André Oliveira Naufel de Toledo

Participação de citocinas relacionadas às respostas Th17/Treg na inflamação periapical inflamatória crônica.

BELO HORIZONTE
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Madre Teresa de Calcutá

Resumo

Participação de citocinas relacionadas às respostas Th17/Treg na inflamação periapical crônica.

A doença periapical inflamatória é uma sequela da infecção e necrose pulpar. Ela representa a resposta de defesa do hospedeiro à agressão proveniente do canal radicular. Assim, lesões periapicais crônicas se desenvolvem como resposta à manutenção da inflamação e à reabsorção óssea. As células T reguladoras (Treg) e células T *helper* (Th) 17 (Th17) têm papel fundamental na regulação da resposta imunológica. *Forkhead box* P3 (FoxP3) é o fator de transcrição para as células Treg, mas a diferenciação e maturação das células T naive em Treg ou Th17 depende também de citocinas específicas como o fator transformador de crescimento β (TGF-β), interleucina 10 (IL-10), interleucina 17 (IL-17), interleucina 6 (IL-6) e interleucina 21 (IL-21). Outras citocinas participam na modulação da atividade de Treg e Th17 como as quimiocinas CCL4 e CCL20 com função de recrutamento destas células para atuação no processo inflamatório. O papel de Treg e Th17 tem sido muito estudado em doenças autoimunes, mas ainda pouco avaliado na patogenia das lesões inflamatórias periapicais crônicas. Para este estudo foram utilizadas oitenta e sete amostras de tecido periapical humano para realização de análises morfológicas e dosagem de citocinas por meio de ensaio imunoenzimático (ELISA) (TGF-β, IL-10, IL-17, CCL4, CCL20). As amostras de tecido periapical foram coletadas de três grupos: grupo controle (dentes hígidos), grupo de dentes com necrose pulpar e lesão periapical e grupo de dentes com necrose pulpar sem lesão

periapical. Observamos alta expressão de CCL4 e TGF-β no grupo com lesão periapical quando comparado com os grupos sem lesão e uma correlação positiva entre CCL20 e IL-17, além de um aumento na expressão de CCL20 no grupo com lesão periapical quando comparado ao controle. Nossas observações implicam que essas duas características, tanto pró-inflamatórias quanto imunossupressoras, estão presentes na lesão periapical crônica, ocorrendo de maneira simultânea e com características de co-estimulação, como resultado do intenso trabalho da resposta imunológica do hospedeiro contra o processo inflamatório proveniente das bactérias intracanais e seus subprodutos.

Palavras-chave: Doença periapical inflamatória, quimiocinas, citocinas, células Treg, Th17.

Abstract

Role of cytokines related to responses Th17 / Treg in chronic inflammatory periapical disease.

Inflammatory periapical disease is a sequel of the infection and pulp necrosis. It represents the host defense response to aggression from the root canal. Chronic periapical lesions are developed in response to the maintenance of inflammation and bone resorption. Regulatory T cells (Tregs) and Th17 cells play a key role in regulating the immune response with opposite functions. Forkhead box P3 (FoxP3) is the master transcription factor for Treg cells, but differentiation and maturation of naive T cells in Treg or Th17 also depend on specific cytokines such as transforming growth factor β (TGFβ), interleukin 10 (IL -10), interleukin-17 (IL-17), interleukin-6 (IL-6) and interleukin-21 (IL-21). Other cytokines participate in the modulation of the Treg and Th17 activity, as the chemokines CCL4 and CCL20 with function of recruitment of these cells to act in the inflammatory process. The role of Treg and Th17 has been better studied in autoimmune diseases, but sparsely evaluated in the pathogenesis of chronic periapical inflammatory lesions. Eighty-six human samples were used to perform histologic analysis and the cytokine dosage by enzyme-linked immunosorbent assay (*ELISA*) (TGF-β, IL-10, IL-17, CCL4, CCL20) in the periapical tissue of three groups: control group, group of teeth with pulp necrosis and periapical lesion and group of teeth with necrosis without periapical lesion. We observed high expression of CCL4 and TGF-β in the group with periapical lesion in comparison with the groups without lesion and demonstrated increased

expression of CCL20 in the group with periapical lesion when compared to control. Our findings imply that these two characteristics, pro-inflammatory and immunosuppressive, are present in the chronic periapical lesion, occurring simultaneously and with co-stimulation characteristics, as a result of the intense actionof the host's immune response against the inflammatory process coming from intracanal bacteria and their by-products.

Key-words: Inflammatory periapical disease, chemokines, cytokines, Treg cells, Th17.

Lista de siglas e abreviaturas

CAPES – Coordenação de Aperfeiçoamento de Pessoal de Nível Superior

CNPQ – Conselho nacional de pesquisas

COEP-UFMG – Comitê de Ética em Pesquisa da Universidade Federal de Minas Gerais

CTLA-4 - Cytotoxic T-lymphocyte antigen 4

DPI – Doença periodontal inflamatória

ELISA - Enzyme-linked Immunosorbent Assay

FAPEMIG – Fundação de amparo à pesquisa de Minas Gerais

FoxP3 - Forkhead box P3

HE – Hematoxilina e eosina

IFN - Interferon

IL – Interleucina

RORc - Fator nuclear orphan

TGF- β - Fator transformador de crescimento β

Th – Células T helper

Treg – Células T regulatórias

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Considerações Iniciais

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A doença periapical inflamatória (DPI) é consequência da resposta de defesa do hospedeiro à agressão proveniente do canal radicular, aparecendo principalmente como uma sequela da infecção e necrose da polpa dentária causada pela cárie (MOLANDER *et al.,* 2007). Bactérias, seus fatores de patogenicidade e seus produtos metabólicos se difundem a partir do canal radicular e, após a resposta aguda inicial inespecífica, estimulam os mecanismos de defesa do hospedeiro, que passam então a ter papel significativo no desenvolvimento das lesões periapicais. (ANDRADE *et al.,* 2013).

As células T efetoras expressam glicoproteínas CD4 e CD8 em sua superfície. Estas proteínas servem como marcadores fenotípicos de diferentes populações linfocitárias. Essas células reconhecem diferentes antígenos, tendo participação central na distinção da resposta imune contra patógenos. Células T podem assim ser subdivididas em células T CD4+, denominadas auxiliares e células T CD8+ conhecidas como linfócitos T citotóxicos (COFFMAN *et al.,* 2006).

As células T CD4+ têm um papel central na resposta imune celular, podendo se diferenciar em pelo menos seis fenótipos diferentes de células T auxiliares (T helper – Th): Th1, Th2, Th9, Th17, Th22, Treg entre outras (SAITO *et al.,* 2010; ARAUJO-PIRES *et al.,* 2014), dependendo do perfil de citocinas produzidas.

As respostas do tipo Th1 e Th2 são caracterizadas principalmente pelos mediadores produzidos e tipo de resposta desencadeada. A resposta Th1, induzida pela produção de IL-12, caracteriza-se pela produção de citocinas pró-

inflamatórias, como o interferon-γ (IFN-γ*),* enquanto que a resposta Th2 caracteriza-se pela produção de citocinas de perfil anti-inflamatório como interleucina-4 (IL-4*)*, IL-5, IL-6, IL-13 e o fator beta de transformação do crescimento (TGF-β). As citocinas produzidas pelas células Th1 estimulam a fagocitose e destruição de micro-organismos patogênicos enquanto as citocinas da resposta Th2, como a IL - 4, geralmente estimulam a produção de anticorpos dirigidos contra grandes parasitas extracelulares. Estas respostas são reguladas por uma família heterogênea de células, conhecidas como células T reguladoras Treg (SAITO *et al.,* 2010; OHKURA *et al.,* 2013). As células Treg desempenham papéis centrais para imunorregulação e indução de tolerância (ZIEGLER *et al.,* 2009). Células Treg inibem a proliferação e produção de citocinas tanto em células T CD4+ quanto em CD8+ resultando em indução de tolerância.

Papel contrário é exercido pelas células Th17, que, entre outros mediadores, produzem a citocina pró-inflamatória, IL-17. A IL-17 tem um papel importante para a indução da inflamação e tem sido alvo de estudos, devido sua participação na patogênese de doenças autoimunes. As doenças autoimunes resultam frequentemente de um desequilíbrio entre as células T reguladoras (Treg) e células Th17 (YANG *et al.,* 2014).

O desenvolvimento de subpopulações Th depende da expressão de fatores de transcrição específicos. O fator de transcrição *forkhead box P3* (FoxP3) e o fator nuclear *orphan* (RORc) são dois importantes fatores de transcrição presentes na resposta imunológica. Foxp3 é indispensável para a diferenciação das células Treg, enquanto o RORc está presente nas células T virgens. Quando estimulado, RORc induz a diferenciação de linfócitos T em células Th17. A indução do gene Foxp3 em células T indiferenciadas, converte-

as em células Treg de função supressora, indicando que FoxP3 desempenha um papel chave no controle da expressão de moléculas supressoras. A elucidação dos alvos moleculares de FoxP3 é fundamental para um completo entendimento das funções supressoras de Treg (ZIEGLER *et al.,* 2009).

Além do FoxP3, o TGF-β também desempenha um papel importante na imunidade controlando a proliferação e diferenciação das células T virgens. Dependente da ação conjunta de outras citocinas (IL-6, IL-10, IL-22), o TGF-β pode induzir a diferenciação de células T em Treg ou células Th17. Células T precursoras expostas ao TGF-β e IL-10 aumentam a síntese de Foxp3 e se diferenciam em células Treg. Por outro lado, células precursoras expostas a TGF-β e IL- 6 se diferenciam em células Th17, que expressam IL-17 e IL-22 sendo RORc o principal fator de transcrição associado às células Th17 em seres humanos. (TESMER *et al.,* 2008; NOAK *et al.,* 2014).

Além da participação das citocinas na diferenciação de células T virgens em células Treg e células Th17, outros fatores como a presença das quimiocinas CCL20 e CCL4 também influenciam diretamente na ação desta resposta inflamatória, uma vez que estão diretamente associadas ao recrutamento de células Treg e células Th17 aos tecidos periapicais cronicamente inflamados. (BYSTRY *et al.,* 2001; SINGH *et al.,* 2008).

O desenvolvimento da lesão periapical está relacionado à resposta do indivíduo frente à agressão. A função da resposta Th17/Treg neste processo ainda é pouco explorada na DPI de origem endodôntica. Assim, o objetivo deste trabalho é avaliar a participação das células Th17/Treg no desenvolvimento da

doença periapical inflamatória crônica através da avaliação das citocinas e quimiocinas associadas a estes tipos subtipos linfocitários.

Revisão de literatura

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Lesões Periapicais Crônicas:

As lesões periapicais crônicas correspondem a reações inflamatórias decorrentes da necrose pulpar após contaminação bacteriana do canal radicular e estão entre as doenças mais frequentes encontradas no osso alveolar. O desenvolvimento de lesões endodônticas periapicais está diretamente associado à migração de micro-organismos e / ou seus subprodutos através do sistema radicular, para a região periapical (KUC *et al.,* 2000)

Os agentes etiológicos principais são as bactérias, predominantemente cocos Gram-positivo (RICUCCI *et al.,* 2016), resultando em uma resposta inflamatória nos tecidos periodontais de suporte. A resposta inflamatória primária na região periapical é caracterizada por uma vasoconstrição rápida, seguida por vasodilatação e hiperemia. Leucócitos migram para as áreas periféricas dos vasos sanguíneos, aderindo às paredes vasculares. Ocorre o extravasamento de plasma e edema, aumento da pressão local e compressão das terminações nervosas, causando dor. A consequência final do processo inflamatório é um infiltrado que contém neutrófilos, linfócitos e macrófagos. Na fase aguda da inflamação, um exsudato é produzido como uma resposta à agressão da polpa e tecido periapical, com predominância de polimorfonucleares neutrófilos. (LÓPEZ-MARCOS *et al.,* 2004; VIRTEJ et al., 2017).

Micro-organismos e seus produtos metabólicos, uma vez dentro do canal radicular, após a necrose do tecido pulpar, ultrapassam o forame apical chegando ao periápice. O que gera um estímulo lesivo, constante e de baixa intensidade, que pode evoluir para um processo crônico, sem sintomatologia

dolorosa (LIMA *et al.,* 2017). No estágio inflamatório crônico ocorre uma proliferação de novas células teciduais, vasos e fibras, numa tentativa de reparar a lesão, resultando na formação de um tecido de granulação. A necrose pulpar e a inflamação periapical podem assim contribuir para o desenvolvimento de lesões periapicais crônicas que são classificadas como: granuloma dentário ou cisto radicular (VIER *et al.,* 2002).

Granulomas dentários consistem em um tecido granulomatoso cronicamente inflamado, caracterizado por células inflamatórias mononucleares, fibroblastos e uma cápsula fibrosa. Restos epiteliais de Malassez, quando presentes neste tecido inflamado, podem levar ao desenvolvimento de um cisto radicular, devido à proliferação epitelial estimulada por citocinas e fatores de crescimento. O cisto radicular é uma lesão inflamatória crônica com cavidade patológica fechada, preenchida com um fluido eosinofílico ou material semi-sólido, revestida parcial ou completamente por epitélio escamoso estratificado não queratinizado. A parede fibrosa subjacente do tecido conjuntivo é geralmente inflamada, com pequenos vasos sanguíneos e um com infiltrado celular consistido principalmente de macrófagos (RICUCCI *et al.,* 2004).

