/- mice compared with WT mice. The exogenous IL-33 significantly downregulated the influx of CD4+ T, CXCR3+ Th1 and CCR6+ Th17 lymphocytes. The percentage of TNF-α-, IL-6-, IFN-γ- and IL-17-producing CD4+ T lymphocytes was significantly lower in the periapical lesions of IL-33-treated mice compared with untreated mice. In contrast, exogenous IL-33 significantly increased the percentage of IL-4-positive CD4+ T lymphocytes (Fig. 1). The obtained findings suggest that IL-33/ST2 signaling negatively regulates the severity of periapical alveolar bone destruction by preventing Th1/Th17 cell-mediated immune responses.

Conclusion

IL-33 presents a new target for therapeutic intervention across a range of diseases. This cytokine mediates signal transduction through the ST2 receptor and potently enhances Th2 immune response. Manipulation of IL-33/ST2 system by using monoclonal anti-ST2 antibodies, ST2 fusion proteins or monoclonal anti-IL-33 antibodies may present new approach in the therapy of Th1/Th17-mediated alveolar bone loss. Future research will implement new therapeuthic strategy in dental practice.

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