A incidência destas lesões é variável, provavelmente influenciada pelos métodos de amostragem e aos critérios histológicos utilizados para o diagnóstico. A incidência relatada de cistos entre lesões periapicais varia de 6% a 73%, tornando o termo “lesão periapical” abrangente o suficiente para se referir tanto ao granuloma periapical quanto ao cisto radicular (SAFI *et al.,* 2008).

O diagnóstico de uma lesão periapical é baseado no exame anatomopatológico das lesões. Exames complementares como radiografia

periapial, testes pulpares e exame clínico também estão entre os procedimentos necessários para um correto diagnóstico (ANDRADE *et al.,* 2013).

Resposta imune periapical

Quando a infecção atinge o periápice, uma microbiota mista predominantemente anaeróbia é estabelecida. Em resposta, o organismo libera mecanismos de defesa, sob a forma de vários tipos de células e anticorpos. Os fatores microbiológicos e os mecanismos de defesa do hospedeiro interagem, reabsorvendo grande quantidade de tecido ósseo periapical (LIAPATAS *et al.,* 2003; SHEN et al., 2017).

Os componentes estruturais de uma lesão periapical dependem do equilíbrio entre os fatores microbiológicos e as defesas do hospedeiro. Estas lesões são uma resposta imune à invasão de micro-organismos, desencadeando o recrutamento de células inflamatórias, produção de mediadores inflamatórios como citocinas e ativação de osteoclastos (STASHENKO *et al.,* 1992; MARTÍN-GONZÁLES et al., 2015).

A função fisiológica das respostas imunológicas é eliminar micro-organismos e outros antígenos e reestabelecer a função do tecido afetado. Esta resposta depende de fatores relacionados ao agressor e ao hospedeiro. Fatores como a quantidade e tipo de citocinas, a natureza do antígeno e constituição genética do hospedeiro, estão associados à modulação dos processos inflamatórios, tais como sua progressão e gravidade (MARC *et al.,* 2010; TEIXEIRA-SALUM et al., 2010).

A resposta imune pode ser dividida em inata ou adaptativa. A imunidade inata está associada às fases iniciais da resposta imune e atua contra o agente

agressor de maneira inespecífica, não havendo alteração com a repetida exposição ao mesmo agente (MEDZHITOV *et al.,* 2000). A imunidade adaptativa utiliza mecanismos efetores similares àqueles da resposta inata, porém os direciona com maior precisão. Essa resposta é determinada pela ativação de linfócitos e pela produção de anticorpos (MOSER *et al.,* 2000).

As citocinas envolvidas nas respostas imunes e no processo inflamatório são proteínas regulatórias que desempenham importante papel na modulação das respostas imunes, incluindo ativação, proliferação, diferenciação, sobrevivência e apoptose dos linfócitos. Estas proteínas são secretadas por diferentes tipos celulares, dentre eles os linfócitos T, e são mensageiros intercelulares pelas quais as células envolvidas nas respostas imunológicas se interagem (SEYMOUR *et al.,* 2004).

Linfócitos T

A população de linfócitos T pode ser subdividida em linfócitos T CD4+ ou T CD8+ de acordo com o tipo de marcador expresso na superfície celular. Essas células se distinguem quanto aos tipos de antígenos que reconhecem e, consequentemente, quanto à participação na resposta imune contra micro-organismos. Os linfócitos T CD4+ estão envolvidos principalmente na ativação e regulação de outras células sendo, portanto, denominados auxiliares (COFFMAN *et al.,* 2006). Esse grupo de células é dividido em subpopulações funcionalmente distintas devido ao repertório de citocinas produzidas. Os linfócitos T CD8+, conhecidos como citotóxicos, também desempenham atividades efetoras importantes para a eliminação de micro-organismos

intracelulares, sendo influenciados diretamente por quimiocinas como CCL4 (SHRESTA *et al.,* 1998; HARLIN *et al.,* 2009).

A imunidade adaptativa ativa mecanismos efetores similares àqueles da resposta inata, porém direciona-os com maior precisão. Na resposta imune celular, em doenças de caráter inflamatório, as células T CD4+ têm um papel central e existem subtipos diferentes de resposta orquestrada por estas células: Th1, Th2, Th17, Treg entre outros (MOSER *et al.,* 2000; HARRINGTON *et al.,* 2006).

Os linfócitos T $CD4^+$ classificados como Th1 produzem mediadores inflamatórios como IL - 2 e IFN-γ, entre outros, e são associados com destruição óssea e progressão de lesão (ARANHA *et al.,* 2012). Os linfócitos Th2 produzem citocinas como IL-4, IL-5 e IL-13, entre outras, comumente associados a tolerância de processos inflamatórios nos tecidos periapicais (ARAÚJO-PIRES et al., 2014). A principal diferença relaciona-se à ação destas células: as células Th1 estão envolvidas com a imunidade celular, enquanto as células Th2 com a imunidade humoral (SAITO *et al.,* 2010).

Embora as respostas Th1/Th2 sejam induzidas por diferentes citocinas, os dois tipos de respostas são reguladas por uma mesma linhagem heterogênea de células, conhecidas como linfócitos T reguladores (Treg). Os linfócitos Treg desempenham um papel importante na modulação, indução e manutenção da tolerância imunológica. Eles controlam as respostas imunes e estão relacionados com a inibição da ativação e expansão de linfócitos auto-reativos nos tecidos periféricos (MARC *et al.,* 2010, NOAK *et al.,* 2014; SINGER *et al.,* 2014).

O paradigma Th1/Th2 foi reavaliado para incluir a população de linfócitos T efetores produtores de IL-17 denominados Th17 (KORN *et al.,* 2007). Os linfócitos Th17 representam uma subpopulação de linfócitos T CD4+ que podem mediar a inflamação crônica (HARRIGTON *et al.,* 2006). Esta população celular também se distingue de outros linfócitos T não apenas por sua expressão gênica e regulação, mas também por suas funções biológicas. Linfócitos Th17, produtores de citocinas como IL-17A, IL-17F dentre outras, são conhecidos por apresentarem perfil pró-inflamatório, possuindo papel importante na defesa do organismo contra infecções, além de induzirem o recrutamento de neutrófilos para o local (DONG *et al.,* 2008).

Considerando que Th1 e Th17 foram associados consistentemente com a destruição óssea e progressão da lesão, seus antagonistas Th2 e citocinas associadas a Treg foram descritos como atenuantes do dano tecidual periapical (GRAVES *et al.,* 2011).

Linfócitos Treg

A conversão de linfócitos T virgens, em linfócitos T de função supressora (Treg), tem sido atribuída a um gene específico, o *Forkhead box* P3 (*FOXP3*). O gene *FOXP3* está localizado na região do cromossomo Xp11.23, responsável pela síntese de FoxP3. Foi demonstrado, em ratos, que a inativação de *FOXP3* resulta na diminuição das células Treg. Atualmente, o fator de transcrição FoxP3 é o melhor e mais confiável marcador para as células Treg (ZHOU *et al.,* 2008; YANG *et al.,* 2014; CAMPOS *et al.,* 2015).

Estudos *in vitro* mostram que células Treg na presença do fator de transcrição FoxP3 podem agir de diferentes maneiras para inibição direta da

replicação de bactérias (Fig. 1), levando à conclusão que esta resposta visa manter a tolerância frente à agressão tecidual provocada por micro-organismos. Além destes mecanismos de supressão direta, a ativação de linfócitos Treg FoxP3^{+} pode induzir a produção de citocinas supressoras como IL-10 que irão agir diretamente na inibição da replicação de bactérias presentes na região periapical de dentes com periodontite crônica. Podem também mediar a apoptose celular através de receptores que irão se ligar a IL-2, uma glicoproteína de ação citolítica (SHEVACH *et al.,* 2009).

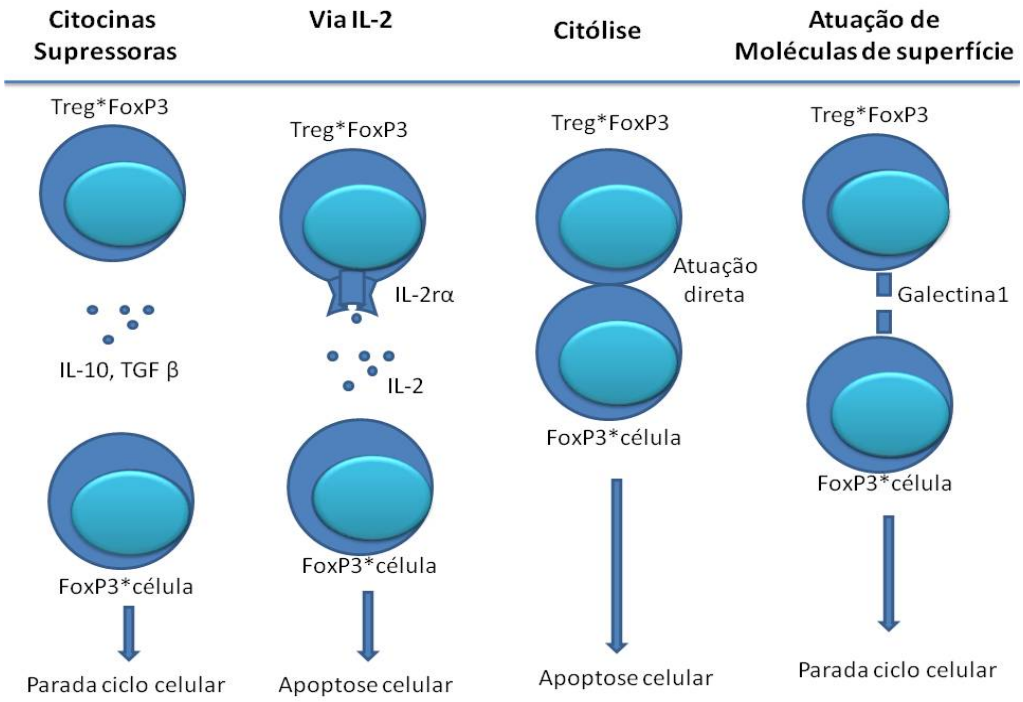

Figura 1: Diferentes maneiras para supressão direta do ciclo celular por Treg/FoxP3. (baseado em SHEVACH et al., 2009).

Linfócitos Th17

Para que ocorra a diferenciação de linfócitos T CD4+ virgens em Th17, um fator de transcrição de linhagem específica, o RORc é indispensável. Esta diferenciação ocorre em três diferentes etapas: iniciação, amplificação e estabilização (LI *et al.,* 2017). Na presença de citocinas como IL-6 e TGF-β no

tecido inflamado, há ativação de RORc que induz a diferenciação de linfócitos T em linfócitos Th17 (MA *et al.,* 2017). IL-1-β, IL-6 e IL-23 são importantes mediadores com ação de amplificação e estabilização de células Th17 para a manutenção da produção da IL-17 e IL-22 (Fig. 2) (IVANOV *et al.,* 2006; NOAK *et al.,* 2014).

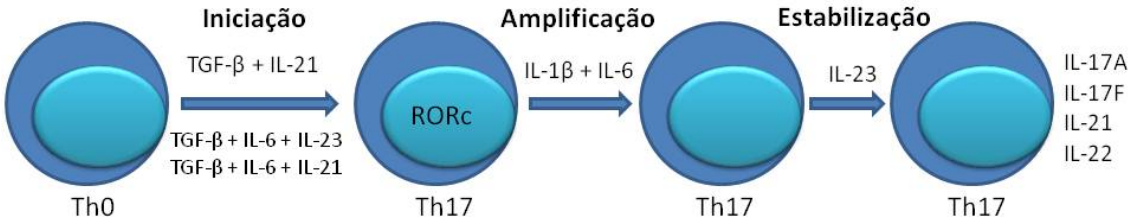

Figura 2 - Iniciação, amplificação e estabilização na diferenciação de linfócitos Th17 (Baseado em NOAK et al., 2014).

Relação Th17/Treg na doença inflamatória periapical

Células Th17 e Treg têm papéis opostos no desenvolvimento da DPI (ANDRADE et al., 2013). Enquanto os linfócitos Th17 estimulam a resposta imune, os linfócitos Treg têm ação regulatória e, portanto, desempenham um papel muito importante na manutenção da tolerância e no controle da expansão e ativação do sistema imune (NOAK et al., 2014).

A expressão de *FOXP3* em populações celulares envolvidas na resposta imune inibe a produção de citocinas pró-inflamatórias, dentre elas a IL-17. Por outro lado, acentua a expressão de citocinas anti-inflamatórias, como IL-10 e TGF-β e de CTLA-4, um inibidor co-estimulatório de Tregs (CHATILA et al., 2003; SHEVACH et al., 2009).

O TGF-β, diferentemente da IL-17 produzida pela população de células Th17, é uma citocina que agrega múltiplas funções regulatórias no sistema imune. Possui potente efeito imunossupressor sabidamente associado aos efeitos inibitórios da reabsorção óssea. Ela induz tanto a expressão de FoxP3 quanto de RORc em células T CD4+. Assim é um fator crítico para indução das células Th17 e células Treg (FU et al., 2004; TEIXEIRA-SALUM et al., 2009).

Apesar da indução destes fatores de transcrição, TGF-β é incapaz de iniciar a diferenciação de células Th17, pois sabidamente, possuem efeitos moduladores opostos no processo inflamatório. É necessário que citocinas pró - inflamatórias, como IL-6 ou IL-21 estejam presentes no microambiente tecidual (MANEL *et al.,* 2008). Assim, na presença de IL-6 e TGF-β, os linfócitos T CD4+ diferenciam-se em células Th17. Durante um processo inflamatório, a expressão de FoxP3, também induzida por TGF-β, é reduzida e a expressão de RORc é aumentada, gerando assim um desequilíbrio na relação Th17/Treg (Fig. 3) (NOAK *et al.,* 2014).

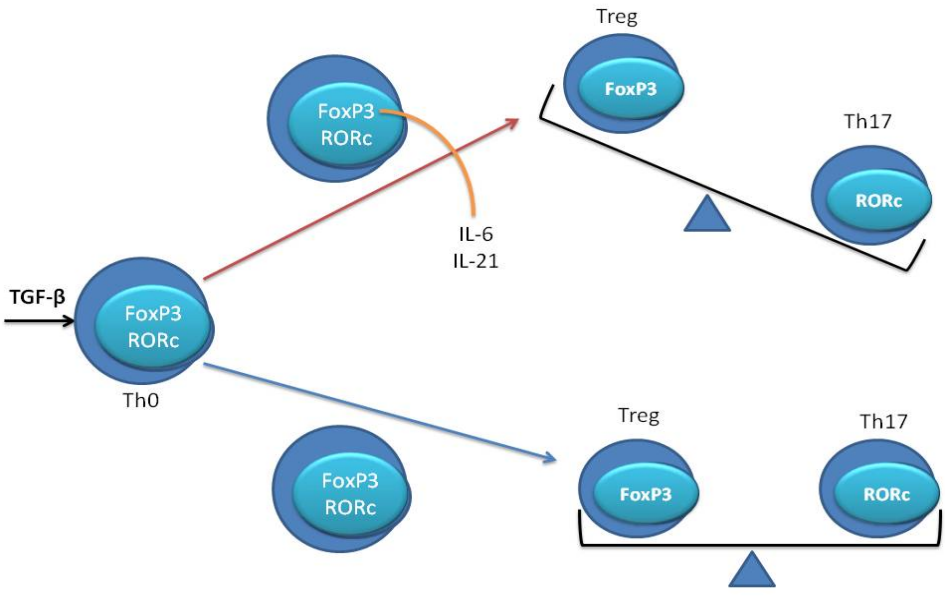

Figura 3 - Equilíbrio entre linfócitos Th17 e Treg. TGF-β é capaz de induzir a expressão de FoxP3 e RORc. Na presença de citocinas pró-inflamatórias, tais como IL-6 ou IL-21, a expressão de FoxP3 é reduzida e a expressão RORc é regulada positivamente. Na ausência de inflamação, o TGF-β promove a diferenciação de Treg; isto é devido à inibição mediada por FoxP3 de RORc (NOAK et al*.,* 2014).

Porém na ausência de um processo inflamatório significativo, marcadores como TGF-β e IL-10 auxiliam a diferenciação de linfócitos T virgens em linfócitos Treg, que mantém a tolerância imunológica. Isto ocorre devido a uma inibição inicial da atividade de RORc mediada por FoxP3 resultando, consequentemente, na inibição da expressão de citocinas pró-inflamatórias como IL-17 e IL-22, pela inibição da diferenciação de linfócitos T precursores em linfócitos Th17. Assim, TGF-β é capaz de induzir a expressão de FoxP3 e RORc. Entretanto, IL-6 ou IL-22 reduzem a expressão de FoxP3 enquanto a expressão de RORc é aumentada, aumentando consequentemente o quadro inflamatório. Na ausência de inflamação, TGF-β promove a diferenciação de linfócitos Treg mediado por FoxP3, suprimindo o quadro inflamatório (Fig. 3). Pouco se conhece, porém, o seu real papel nas lesões periapicais (TEIXEIRA-SALUM *et al.,* 2009; NOACK *et al.,* 2014).

Até o momento, estudos têm demonstrado (PEIXOTO *et al.,* 2009; FUKADA *et al.,* 2009; ANDRADE *et al.,* 2013; ARAUJO-PIRES *et al.,* 2014; CAMPOS *et al.,* 2015) que FoxP3 é um interruptor principal que regula a progressão e função das células Treg, cujas funções incluem a inibição das respostas imunes. Por outro lado, a resposta imune Th17 parece desempenhar um papel dominante na exacerbação da inflamação (COLIĆ et al., 2009). Além disso, marcadores distintos, como IL-1β, IL-6 ao lado de IL-17 contribuem para

a atividade pró-inflamatória de linfócitos Th17 (MARÇAL et al., 2010). Enquanto a associação de FoxP3, TGF- β, IL-10, IL-9 e IL-4 contribuem para a inatividade das lesões (ARAUJO-PIRES *et al.,* 2014). Assim, a participação de Treg e Th17 tem sido associada à modulação de lesões periapicais humanas e reabsorção óssea.

Durante a homeostase do organismo, o desenvolvimento de células T reguladoras a partir de células T virgens, parece ser favorecido. Porém, mesmo TGF-β, sendo uma potente citocina com características imunossupressoras, quando em condições ideais no sítio inflamatório, pode induzir a diferenciação de células T em células Th17, aumentando, consequentemente, a produção de IL-17 e mediando, de forma indireta, a inflamação. As quimiocinas CCL4 e CCL20, já foram descritas (LIU JY *et al.,* 2015) como recrutadoras de células Treg e Th17 respectivamente. Em lesão periapical humana, o tema ainda carece de elucidação. Entretanto, as relações das citocinas e quimiocinas, envolvidas no processo inflamatório das lesões periapicais, ainda necessitam de maiores esclarecimentos (YAGI *et al.,* 2004).

Justificativa

Justificativa

Os mecanismos de desenvolvimento das lesões perirradiculares ainda não são totalmente compreendidos (FUKADA *et al.,* 2009). São necessários mais estudos para dissecar completamente a rede de citocinas envolvida na patogênese das lesões periapicais, visando desvendar as vias protetora e destrutiva e, portanto, contribuir para melhorar o diagnóstico e o tratamento dessas doenças (ARAUJO-PIRES *et al.,* 2014).

A participação de citocinas de perfil Th17 ou Treg no desenvolvimento da lesão periapical inflamatória crônica humana é ainda muito pouco avaliada. Por outro lado, o conhecimento das citocinas e quimiocinas envolvidas no processo, pode vir a ser uma abordagem terapêutica em relação à inflamação periapical e à reabsorção óssea, merecendo portanto, ser amplamente explorada.

Objetivos

Objetivos

Objetivo geral:

Avaliar a expressão das citocinas IL-17, IL-10, TGF-β, CCL4 e CCL20 em tecido periapical de dentes humanos hígidos e de dentes com necrose pulpar, com e sem lesão periapical.

Objetivos Específicos:

1- Fazer a avaliação clínica e histopatológica da região periapical de dentes com lesão.

2- Quantificar e comparar as citocinas TGF-β, IL-10, IL-17, CCL4 e CCL20 em tecidos periapicais de dentes hígidos e de dentes com necrose pulpar com e sem lesão periapical pela técnica de ELISA.

3- Verificar a correlação das citocinas nos diferentes grupos.

4- Elaborar uma revisão sistemática sobre participação dos linfócitos Th17/Treg no desenvolvimento das lesões periapicais inflamatórias crônicas.

Metodologia Expandida

Metodologia Expandida

Considerações éticas:

Este trabalho foi submetido à avaliação do Comitê de Ética em Pesquisa da Universidade Federal de Minas Gerais e foi aprovado segundo parecer (CAAE – 398983.14.1.0000.5149) (Apêndice 1).

Amostra

Foram incluídas neste estudo oitenta e seis amostras de tecido periapical obtidas de dentes humanos, com e sem necrose pulpar, sem tratamento endodôntico e com indicação para exodontia e tecidos periapicais de dentes hígidos. Os indivíduos foram pacientes diagnosticados com lesões periapicais caracterizadas radiograficamente como lesões radiolúcidas, com ausência do espaço do ligamento periodontal e descontinuidade da lâmina dura. Todas as amostras do grupo com necrose, foram obtidas a partir de dentes sem sensibilidade pulpar e sem tratamento endodôntico, que foram encaminhados para extração dentária ou cirurgia apical endodôntica. Pacientes com condições médicas que exigem o uso de modificadores sistêmicos do metabolismo ósseo ou outra terapia medicamentosa assistida (antibióticos sistêmicos, terapia antiinflamatória ou hormonal), nos últimos 6 meses antes do início do estudo, pacientes com condições pré-existentes, como traumas oclusais e gestantes ou lactantes foram excluídas do estudo. No caso de dentes multiradiculares diagnosticados com lesão periapical, foram incluídos no estudo apenas os tecidos da raiz afetada pela lesão observada radiograficamente. O diagnóstico de necrose pulpar foi confirmado por exame clínico, testes de vitalidade pulpar

(térmico, elétrico, cavidade) e radiográfico (ROMANOS *et al.,* 2014). O exame clínico incluiu os dados objetivos colhidos pelo profissional, por exames como palpação, percussão, inspeção e exploração. A palpação, a partir do tato e compressão ou preensão digital, fornece impressões sobre uma determinada área, podendo definir forma, limites, consistência, modificações de textura, espessura, sensibilidade, volume, mobilidade, conteúdo, flutuação, temperatura e elasticidade. Foi feito o preenchimento de ficha clínica com todos os dados do participante relacionados a sua saúde geral e a presença ou não da lesão periapical (Apêndice 2). Tecidos periapicais de dentes hígidos foram obtidos a partir de dentes com indicações ortodônticas para extração, e terceiros molares hígidos sem diagnostico de processos inflamatórios, sujeitos aos mesmos critérios de exclusão estabelecidos acima. As lesões periapicais foram seccionadas, uma metade foi fixada em formol a 10% para diagnóstico histopatológico e a outra foi mantida Ultra-Freezer Modelo IULT 9504D – Faixa de Temperatura: -50 a -86. Todos os participantes foram devidamente esclarecidos e informados sobre a pesquisa, seus métodos e objetivos, sendo incluídos somente após a obtenção de um consentimento livre e informado devidamente assinado (Apêndice 3).

ELISA

As amostras de tecido foram maceradas em um homogeneizador próprio (PowerGen 1000, Fisher Scientific) na presença de 1 mL (para cada 100 mg de tecido) de solução PBS (0,4 mM NaCl, 10 mM $NaPO_4$) com inibidores de proteases (0.1mM PMSF, 0,1nM benzethonium clorídrico, 10mM EDTA e 20KI aprotinina A) e 0,05% Tween20. A solução resultante foi centrifugada por 10 min

a 10000 r.p.m. a 4°C e o sobrenadante recolhido para a dosagem de TGF-β, IL-10, IL-17, CCL4 e CCL20 por ELISA, utilizando kits comerciais (TGF-β e IL-10 R&D Systems Europe Ltd., Abington, UK; IL-17, CCL4 e CCL20 Abcam Plc Europe Ltd., Cambridge, UK). Foram seguidas as instruções do fabricante para a realização dos ensaios. Os resultados foram expressos em picogramas de citocinas (+SD) por 100 mg de tecido periodontal e a absorbância obtida em leitor de ELISA a 492 nm.

Análise estatística

Após obtenção dos resultados, foi aplicado o teste Shapiro-Wilk para análise da normalidade de distribuição da amostra e o teste de Levene para homogeneidade. Para análise das variáveis independentes foi utilizado o teste Mann – Whitney (para distribuição não normal). A relação entre as variáveis foi obtida através da Correlação de Spearman.

Grupos avaliados: Grupo com necrose e lesão periapical, grupo com necrose sem apresentar lesão periapical, e tecidos periapicais de dentes hígidos.

Os dados foram analisados usando SPSS - Statistical Package for Social Sciences, versão 19.0 para Macbook - Chicago, IL, USA e o GraphPad Prism 5® versão 5.01. O nível de significância após aplicada a Correção de Bonferroni foi estabelecido em ($p < 0,017$).

Revisão sistemática

Foi feita uma revisão sistemática da literatura existente sobre o tema "Participação de linfócitos Th17 e Treg no desenvolvimento das lesões

periapicais inflamatórias crônicas". Utilizando as bases de dados: Pubmed, Web of Science, Scopus e Medline. Foram seguidas as recomendações do cheklist PRISMA para todo o desenvolvimento da revisão sistemática. (Apêndice 4).

Artigos

Artigo 1

Role of cytokines related to responses Th17 / Treg in chronic inflammatory periapical disease.

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Artigo a ser submetido para publicação no periódico *Journal of Endodontics* (Apêndice 5).

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Summary

Introduction: Inflammatory periapical disease is a sequel of pulp necrosis and represents the host defense response to aggression from the root canal. Cytokines have a fundamental role in the maintenance of inflammation and bone response, culminating with the developing of chronic periapical lesions Regulatory (Treg) and Th17 cytokines play a key role in regulating the immune response. This study aimed comparatively investigates TGF-B, IL-17, IL-10, CCL20, and CCL4 in human periapical tissues. **Methods**: Enzyme-linked immunosorbent assay (*ELISA*) measured the levels of TGF-β, IL-10, IL-17, CCL4, CCL20 in periapical tissues of three groups: higid teeth (27); teeth with pulp necrosis and periapical lesion (27), and, teeth with necrosis without periapical lesion (32). **Results:** The group with periapical lesion showed a high expression of CCL4 and TGF-β in comparison with the groups without lesion. CCL20 was also higher in the group with periapical lesion when compared to control. **Conclusion:** Our findings imply that both types of cytokines, pro-inflammatory and immunosuppressive, occurs simultaneously in human chronic periapical lesions and are important in their maintenance.

Introduction

The inflammatory periapical disease is a consequence of the host's defense response to aggression originating from the root canal, mainly appearing as a sequel of infection and necrosis of the dental pulp caused by cavities (1). Bacteria, their pathogenic factors and their metabolic products diffuse from the root canal and, after the initial non-specific immune response, stimulate host defense mechanisms, which then play a significant role in the development of periapical lesions. (2).

Treg cells play central role for immunoregulation and induction of tolerance. (3). These cells are regulated by cytokines like TGF-β and IL-10 also which control the proliferation and differentiation of naive T cells in Treg cells (4,5). TGF-β and IL-10 show a potent immunosuppressive effect associated with an inhibitory effect on bone resorption in chronic periapical granulomas and radicular cysts (6,7). CCL4 also plays an important role in this immunoregulation process, being main chemoattractant responsible for recruiting treg cells into inflammatory sites. (8,9).

Contrary role is exerted by Th17 cells, which, among other mediators, produce the pro-inflammatory cytokine, IL-17. IL-17 plays an important role in the induction of inflammation and due this, its role in the pathogenesis of autoimmune diseases has been explored a lot (10). Data suggest that up-regulation and stable expression of CCR6 is a fundamental feature of Th17 differentiation (11). CCR6 is expressed on subsets of T cells and dendritic cells and has CCL20 as its unique ligand. CCL20 acts as chemoattractant recruiting Th17 cells. (12)

However, in respect to periapical inflammatory disease, the up/down regulation of both Treg and Th17 cells have been mainly explored in animal models (13,14,10), with few studies conducted in human (2,15). The results of these studies stress the antagonism

of these cells, and this relationship is commonly described as a result of an imbalance between them. Studies exploring the regulatory mechanisms in this relationship are crucial. This study aimed to investigate the role of Treg and Th17 cells in inflammatory periapical disease, comparing the expression of the immunoregulatory cytokines TGF-β, IL-10, CCL4 and the proinflammatory IL-17 and CCL20 in tissues removed from teeth with pulp necrosis, with and without lesions and controls.

Materials and Methods

This study was approved by the Institutional Committee of Ethics (CAAE – 398983.14.1.0000.5149) and all participants signed an informed consent. All samples were collected at the Oral Sugery Clinic and all participants were indicated for tooth extraction. The samples were composed of 32 periapical lesions (patients aged 19– 51 years), 27 periapical tissue whitout lesion from teeth with diagnosis of pulp necrosis (obtained by the curettage of periodontal apical tissue after extraction of the dental element, patients aged 22–58 years) and 27 controls (healthy periapical tissue obtained from third molars extractions, patients aged 18– 27 years). No distinctions between specimens were made regarding the etiology or the tooth type. The periapical lesions were characterized radiographically as radiolucent lesions showing absence of the periodontal ligament space and discontinuity of the lamina dura (16). The criteria for exclusion were patients with medical conditions requiring the use of systemic modifiers of bone metabolism or other assisted drug therapy (systemic antibiotics, anti-inflammatory, or hormonal therapy) during the last 6 months before initiation of the study, patients with pre-existing conditions such as periodontal disease, and pregnant or lactating women.

The periapical lesions were divided into 2 roughly similar fragments. The first fragment was collected to perform the enzymelinked immunosorbent assay (ELISA) to detect chemokines. The second fragment was set in 10% buffered formalin, histologically processed, sectioned, and stained with hematoxylin and eosin (HE) for histologic diagnosis.

Morphological analysis

Granulomas were histologically defined by the inflammatory chronic tissue characterized by capillaries, inflammatory cells, fibroblasts, collagen, and absence of an epithelial lining. Periapical cysts comprised lesions in which cavities were further developed and lined by stratified squamous epithelium. Because of losses during the processing of samples from periapical lesion group, 32 samples were used for analyses of chemokines, and 26 samples were stained in HE for morphological analysis.

Detection of Tissue Chemokines

Collected samples were weighed and homogenized in a buffer (0.4 mM NaCl, 10 mM NaPO, pH 7.4) containing inhibitors of proteases (0.1 mM phe- nylmethylsulfonyl fluoride, 0.1 mM benzethonium chloride, 10 mM EDTA, and 0.01 mg/mL aprotinin A) and polysorbate 20 (0.05%), pH 7.4, at a ratio of 1 mL solution per 100 mg tissue. The homogenate was centrifuged (8,946 g) at 4 C for 10 minutes. The supernatant was then collected and used for the quantification of chemokines. The levels of TGF-B, IL-17, IL-10, CCL20 and CCL4 were evaluated by ELISA, using commercially available kits (TGF-β and IL-10 from R&D Systems Europe Ltd., Abington, UK; IL-17, CCL4 and CCL20 from Abcam Plc Europe Ltd., Cambridge, UK). All assays were carried out

according to manufacturer instructions. The results were expressed as picograms of chemokine per 100 mg tissue.

Statistical Analyses

Statistical analyzes were performed using Statistical Package for Social Sciences (SPSS), version 19.0 (IBM, Armonk, NY, USA). Normal distribution was tested using the Shapiro–Wilks procedure. Kruskal–Wallis and Mann–Whitney U tests were used for analyses of the samples with non-normal distributions. After applying the Bonferroni correction, statistical significance was achieved when P values were <0.017. Chemokine levels expressed as picograms of chemokine/100 mg tissue were correlated with the number of DCs expressed in densities (cells/mm). Chemokine levels were compared between individuals diagnosed with periapical lesion, pulp necrosis without periapical lesion and controls.

Results

From thirty-two lesions assigned to immunoassay procedures, 26 samples were stained in HE for morphological analysis with seven periapical cysts (26,9%) and nineteen periapical granulomas (73,1%).

Eighty-six samples were used to perform the cytokine dosage by ELISA (TGF-β, IL-10, IL-17, CCL4, CCL20), among the three groups: control group (27), group with necrosis without periapical lesion (27) and group with necrosis and periapical (32) lesion.

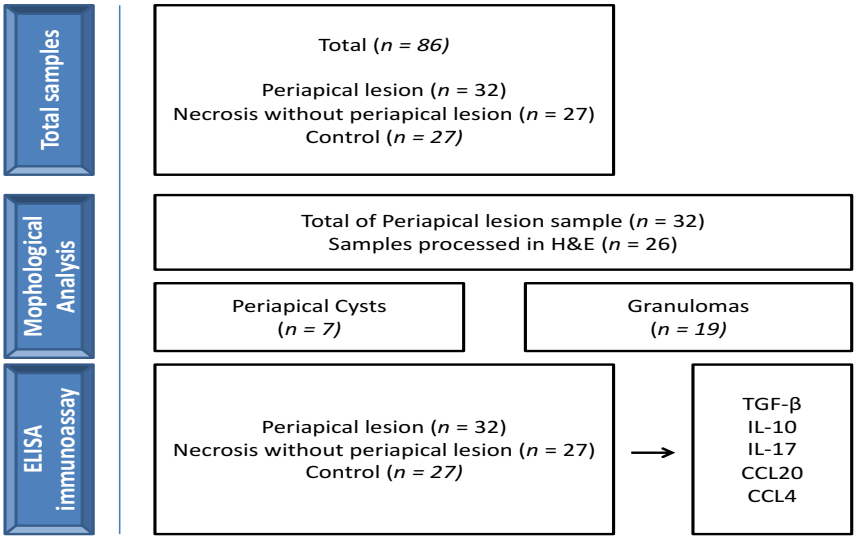

Figure 1: Flowchart representing the distribution of the samples in the three groups and the cytokines used in the immunoassay.

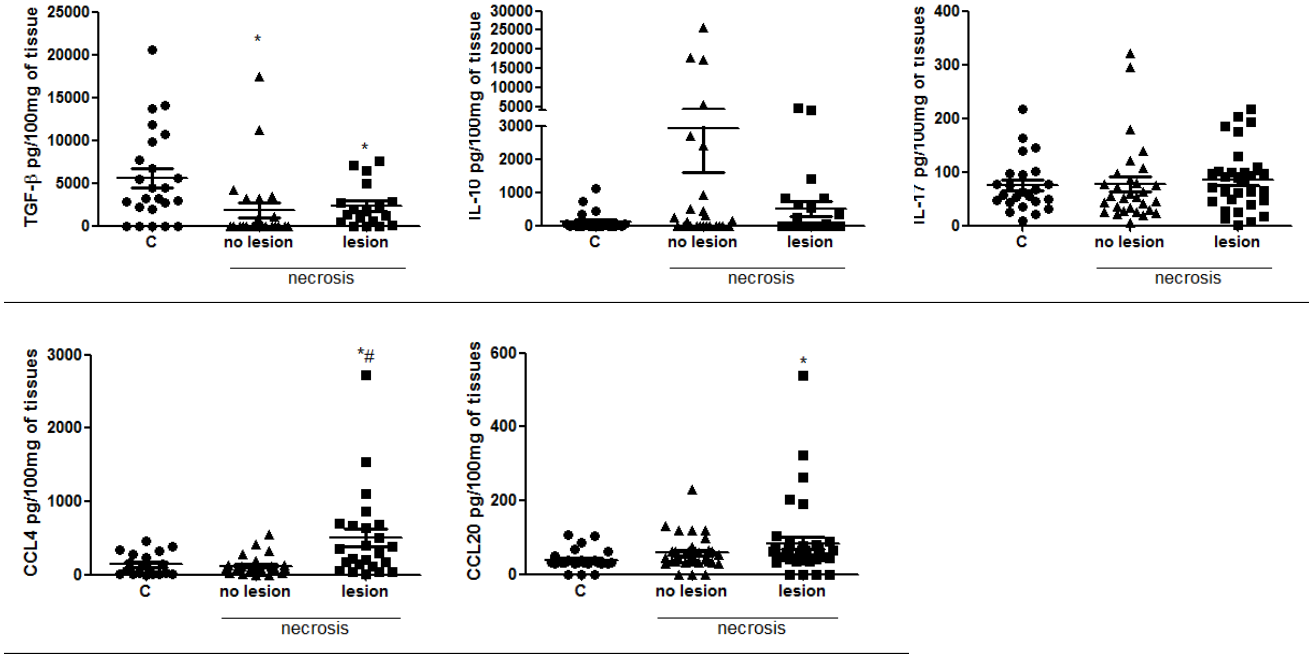

Figure 2: The expression of cytokines TGF-β, IL-10, IL-17, CCL4, CCL20 among the three groups: control group (n=27), group with necrosis without periapical lesion (n=27) and group with necrosis and periapical lesion (n=32). The levels of expression were determined by the Mann-Whitney U test).

TGF-β, CCL4 and CCL20 showed statistical differences between the groups evaluated. TGF-β and CCL4 showed higher expression in the group with periapical lesion

than the necrosis group without lesion ($p<0.017$). TGF-β also showed a difference between necrosis without lesion and control with a high expression in necrosis without lesion group ($p \leq 0.017$). CCL20 and CCL4 presented similar results. Both have a higher expression in the periapical lesion group than control (p=,000). The expression of CCL20 and CCL4 did not show difference between the necrosis without lesion and control (p=,036 and p=,378 respectively). TGF-β levels were also similar between the group with periapical lesion and control ($p> 0.017$) and the same was found for CCL20 comparing the groups necrosis whit and without lesion (p=,065) as shown in Figure 2. The data analysis showed a positive correlation between CCL20 and IL-17 between the entire sample (r=0.76). The same positive correlation was observed between IL-10 and CCL4 (r=0.263). The control group show the higher correlation between evaluated groups whit IL17/CCL20 (r=0,251) and IL10/CCL4 (r=0,384). As shown in table 1. A negative correlation between IL-17 and TGF-β was also observed (r= -0.002).

Discussion

Periapical lesions develop as a response to the chronic presence of antigens in periapical tissues caused by bacterial infection of the root canal system (17). As a consequence, innate and adaptive responses are activated, compromising the vascular and cellular system, culminating in bone resorption in the root apex region of the necrotic teeth (18). Different cells are involved in this process, including neutrophils, macrophages, T lymphocytes and B lymphocytes, as well as Treg cells that regulate the immune response (19). A strong combination of antigens and maximum co-stimulation is required for the development and maturation of Treg cells (20). The host response is certainly related to this course. Several cytokines and chemokines like TGF-β, IL-10, IL-17, CCL4 and CCL20, are involved in the immune responses that take place during

periapical lesions development (21). In this sense, Colić *et al.* (2009) (17), showed, for the first time, the presence of Tregs cells in periapical lesions that also express IL-10 and TGF-β, and are suppressive *in vitro.* While Marçal et al. (2010) (22), showed that the production of IL-17 was associated with an exacerbation of the inflammatory response, an elevated number of neutrophils, and bone resorption in human tissue. Our study evaluated the role of both the Treg and Th17 in periapical tissues evaluating some cytokines that are key elements controlling this response.

The quantitative analysis by ELISA showed a higher expression of TGF-β and CCL4 in periapical lesion in comparison with the other two groups (necrosis without periapical lesion and controls). Similar results were described by Bystry *et al.* (2001) (8), showing CCL4 as the most potent chemoattractant of Treg cells, suggesting that the recruitment of regulatory T cells by CCL4 plays a central role in the start of humoral responses. The immunosuppressive mechanisms of Treg cells mediated by TGF-β and IL-10 are responsible for the healing process and control of the inflammatory mechanisms in the periapical region (23). Our results corroborate with studies that showed a positive correlation of these markers with the inactivity of the periapical lesions (17,24). It is possible that this immunosuppressive feature represents a protective mechanism in an attempt to avoid the progression of bone destruction promoted by the periapical lesion. The high expression of CCL4 and TGF-β d in the group with periapical lesion, suggests that the maintenance and induction of Treg cells by these cytokines occur in the immune regulatory process of periapical lesions.

When comparing only the two groups, necrosis with periapical lesion and necrosis without lesion, we also observed a greater expression of TGF-β and CCL4 cytokines in the lesion group. Inflammatory chronic lesions can present an immunosuppressive

profile, explaining the levels of those cytokines (25). This correlation was also observed by Colić *et al.* (2009) (17) suggesting a positive association between TGF-β and Treg cells acting on the development of chronic periapical lesions.

Th17 and Treg cells have opposite roles in the development of the periapical lesion. The cytokines that represent the action of those cells are especially represented by TGF, IL-10, CCL4, CCL20 and IL-17. While Treg cells have a regulatory action mediated by cytokines like TGF, IL-10 and CCL4 (2,8) Th17 cells stimulate the local inflammatory response by the production of the cytokine IL-17, which plays a very important role maintaining tolerance and controlling the expansion and activation of the immune system (4). Even if TGF-β is a potent cytokine with immunosuppressive characteristics, T cell differentiation in Th17 cells can occur increasing IL-17 production and indirectly mediating inflammation (26). CCL20 was specifically expressed in Th17 cells, but not in Treg and other effector Th subsets (27). Our study demonstrated a positive correlation in the expression of CCL20 and IL-17. Among these, the increased expression of CCL20 in the group with periapical lesion occurred when compared to the groups necrosis without periapical lesion and control. The positive correlation and the high presence of pro-inflammatory chemokines CCL20 and IL-17 in the group with periapical lesion shows an inflammatory activity present in the periapical region of the collected samples, probably as a response to the presence of bacteria from the root canal system.

Our study showed the simultaneous immunosuppressive and pro-inflammatory features in chronic periapical lesions represented respectively by IL-10/TGF-β/CCL4 and IL-17/CCL20. A positive correlation between IL-17/CCL20 and IL-10/CCL4 was also found in all groups. These results suggest that the response Treg/Th17 in chronic periapical lesions occurs not as a balance but as a co-stimulation mechanism.

Conclusion

The role of Treg/Th17 response has been described in the recent literature as a antagonistic balance between the immunosuppressive characteristics of Treg and the pro-inflammatory capacity of Th17. This study demonstrates that both characteristics of Treg/Th17 cells are simultaneously present in the chronic periapical lesions caused by endodontic factors, probably contributing for the persistence of same lesions.

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Conflict of interest

The authors declare no conflict of interest.

Tables

	PRO-INFLAMMATORY		ANTI-INFLAMMATORY	
ALL SAMPLES (N=86)	**IL-17**	**CCL20**	**IL-10**	**CCL4**
IL-17	1	,076		
CCL20	,076	1		
IL-10			1	,263
CCL4			,263	1
LESION (N=32)	**IL-17**	**CCL20**	**IL-10**	**CCL4**
IL-17	1	,011		
CCL20	,011	1		
IL-10			1	,003
CCL4			,003	1
NO LESION (27)	**IL-17**	**CCL20**	**IL-10**	**CCL4**
IL-17	1	,008		
CCL20	,008	1		
IL-10			1	,078
CCL4			,078	1
CONTROLS (27)	**IL-17**	**CCL20**	**IL-10**	**CCL4**
IL-17	1	,251		
CCL20	,251	1		
IL-10			1	,384
CCL4			,384	1

Table 1 - Spearman correlation of the citokynes and chemokynes IL-17/CCL20 (Pro-inflammatory) and IL-10/CCL4(Anti-inflammatory in all evaluated sample).

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Artigo 2

Treg and Th17 cells in inflammatory periapical disease: a systematic review.

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Treg and Th17 cells in inflammatory periapical disease: a systematic review.

Abstract

Periapical lesions occur as an outcome of teeth's pulpal necrosis. This process is regulated by the immune system including the Treg and Th17 response. The objective of this study was to conduct a systematic review to determine the role of Treg/Th17 cells in the progression of chronic inflammatory periapical lesions in humans. Systematic computerized searches were carried out using the electronic databases Pubmed, Medline, Web of Science and Scopus from their date of inception through the first week of May 2017. Hand searches of the reference list of the included article and grey literature search were also carried out. Articles that evaluated the role of Th17 and Treg lymphocyte in the progression of human periapical lesions were included. Study selection and the quality assessment of the included articles using the Newcastle-Ottawa scale were carried out by two authors. Fifty seven titles/abstracts were screened and eight studies met the eligibility criteria and were included in this systematic review. The included studies showed large variation in type of periapical lesion assessed, mean age, age range, type of experiment used in the included study and findings regarding the participation of Th17 and Treg cells in the progression of periapical lesions. The studies suggested a greater participation of regulatory T cells in the modulation of the inflammatory response in periapical lesions. This systematic review highlights the relationship between the inhibitory characteristic of Treg cells, and the pro-inflammatory feature of Th17 cells in the progression of human periapical lesions.

Keywords:

Periapical lesions, T cells, T reg cells, Th17 cells, Th17, FoxP3.

Introduction

Apical chronic periodontitis is a common inflammatory osteolytic disease of the jaws. This process is regulated by several inflammatory mediators including cytokines released by leukocytes[1]. Some leukocytes are recruited from the periapical tissues in response to intracanal bacterial infection, and the persistence of the antigenic stimulus can results in chronic periapical lesions such as periapical granuloma and radicular cyst[2].

Evidence indicating the role of immunologic mechanisms in the pathogenesis of periapical lesions has been widely documented[3]. The interplay between immune regulatory mechanisms and effector T cell responses is a crucial determinant of innate and adaptive immunity[4]. $CD4^+$ T cells play a regulatory role and help to constrain the effector function of others cell types[5]. More recently[6], the identification of CD4+ Foxp3+ Tregs and Th17 modified the paradigm Th1-Th2. The Treg-Th17 dicotomy provides a way to comprehension of immunity/inflammation in an increasing number of diseases, including periodontal disease and others involving the progressive bone resorption[7].

Treg cells, defined by the expression of the lineage specific transcription factor FoxP3 (Forkhead box P3), are required cells for immune induction[8]. Th17 cells play critical roles in the progression of autoimmunity and inflammation by the production of IL-17 and require specific cytokines for their differentiation, such as the transforming growth factor-β (TGF-β), which may be combined with interleukin-6 (IL-6) or IL-21 and the transcription factor RORγt[9]. The idea of Treg and Th17 participating in the inflammatory response may help explain many unresolved mysteries in the immunobiology of a periapical lesions, such as bone resorption occurring as a reflection of intracanal infection in different proportions in similar cases of pulpal necrosis[10]. However, in respect to periapical inflammatory disease, the up/down regulation of both

Foxp3 and Th17 have been explored in animal models[11,12,13], with few studies conducted in human, and considering the different types of chronic lesions, radicular cyst or periapical granuloma. Thus, defining the role of Treg/Th17 is crucial for the entire understanding of the immune system regulation in these lesions.

This systematic review aims to investigate the response of the regulatory T cells (Tregs) and Th17 cells in human inflammatory chronic disease and to describe the role of these subsets of cells in the progression of periapical lesions in humans.

Methods

Protocol and registration

This systematic review was described using the Preferred Reporting Items for Systematic Reviews and Meta-Analyses checklist (PRISMA) as a template[14]. Neither a protocol nor a systematic review registration was considered.

Eligibility criteria

Studies which evaluated the role of Th17 and Treg cells in the progression of human periapical lesions were included. An illustration of the pathway of the Treg/Th17 cells and the involved biomarkers is provided in Figure 1. The following PECO question has been applied:

P = Periapical lesions in humans

E = High levels of expression of Treg/Th17 cells and/or FoxP3/IL-17

C = Low levels of expression of Treg/Th17cells and/or FoxP3/IL-17

O = Progression of periapical lesions

S = Observational studies

Reviews, case reports, letters to editors, studies involving animals and meeting abstracts were excluded. No restriction regarding language, date and status of publication was applied.

Information sources

Systematic computerized searches were carried out using the electronic databases Pubmed, Medline (Ovid SP), Web of Science (Thomson Reuters) and Scopus (Elsevier) from their date of inception through the first week of October 2015. These computerized searches were updated in first week of May 2017. Hand searches of the bibliographic references of the included articles were also carried out in order to identify relevant articles that might have been missed in the electronic search. Finally, a grey literature search was conducted by using Google Scholar search engine. This gray literature search was limited to the first 100 hits. If additional information was needed, efforts would have been made to contact the authors of the article. References were managed using the EndNote software (EndNote™ Thomson Reuters, Philadelphia, PA, USA). Duplicate hits were removed upon identification.

Search

The searches were conducted with the assistance of a senior librarian. The keywords and appropriate truncations used during the search were specific to Pubmed and Medline. The search strategy used for Pubmed was also used for Web of Science, Scopus and Google Scholar. The keywords and the number of hits found in each electronic search are presented in Table 1.

Study selection

Study selection was carried out in two phases. During the first selection phase, two authors (AONT, MCFA) independently reviewed the list of titles/abstracts for inclusion. Titles/abstracts that did not meet the eligibility criteria were excluded. Once potentially adequate abstracts were selected, full text articles were retrieved for a second selection process. During the second selection phase, the same reviewers (AONT, MCFA) independently evaluated full-texts, and those studies that met all eligibility criteria were included in the present systematic review. In both phases, disagreements between reviewers were resolved through discussion until achievement of a consensus. A third reviewer was involved when this attempt failed to make a final decision (MFMM).

Data collection process

Data collection was independently performed by two authors (AONT, MCFA). Discrepancies between authors were discussed until a consensus was reached. Again, a third party was involved in the process when necessary (MFMM). If any included article presented missing information, contact with authors would have been made.

Data items

Data on the following items were collected: author and date of publication, country where the study was conducted, study design, sample size, type of periapical lesion assessed, mean age, age range, type of experiment used in the included study and findings regarding the participation of Th17 and Treg cells in the progression of periapical lesions. Data items were organized in a standardized table.

Risk of bias in individual studies

Risk of bias in individual studies was evaluated according the Newcastle-Ottawa scale[15]. The scale scores ranged depending on the study design. For case control studies, a quality score was calculated based on group selection (four items), comparability between groups (one item), and outcome and exposure assessment (three items) categories. A maximum of 1 point was awarded for each item in the group selection and outcome and exposure assessment categories. A maximum of two points was awarded for comparability. Thus, the maximum score was nine points representing the highest methodological quality. A study was categorized as high quality if the total score was 7 or higher. For cross-sectionals studies, the score was calculated based on the same three categories. Nevertheless, those categories had a different number of items: group selection (two items), comparability (one item), and outcome and exposure assessment (two items). Therefore, the maximum score was 6 points and also denoted the highest methodological quality. A study was categorized as high quality if the total score was 4 or higher.

Summary measures

Any type of measurement (e.g.: *P* value) examining the participation of Th17 and Treg cells in the progression of periapical lesions was considered.

Synsthesis of results

Homogeneous and appropriate data for pooling would be combined in a meta-analysis. On the other hand, if data was heterogeneous, a qualitative summary of results would be carried out instead. Heterogeneity was evaluated by means of the assessment of the variability across included articles regarding study design, type of test used and type

of statistical measurement examining the role of Th17 and Treg cells in the progression of periapical lesions.

Risk of bias across studies

Bias regarding multiple publications was investigated in the present systematic review. The unit of analysis considered was the study and not the publication.

Results

Study selection

A total of 121 records were initially identified through the electronic databases. The updated search retrieved 18 records. Following the removal of 82 duplicates, 57 titles/abstracts were screened. Of the 57 titles/abstracts evaluated in phase 1, 39 did not meet the eligibility criteria and were excluded. In phase 2, the full text of 18 studies were retrieved and evaluated. Of the 18 full text articles read, only eight [16,17,18,19,20,21,22,23] met the eligibility criteria. A list of the 10 articles excluded in phase 2 and the reason for their exclusion is presented in Appendix 1. No additional study was identified in the reference lists of the included articles and in the grey literature search. A flowchart illustrating the selection of studies for this systematic review is depicted in Figure 2.

Study characteristics

Among the eight included articles, four were cross-sectional studies[18,19,21,22] and four were case-control studies[16,17,20,23]. All studies involved human periapical lesions fragments. Samples of six included articles[16,17,20,21,22,23] were obtained directly through

surgery[16,17,20,21,22,23] and samples of two studies were obtained through institutional archives that were collected from previous surgical procedures[18,19].

Four articles reported the mean age of participants[20,21,22,23] and five studies defined the range between 17 and 64 years[16,17,21,22,23]. The language of the articles was predominantly English, although the studies were conducted in different countries (Brazil, United States and Serbia). The method of investigation used in two studies was the ELISA method[20,22]. Real-time polymerase chain reaction (RT-PCR) was used in three studies[16,17,23], flow cytometry in two studies[21,22], confocal microscopy in one study[21] , and immunohistochemistry was used in four studies[18,19,20,22]. The entire description of studies' characteristics is shown in Table 2.

Risk of bias within studies

The quality assessment of the studies is shown in Appendix 2 and Appendix 3. The cross-sectional studies[18,19,21,22] received between two and four points. The case-controls studies[16,17,20,23] received between six and nine points.

Results of individual studies

Different factors were considered in the studies, demonstrating considerable diversity among the indices employed. The type of periapical lesion was analyzed based on histologic and radiographic characteristics to differentiate periapical granulomas[16,17,18,19,20,23], periapical cysts[16,23] and residual radicular cysts[18,19,20]. Two articles classified only as periapical lesion without differentiate in granuloma or periapical cysts[21,22]. A statistically significant association was found between Treg/Th17 markers and clinicopathologic features of chronic inflammatory periapical lesions in human tissues[16,17,18,19,20,21,22,23]. Those significant associations are described below.

Findings regarding the relation between Treg/Th17 in periapical lesions

All the studies that assessed the Treg/Th17 presence in periapical lesions, showed the influence of some immunological markers (FoxP3, IL-4, IL-9, IL-10, IL-17, IL-21, IL-22, TGF-ß, TNF-α, IFN-γ) in the modulation of the inflammatory response and progression of periapical lesions. The effects of this influence are showed in table 2.

The expression of *FOXP3* mRNA and Tregs function was observed in one study (18), *FOXP3* mRNA expression was positively correlated with IL-10 and TGF-β levels in inactive lesions. It is possible that such correlation represents a protective mechanism to avoid lesion progression and extensive bone destruction. Treg cells may exert their regulatory functions by controlling the immune and inflammatory processes seen in periapical lesions.

Similar methods of analysis were further tested in another study[19], and data demonstrated distinct patterns of cytokine expression in active and inactive periapical granulomas. In active lesions, pro-inflammatory Th1 and Th17-related mediators, along with IL-21, are supposed to independently drive lesion progression.

Peixoto et al. 2012[19], suggested that the participation of Treg cells in the modulation of the inflammatory response, associated with high levels of inflammatory mediators, contribute to the greater potential growth of these lesions. Similar results were observed by Campos *et al*., 2015[16]. FoxP3 acts as a master switch governing the progression and function of T-regulatory cells, whose functions include the inhibition of immune responses in periapical lesions. However, Marçal et al. 2010[20], showed that the production of IL-17 was associated with an exacerbation of the inflammatory response, an elevated number of neutrophils, and bone resorption in human tissue. The results using immunohistochemical analysis, showed a larger number of IL-17–positive cells in lesions

that had sinus tract. Significantly higher IL-17 levels were also observed by Colić et al. 2009[21]. The chronic periapical lesions might experience a reagudization process, with the increased presence of IL-17

Using confocal microscopy, flow cytometry and ELISA, Colić et al. 2009[22], showed, for the first time, the presence of $CD4^{+}CD25^{hi}Foxp3^{+}$ Tregs in periapical lesions that also express IL-10 and TGF-β, and are suppressive *in vitro*. They suggest that the progression of human periapical lesions is a dynamic process in which different inflammatory cells and their secreted products are involved. Furthermore, Fukada et al. 2009[23] demonstrated that the role of immune and osteoclastic cell activity in cysts and granulomas seems to be critically regulated by Treg/Th17. However, the mechanisms of periapical lesion progression still have not been fully understood.

Synthesis of results and risk of bias across studies

Although meta-analysis was planned, the high degree of heterogeneity across the included studies precluded the grouping of data for this type of analysis. The included studies were heterogeneous in terms of study design [case control studies[16,17,20,23] and cross-sectional studies[18,19,21,22]], method of molecular analysis used [ELISA method[20,22], RT-PCR[16,17,23], flow citometry[21,22], confocal microscopy[21] and immunohistochemistry[18,19,20,22]] and type of summary measures examining the presence of Th17 and Treg cells in the progression of periapical lesions [*p* value and r value[16,21], *p* value, mean and standard deviation[17,22], *p* value, r value, mean, standard error of mean and median[18], *p* value, median and interquartile range[19], *p* value, median, interquartile range and z value[20], *p* value[23]]. Moreover, the studies in which immunohistochemical analysis was used varied in terms of methods adopted [computerized program[20], three microscopic fields of each specimen[18] and one microscopic field of each specimen[19]].

Therefore, our intent was to carry out only a qualitative summary. The data extracted were organized in Table 2 to facilitate a narrative synthesis in this systematic review.

Risk of bias across studies

The objective of this systematic review was to identify studies and not publications. Nevertheless, multiple publications[21,22] using the same data pool were selected because they measured the presence of Th17 and Treg cells in the progression of periapical lesions using different strategies. One article used FoxP3 as the main target of the study[21], while the second focused in Th17 cells markers[22].

Discussion

Summary of evidence

This systematic review collected contributions from different studies enabling researchers to understand the key role of the Treg/Th17 in the progression of periapical lesions. Research on the pathogenesis of autoimmune and inflammatory diseases has made significant progress in the past few years and Th17 and Treg cells have emerged as major players in autoimmunity. Moreover, the present study allows investigators to be aware of the influence of different immunological markers, such as FoxP3 and IL-17 in such an outcome[24]. Treg and Th17 cells are both present in inflammatory sites, but they seem to have opposing roles in the progression of inflammatory periapical lesions. While Th17 cells stimulate the immune response, Treg cells have a regulatory activity and, therefore, play a very important role in the maintenance of tolerance and in the control of the expansion and activation of the immune system[25]. The information provided by the studies included in this systematic review may

be also relevant for clinical purposes. The Treg/Th17 relationship may be useful in the understanding of the progressive bone resorption related to periapical lesions[26].

Studies have demonstrated[16,17,18,19,21,23] that FoxP3 is a master switch governing the progression and function of Treg cells, whose functions include the inhibition of the immune responses. On the other hand, Th17 immune response seems to play a dominant role in exacerbating inflammation[22]. In addition, distinct markers, such as IL-1, IL-6 and TNF-α alongside TGF-β contribute to the production of IL-17[20]. On the other hand, the association of FoxP3, IL-10, IL-9, IL-4 and IL-22 contributes to lesions inactivity[17]. Thus, the participation of Treg and Th17 has been associated with the modulation of human periapical lesions and bone resorption.

Different methods of analysis (RT-PCR, immunohistochemistry analysis, confocal microscopy, flow cytometry, ELISA) were used to evaluate the role of the celular immune response in the progression of periapical lesions. Each of these approaches has both advantages and disadvantages[27]. Confocal microscopy displays cell images in high resolution, but the absolute fluorescence sensitivity is substantially lower than other techniques. Flow citometry, though, deteriorates imaging fully in favor of fluorescence sensitivity and the method is also strengthened by the rapid analysis of a great amount of cells[28]. RT-PCR is a quantitative method which enables researchers to identify many types of cytokines in small samples. However, the presence of RNA may reflect protein levels with low levels of accuracy[29]. ELISA, on the other hand, allows the researcher to detect cytokines at protein level. This method is also quantitative. While the afore-mentioned protocols cannot identify the cytokine-producing cell types, immunohistochemistry overcomes this shortcoming. The identification of those cells may be carried out over tissues that have not produced sufficient cytokines to be detected by

the other approaches. On the other hand, immunohistochemistry does not provide a quantitative analysis as precise as the other methods do.[27]

The role of Treg/Th17 response in inflammatory immune bone resorption has been explored in other diseases[30]. In Rheumatoid Arthritis (RA), studies described an excessive production of IL17 followed by bone and cartilage destruction. Moreover in RA, Treg cells in RA are not entirely efficient to control inflammation. A similar mechanism is found in periodontal disease[25,31]. With respect to the periapical lesion progression, clinical and experimental studies suggest that the balance between Treg and Th17 cells is critical to the outcomes of periapical lesions in terms of activity (lesion expansion), or inactivity (healing)[17,20]. The intensity of inflammatory infiltrate was found to be associated with a higher expression of FoxP3 and Th17 cells[18,19]. Higher IL-17 levels were also observed in cases of patients with sinus tract[20]. The chronic periapical lesions might experience a reagudization process, with the increased presence of IL-17. These data are shown in table 2.

Limitations

The lack of quantitative data for meta-analysis could be considered a drawback of the present systematic review. Meta-analysis is not feasible when the data present high levels of heterogeneity. This heterogeneity among the included studies emerged mainly due to the differences in study design, methods of molecular analysis and type of summary measures used to report the presence of Th17 and Treg cells in the progression of periapical lesions precluding any possibility of a pooled estimation of the results Meta-analysis would have allowed the authors to determine the strength of evidence more precisely[32,33,34]. Moreover, the literature has not recommended any tool for quality evaluation of in vitro studies yet[35]. Thus, the Newcastle Ottawa scale was adapted to assess the risk of bias within the included articles. Finally, even though the included case

control studies[16,17,20,23] were classified as high quality assessments, this study design along with the cross sectional design of the other four included articles[18,19,21,22] demand careful interpretation of their results[36]. Caution should also be taken in the interpretation of findings presented herein because the present systematic review focused on the FoxP3 and Il-17, which are considered reliable and specific biomarkers used to detect Treg and Th17. However, it has been recognized that other biomarkers are involved in this Treg/Th17 imbalance[16,17].

Suggestions for future research

In conclusion, this systematic review identified the presence of Treg and Th17 cells in the progression of periapical lesions. It also highlighted the relationship between the inhibitory characteristic of FoxP3 and the pro-inflammatory feature of IL-17 in the progression of periapical lesions. However, a significant number of the existing articles[37,38] addressing this issue were excluded from this systematic review because they used animal experiments in their methodology, which limits the extension of the results for the human immune response analysis. Few scientific reports with humans are available in research databases. Therefore, we suggest that further studies evaluating the influence of Treg / Th17 in the progression of human periapical lesions and bone resorption are conducted to create more comparable data allowing a quantitative analysis of the findings[39]. Additionally, strong future research should also be carried out to overcome the limitations of the included studies presented herein and to translate research into clinical practice[34].

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Figure and Table legends

Figure 1: Imbalance between Th17 and Treg lymphocytes, mediated by RORc and FoxP3.

Figure 2: A flowchart illustrating the selection of studies for this systematic review.

Table 1: Search strategy for each each electronic database.

Table 2: Summary of characteristics of the included studies.

Appendix 1: List of studies excluded following full text reading and reasons for the exclusion

Appendix 2: Quality assessment of included cross-sectional studies based on the Newcastle-Ottawa scale

Appendix 3: Quality assessment of included case control studies based on the Newcastle-Ottawa scale

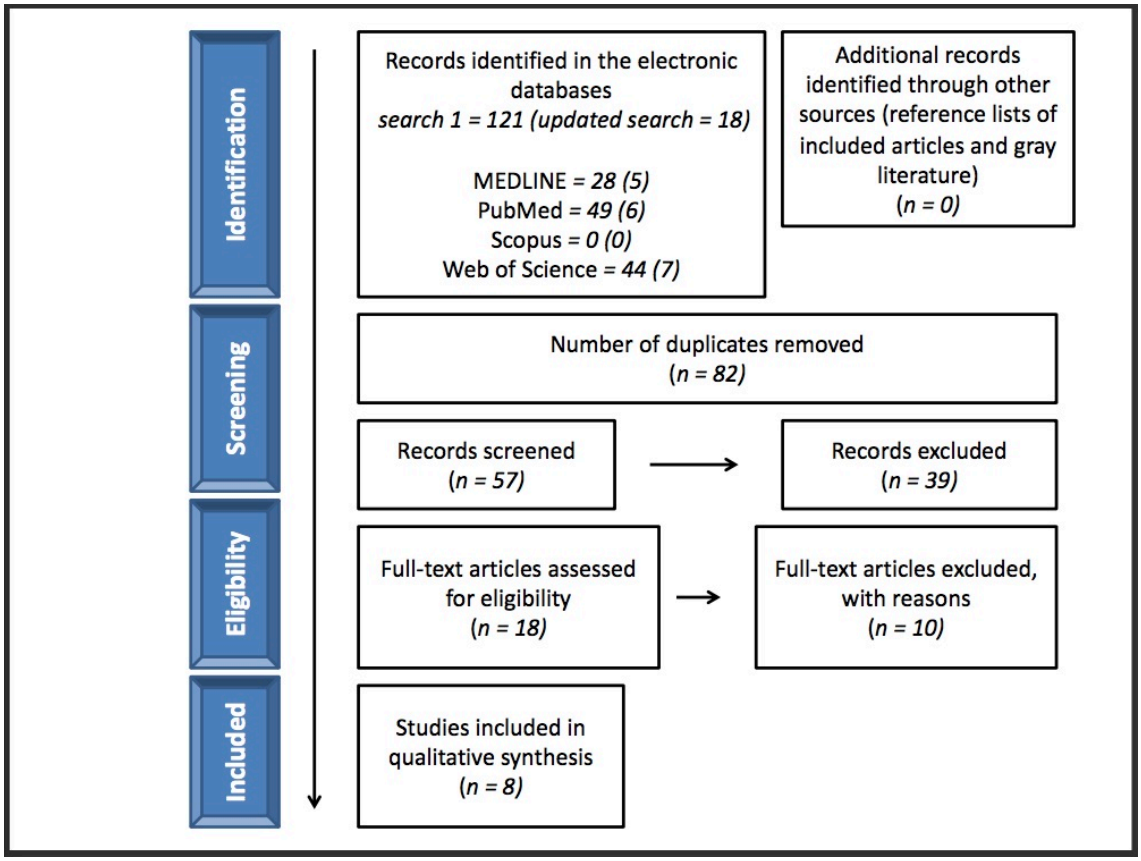

Figure 1: A flowchart illustrating the selection of studies for this systematic review.

Table 1: Search strategy for each each electronic database

Database	Keywords	Results Search 1*	Results Updated Search **
Pubmed	1 - periapical lesions OR periapical diseases (Mesh) OR radicular cyst (Mesh) OR radicular cysts OR periapical granuloma (Mesh) OR periapical granulomas 2 - t cells, T-lymphocytes (Mesh) OR T lymphocytes OR cd4-Positive T-lymphocytes (Mesh) OR T-Lymphocytes, Regulatory (Mesh) OR regulatory T cells OR regulatory cells OR T reg OR T reg cells OR Th17 cells (Mesh) OR Th17 OR FOXP3 protein, human (Mesh) OR FoxP3 OR forkhead box P3 OR Foxp3+ OR RORC protein, human (Mesh) OR RORγt OR RORγt OR RORγt OR ror gamma t OR ror gamma OR ror gammat 3 – 1 AND 2	49	06
Medline	1 - periapical diseases OR periapical lesions (mp) OR radicular cyst OR periapical granuloma 2 - T-Lymphocytes, Regulatory OR regulatory T cells (mp) OR Th17 Cells OR Th17 (mp) OR FOXP3 protein, human (mp) OR FoxP3 (mp) OR forkhead box P3 (mp) OR Foxp3+ (mp) OR ror gamma t (mp) OR ror gamma (mp) 3 – 1 AND 2	28	05
Scopus	same as Pubmed	00	00
Web of Science	same as Pubmed	44	07

*Up to the first week of October 2015
**From the second week of October 2015 to the first week of May 2017

				lesion	Age		analysis	Treg\Th17 in periapical lesions
Campos *et al*., 2015.	Brazil and United States	Case-control	Brazilian sample: 115 periapical lesions and 27 controls American sample: 35 periapical lesions and 6 controls	Brazilian sample Periapical granulomas: 115 American sample Periapical granuloma: 27 Periapical cysts: 8	Not reported.	Brazilian sample Cases: Participants aged between 19 and 59 years old Controls: Participants aged between 17 and 27 years old American sample Cases: Particpants aged between 18 and 53 years old Controls: Participants aged between 19 and 29 years old	Real-time polymerase chain reaction (RT-PCR).	FOXP3 mRNA levels were positive correlated with the expression of immunoregulatory molecules IL- (P < .001, r = 0.5183) and TGF-ß (P < .001, r = 0,4564) FOXP3 gene promoter showed the highest level of methylation in both periapical granulomas and apical c (P < .001) Results suggest that FoxP3 acts as master switch governing the development and function of T-regulatory cells, whose functions include the inhibition of immune responses and temper inflammatio periapical lesions.
Araujo-Pires *et al*., 2014.	Brazil	Case-control	110 periapical lesions and 26 controls	Periapical granulomas: 110	Not reported.	Cases: Participants aged between 19 and 59 years old Controls: Participants aged between 19 and 24 years old	Real-time polymerase chain reaction. (RT-PCR).	*P<.05 (unpaired t-test) between controls and periapical lesions Analysis demonstrates the associa of TNF-α, IL-21, IL-17 and IFN-γ w periapical lesions activity, and the association of FOXP3, IL-10,IL-9, I and IL-22 with periapical lesions inactivity.
Andrade *et al*., 2013.	Brazil	Cross Sectional	60 periapical lesions	Periapical granulomas: 20 Radicular cysts: 20 Residual radicular cysts: 20	Not reported.	Not reported	Immunohistoche mical analysis	Periapical lesions with inflammator infiltrate grade III exhibited a highe number of FoxP3+ cells (P = .002).Lesions with inflammatory infi grade I showed a tendency for a lo

								expression of IL-17 and TGF- ß 1 (.085 and P = .051, respectively). Th17 and Treg cells participate in t process, exerting opposite modula effects in periapical lesions.
Peixoto *et al*., 2012.	Brazil	Cross Sectional	60 periapical lesions	Periapical granulomas: 30 Radicular cysts: 30	Not reported.	Not reported	Immunohistoche mical analysis	Comparison of the median number FoxP3+ cells in relation to the inter of the inflammatory infiltrate reveal no statistically significant difference between groups (P = .465) The present results suggest a grea participation of regulatory T cells in modulation of the inflammatory response in periapical granuloma.
Marçal *et al*.,2010.	Brazil	Case-control	50 periapical lesions and 5 controls	Periapical granulomas: 25 Radicular cysts: 25	Cases: 34.8 years Controls: Not reported	Not reported	Immunohistoche mical analysis	Positive correlation between FoxP3 TGF-ß was observed in periapical lesions (Spearman correlation: P = .018, z = 3.12). Significantly higher IL-17 levels we also observed in cases of patients sinus tract. The chronic periapical lesions might experience a reagudization process, with the increased presence of IL-17.
Colić *et al*., 2009.	Serbia	Cross Sectional	28 periapical lesions	Not reported.	38.8 years	Participants aged between 18 and 64 years old	Confocal microscopy Flow cytometry ELISA	p < .05 compared with correspondi control. These findings suggest that $CD4^{+}CD25^{hi}Foxp3^{+}$ cells in periapi lesions may play regulatory roles in controlling local immune/inflammat processes.
Colić *et al*., 2009.	Serbia	Cross Sectional	96 periapical lesions	Not reported	34 years	Participants aged between 18 and 62 years old	Flow cytometry Immunohistoche mical analysis	p < .005 compared to correspondir control groups (ANOVA). Symptomatic lesions are character by high production of IL-17, positiv

							ELISA	correlation between IL-17 and IFN- periapical lesions. Th17 immune response seems to play a dominar role in exacerbating inflammation.
Fukada *et al*., 2009.	Brazil	Case-control	38 sample: (30 periapical lesions and 8 controls)	Periapical cysts: 10 Granulomas: 20	45 yr	Participants aged between 32 and 62 years old	Real-time polymerase chain reaction (RT-PCR).	p < .05 compared to controls, p <0 comparing two lesion types. Greater expression of FoxP3 was s in periapical granulomas. The concomitant expression of Treg ce markers suggests a possible suppression of the Th1 response ir granulomas.

Table 2: Summary of characteristics of the included studies

Appendix 1: List of studies excluded following full text reading and reasons for the exclusion

1 - He M, Song G, Yu Y, Jin Q, Bian Z.LPS-miR-34a-CCL22 axis contributes to regulatory T cell recruitment in periapical lesions . Biochem Biophys Res Commun. 2015; 460(3):733-40.

Reason for exclusion: Study using animal samples.

2 - Velickovic M, Pejnovic N, Mitrovic S, Radosavljevic G, Jovanovic I, Kanjevac T, Jovicic N, Lukic A. ST2 deletion increases inflammatory bone destruction in experimentally indeced periapical lesions in mice. .J Endod. 2015 Mar; 41(3):369-75.

Reason for exclusion: Study using animal samples.

3 - Xiao L, Zhu L, Yang S, Lei D, Xiao Y, Peng B. Different correlation of sphingosine-1- phosphate receptor 1 with receptor activator of nuclear factor kappa Bligand and regulatory T cells in rat periapical lesions. J Endod. 2015; 41(4):479-86.

Reason for exclusion: Study using animal samples.

4 - Wei S, Kawashima N, Suzuki N, Xu J, Takahashi S, Zhou M, Koizumi Y, Suda H.Kinetics of Th17 related cytokine expression in experimentally induced rat periapical lesions. Aust Endod J. 2013; 39(3):164-70.

Reason for exclusion: Study using animal samples.

5 - AlShwaimi E, Berggreen E, Furusho H, Rossall JC, Dobeck J, Yoganathan S, Stashenko P, Sasaki H. IL-17 receptor A signaling is protective in infection-stimulated periapical bone destruction. J Immunol. 2013; 191(4):1785-91.

Reason for exclusion: Study using animal samples.

6 - AlShwaimi E, Purcell P, Kawai T, Sasaki H, Oukka M, Campos-Neto A, Stashenko P.Regulatory T cells in mouse periapical lesions. J Endod. 2009; 35(9):1229-33.

Reason for exclusion: Study using animal samples.

7 - Xiong H, Wei L, Peng B. Immunohistochemical localization of IL-17 in induced rat periapical lesions. J Endod. 2009; 35(2):216-20.

Reason for exclusion: Study using animal samples.

8 - Yang S, Zhu L, Xiao L, Shen Y, Wang L, Peng B, Haapasalo M. Imbalance of interleukin-17+ T-cell and Foxp3+ regulatory T cell dynamics in rat periapical lesions.J Endod. 2014; 40(1):56-62.

Reason for exclusion: Study using animal samples.

9 - Ferreira LG, Rosin FC, Corrêa L. Analysis of Interleukin 17A in periapical abscess and granuloma lesions. BOR. 2016; vol30.0034.

Reason for exclusion: Not reviewed FoxP3.

10 - Campos K; Franscisconi CF; Okehie V; de Souza LC; Trombone AP; Letra A; Garlet GP; Gomez RS; Silva RM. 4. FOXP3 DNA methylation levels as a potential biomarker in the development of periapical lesions.
J Endod. 2015; 41(2):212-8.

Reason for exclusion: Not reviewed Th17 cells.

Appendix 2: Quality assessment of included cross-sectional studies based on the Newcastle-Ottawa scale

Author	Selection*		Comparability**	Outcome***		Score****
	Definition of test[1]	Representativeness and selection of individuals with periapical lesions[2]	Control for confounders[3]	Diagnosis of periapical lesions[4]	Response rate[5]	
Andrade *et al*., 2013.	✯	✯				2
Peixoto *et al*., 2012.	✯	✯		✯		3
Colić *et al*., 2009.	✯	✯				2
Colić *et al*., 2009.	✯	✯				2

*a maximum of 1 point for each item; **a maximum of 2 points for each item; ***a maximum of 1 point for each item
****a maximum of 6 points
✯ 1 point
[1] a) secure record (eg Real-time polymerase chain reaction, Immunohistochemical analysis, Confocal microscopy ,Flow cytometry , ELISA) ✯, b) written self report or medical record only, c) no description
[2] a) individuals with periapical lesion in a defined catchment area or community, random sample, sample calculation ✯, b) not satisfying requirements in part (a) fully, c) not stated
[3] a) study control for periapical lesion ✯, b) study control for 2 or more confounding variables ✯✯
[4] a) periapical lesions diagnosis was performed based on histopathological and radiographic analysis ✯, b) based on self reports or not satisfying requirements in part (a) fully, c) no description
[5] a) rate of sample loss ≤20% ✯, b) rate of sample loss > 20%, c) not stated

Appendix 3: Quality assessment of included case control studies based on the Newcastle-Ottawa scale

Author	Selection*				Comparability**		Outcome***		Score****
	Is the case definition adequate?[1]	Representativeness of the cases[2]	Selection of Controls [3]	Definition of Controls [4]	Comparability of case controls on the basis of the design or analysis[5]	Assessment of exposure[6]	Same method of ascertainment for cases and controls [7]	Non-Response rate [8]	
Campos *et al*., 2015.	✯	✯	✯	✯	✯	✯	✯	✯	8
Araujo-Pires *et al*., 2014.	✯	✯	✯	✯	✯	✯	✯	✯	8
Marçal *et al*.,2010.	✯	✯		✯	✯	✯	✯	✯	7
Fukada *et al*., 2009.	✯	✯	✯	✯	✯	✯	✯	✯	8

*a maximum of 1 point for each item; **a maximum of 2 points for each item; ***a maximum of 1 point for each item
****a maximum of 9 points
✯ 1 point
[1] a) yes, with independent validation ✯, b) yes, eg record linkage or based on self reports, c) no description
[2] a) consecutive or obviously representative series of cases ✯, b) potential for selection biases or not stated
[3] a) community controls ✯, b) hospital controls, c) no complete description
[4] a) no history of disease (endpoint) ✯, b) no description of source
[5] a) study control for periapical lesion ✯, b) study control for 2 or more confounding variables ✯✯
[6] a) secure record (eg Real-time polymerase chain reaction, Immunohistochemical analysis, Confocal microscopy ,Flow cytometry , ELISA) ✯, b) written self report or medical record only, c) no description
[7] a) yes ✯, b) no
[8] a) same rate for both groups ✯, b) non respondents described, c) rate different and no designation

Considerações Finais

Considerações Finais

Como consideração final da pesquisa, nosso trabalho através da análise da participação de citocinas e quimiocinas na lesão periapical, identificou a presença e atuação de células Treg e Th17 nessas lesões. Também destacamos através de uma revisão sistemática, a relação entre a característica inibidora da FoxP3 (PEIXOTO *et al.,* 2012; ANDRADE *et al.,* 2013; CAMPOS *et al.,* 2015), e a característica pró-inflamatória da IL-17 (COLIĆ *et al.,* 2009; MARÇAL *et al.,* 2010), nos tecidos periapicais humanos. O papel de Treg / Th17 tem sido descrito na literatura recente com o enfoque em um equilíbrio antagônico entre as características imunossupressoras de Treg e a capacidade pró-inflamatória de Th17 (NOACK *et al.,* 2014). Porém nosso estudo além de uma análise minuciosa da literatura realizada através da revisão sistemática, demonstrou através de testes laboratoriais em amostras humanas que citocinas como IL-17, IL-10, TGF-β, CCL4 e CCL20 influenciam diretamente a atuação das células Treg e Th17, e são expressas simultaneamente nas lesões periapicais inflamatórias, mediando uma resposta persistente do sistema imune frente a agressão crônica oriunda de bactérias intracanais e seus subprodutos.

Portanto, mediadores imunossupressores e pró-inflamatórios se apresentam de maneira simultânea e aumentada quando há presença de lesão periapical quando comparados aos tecidos periapicais de dentes hígidos e dentes com necrose pulpar sem o desenvolvimento de lesão. Este resultado sugere que a resposta Treg/Th17 nas lesões periapicais crônicas ocorre como uma relação de co-estimulação. Se este equilíbrio é responsável pela resistência de algumas lesões aos tratamentos convencionais, é algo que permanece para ser pesquisado.

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Apêndices

Apêndice 1

UNIVERSIDADE FEDERAL DE MINAS GERAIS
COMITÊ DE ÉTICA EM PESQUISA - COEP

Projeto: CAAE – 39898314.1.0000.5149

Interessado(a): Profa. Maria Cássia Ferreira de Aguiar
Departamento de Clínica, Patologia e Cirurgia Odontológicas
Faculdade de Odontologia - UFMG

DECISÃO

O Comitê de Ética em Pesquisa da UFMG – COEP aprovou, no dia 10 de abril de 2015, o projeto de pesquisa intitulado **"Participação de linfócitos Th17 e Treg no desenvolvimento das lesões periapicais inflamatórias crônicas"** bem como o Termo de Consentimento Livre e Esclarecido.

O relatório final ou parcial deverá ser encaminhado ao COEP um ano após o início do projeto através da Plataforma Brasil.

Telma Campos Medeiros Lorentz

Profa. Dra. Telma Campos Medeiros Lorentz
Coordenadora do COEP-UFMG

Av. Pres. Antonio Carlos, 6627 – Unidade Administrativa II - 2º andar – Sala 2005 – Cep:31270-901 – BH-MG
Telefax: (031) 3409-4592 - e-mail: coep@prpq.ufmg.br

Apêndice 2

FICHA CLÍNICA

NOME:		NASC.:	
R.G.:		C.P.F.:	SEXO:
ENDEREÇO:			
BAIRRO:	CEP	CIDADE	
TEL. RES.:		PROFISSÃO:	
TEL. CEL:		TEL.COM.:	
RESPONSÁVEL (MENORES 18):		PARENTESCO:	

	SIM / NÃO		**ESPECIFICAÇÕES**	
ESTÁ SOB CUIDADOS MÉDICOS			QUAL(IS)?	
TOMA ALGUM MEDICAMENTO			QUAL(IS)?	
JÁ SOFREU CIRURGIAS/EXTRAÇÕES			QUAL(IS)?	
JÁ FOI ANESTESIADO(A)			HOUVERAM PROBLEMAS?	
SOFRE(U) DE DOENÇA SEVERA			QUAL(IS)?	
TEM ALERGIA / ASMA			A QUE?	
TEM PROBLEMAS CARDÍACOS			QUAL(IS)?	
USA MARCAPASSO			DESDE?	
TEM PRESSÃO ALTA			CONTROLADA?	
TEM DIABETES			TIPO:	DESDE:
JÁ TEVE HEPATITE			TIPO:	
É PORTADOR DO VÍRUS HIV			DESDE:	
FUMA REGULARMENTE			O QUE?	FREQUÊNCIA:
CONSOME BEBIDAS ALCOÓLICAS			QUAIS?	FREQUÊNCIA:
USA ALGUM TIPO DE DROGA (MESMO QUE OCASIONALMENTE)			QUAL?	FREQUÊNCIA:
MULHERES — ESTÁ GRÁVIDA			MÊS:	
MULHERES — USA ANTICONCEPCIONAIS			QUAL?	DESDE:

ELEMENTOS DENTAIS:					
EXAME SUBJETIVO					
A – DOR PROVOCADA:			**B – DOR ESPONTÂNEA**		
( ) INTERMINENTE	( ) CONTÍNUA	( ) CESSA COM ANALGÉSICO	( ) _____		
EXAME OBJETIVO					
1 – INSPEÇÃO	( ) HÍGIDO	( ) RESTAURADO	( ) PRÓTESE	( ) EXPOSIÇÃO	
( ) CÁRIE	( ) EDEMA	( ) FÍSTULA	( ) _____		
2 – PERCUSSÃO	**A - VERTICAL**		**B - HORIZONTAL**		
	( ) AUSÊNCIA	( ) SENSIBILIDADE	( ) DOR		
3 – FRIO	( ) INDOLOR	( ) SENSÍVEL	( ) DOR	( ) CESSA RÁPIDO	( ) PROLONGADA
4 – PALPAÇÃO	( ) INDOLOR	( ) SENSIBILIDADE	( ) DOR	( ) EDEMA	
EXAMES COMPLEMENTARES					
1 – EXAME RADIOGRÁFICO	( ) LESÃO PERIAPICAL	( ) ESPESSAMENTO PERIODONTAL			
( ) CÁRIE	( ) PERDA DA LÂMINA DURA	( ) _____	( ) _____		
DIAGNÓSTICO					
( ) POLPA NORMAL	( ) PULPITE REVERSÍVEL	( ) PULPITE IRREVERSÍVEL	( ) NECROSE		
( ) PERICEMENTITE	( ) ABSCESSO_____	( ) OUTRO_____			

Belo Horizonte, _____ de _____ de 200__.

_____ _____

Assinatura do Paciente ou responsável Assinatura do Pesquisador

Apêndice 3

UNIVERSIDADE FEDERAL DE MINAS GERAIS – FACULDADE DE ODONTOLOGIA

COMITÊ DE ÉTICA EM PESQUISA

TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO

Você está sendo convidado a participar de uma pesquisa com o título "**Participação de citocinas relacionadas às respostas Th17/Treg em lesões periapicais inflamatórias crônicas.**". O objetivo deste trabalho é estudar a resposta Treg/Th17 no desenvolvimento da doença periapical inflamatória crônica através da avaliação dos seus componentes principais, IL17, IL-10, TGFβ, CCL4 e CCL20. Não há nenhum risco para a sua saúde em fazer o exame. Mas se for encontrada alguma alteração bucal, você será informado(a) e poderá ser encaminhado(a) para tratamento nas clínicas da Faculdade de Odontologia-UFMG. Não haverá despesas para você ao participar da pesquisa. Você tem inteira liberdade para retirar o seu consentimento a qualquer tempo, sem nenhum prejuízo. Os dados serão coletados apenas para esta pesquisa e os resultados serão tornados públicos a partir da defesa da tese de doutorado. Deve ficar claro que a sua **identidade será preservada.**

Faculdade de Odontologia/UFMG, Av. Antônio Carlos, 6627, Pampulha, Belo Horizonte-MG, Tel: 3409-2424. COEP - Comitê de Ética em Pesquisa. Av. Antônio Carlos, 6627, Administrativa II - 2º andar, Campus Pampulha, CEP 31270-901. Tel: 3409-4592

AUTORIZAÇÃO

Eu, _____, aceito em participar da pesquisa intitulada provisoriamente "**Participação de citocinas relacionadas às respostas Th17/Treg em lesões periapicais inflamatórias crônicas.**". Declaro ter sido informado dos riscos e benefícios da pesquisa. Minha participação reflete meu interesse em colaborar para o desenvolvimento do projeto, tendo sido a mim facultada a possibilidade de aceitar ou não participar desse projeto de pesquisa, podendo retirar o meu consentimento a qualquer momento. Fui devidamente esclarecido (a) que os dados serão examinados e publicados pela equipe de pesquisadores, sendo preservada minha identidade.

Belo Horizonte, _____ de _____ de 200__.

_____ _____

Assinatura do Paciente ou responsável Assinatura do Pesquisador

Apêndice 4

PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	

PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	

From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(6): e1000097. doi:10.1371/journal.pmed1000097

For more information, visit: www.prisma-statement.org.

Apêndice 5

Guidelines for Publishing Papers in the JOE

Writing an effective article is a challenging assignment. The following guidelines are provided to assist authors in submitting manuscripts.

The *JOE* publishes original and review articles related to the scientific and applied aspects of endodontics. Moreover, the *JOE* has a diverse readership that includes full-time clinicians, full-time academicians, residents, students and scientists. Effective communication with this diverse readership requires careful attention to writing style.
General Points on Composition
Organization of Original Research Manuscripts
Manuscripts Category Classifications and Requirements
Available Resources

1. **General Points on Composition**
    1. Authors are strongly encouraged to analyze their final draft with both software (*e.g.*, spelling and grammar programs) and colleagues who have expertise in English grammar. References listed at the end of this section provide a more extensive review of rules of English grammar and guidelines for writing a scientific article. Always remember that clarity is the most important feature of scientific writing. Scientific articles must be clear and precise in their content and concise in their delivery since their purpose is to inform the reader. The Editor reserves the right to edit all manuscripts or to reject those manuscripts that lack clarity or precision, or have unacceptable grammar or syntax. The following list represents common errors in manuscripts submitted to the *JOE*:
    2. The paragraph is the ideal unit of organization. Paragraphs typically start with an introductory sentence that is followed by sentences that describe additional detail or examples. The last sentence of the paragraph provides conclusions and forms a transition to the next paragraph. Common problems include one-sentence paragraphs, sentences that do not develop the theme of the paragraph (see also section “c” below), or sentences with little to no transition within a paragraph.
    3. Keep to the point. The subject of the sentence should support the subject of the paragraph. For example, the introduction of authors’ names in a sentence changes the subject and lengthens the text. In a paragraph on sodium hypochlorite, the sentence, “In 1983, Langeland et al., reported that sodium hypochlorite acts as a lubricating factor during instrumentation and helps to flush debris from the root canals” can be edited to: “Sodium hypochlorite acts as a lubricant during instrumentation and as a vehicle for flushing the generated debris (Langeland et al., 1983)." In this example, the paragraph’s subject is sodium hypochlorite and sentences should focus on this subject.
    4. Sentences are stronger when written in the active voice, *i.e.*, the subject performs the action. Passive sentences are identified by the use of passive verbs such as “was,” “were,” “could,” etc. For example: “Dexamethasone was found in this study to be a factor that was associated with reduced inflammation,” can be edited to: “Our results demonstrated that dexamethasone reduced inflammation.” Sentences written in a direct and active voice are generally more powerful and shorter than sentences written in the passive voice.
    5. Reduce verbiage. Short sentences are easier to understand. The inclusion of unnecessary words is often associated with the use of a passive voice, a lack of focus or run-on sentences. This is not to imply that all sentences need be short or even the same length. Indeed, variation in sentence structure and length often helps to maintain reader interest. However, make all words count. A more formal way of stating this point is that the use of subordinate clauses adds variety and information when constructing a paragraph. (This section was written deliberately with sentences of varying length to illustrate this point.)
    6. Use parallel construction to express related ideas. For example, the sentence, “Formerly, endodontics was taught by hand instrumentation, while now rotary instrumentation is the common method,” can be edited to “Formerly, endodontics was taught using hand instrumentation; now it is commonly taught using rotary instrumentation.” The use of parallel construction in sentences simply means that similar ideas are expressed in similar ways, and this helps the reader recognize that the ideas are related.
    7. Keep modifying phrases close to the word that they modify. This is a common problem in complex sentences that may confuse the reader. For example, the statement, “Accordingly, when conclusions are drawn from the results of

this study, caution must be used," can be edited to "Caution must be used when conclusions are drawn from the results of this study."

8. To summarize these points, effective sentences are clear and precise, and often are short, simple and focused on one key point that supports the paragraph's theme.

9. Authors should be aware that the *JOE* uses iThenticate, plagiarism detection software, to assure originality and integrity of material published in the *Journal*. The use of copied sentences, even when present within quotation marks, is highly discouraged. Instead, the information of the original research should be expressed by new manuscript author's own words, and a proper citation given at the end of the sentence. Plagiarism will not be tolerated and manuscripts will be rejected, or papers withdrawn after publication based on unethical actions by the authors. In addition, authors may be sanctioned for future publication.

2. **Organization of Original Research Manuscripts**

Please Note: *All abstracts should be organized into sections that start with a one-word title (in bold),* i.e.*, Introduction, Methods, Results, Conclusions, etc., and should not exceed more than 250 words in length.*

1. **Title Page:** The title should describe the major emphasis of the paper. It should be as short as possible without loss of clarity. Remember that the title is your advertising billboard—it represents your major opportunity to solicit readers to spend the time to read your paper. It is best not to use abbreviations in the title since this may lead to imprecise coding by electronic citation programs such as PubMed (*e.g.*, use "sodium hypochlorite" rather than NaOCl). The author list must conform to published standards on authorship (see authorship criteria in the Uniform Requirements for Manuscripts Submitted to Biomedical Journals at *www.icmje.org*). The manuscript title, name and address (including email) of one author designated as the corresponding author. This author will be responsible for editing proofs and ordering reprints when applicable. The contribution of each author should also be highlighted in the cover letter.

2. **Abstract:** The abstract should concisely describe the purpose of the study, the hypothesis, methods, major findings and conclusions. The abstract should describe the new contributions made by this study. The word limitations (250 words) and the wide distribution of the abstract (*e.g.*, PubMed) make this section challenging to write clearly. This section often is written last by many authors since they can draw on the rest of the manuscript. Write the abstract in past tense since the study has been completed. Three to ten keywords should be listed below the abstract.

3. **Introduction:** The introduction should briefly review the pertinent literature in order to identify the gap in knowledge that the study is intended to address and the limitations of previous studies in the area. The purpose of the study, the tested hypothesis and its scope should be clearly described. Authors should realize that this section of the paper is their primary opportunity to establish communication with the diverse readership of the *JOE*. Readers who are not expert in the topic of the manuscript are likely to skip the paper if the introduction fails to succinctly summarize the gap in knowledge that the study addresses. It is important to note that many successful manuscripts require no more than a few paragraphs to accomplish these goals. Therefore, authors should refrain from performing extensive review or the literature, and discussing the results of the study in this section.

4. **Materials and Methods:** The objective of the materials and methods section is to permit other investigators to repeat your experiments. The four components to this section are the detailed description of the materials used and their components, the experimental design, the procedures employed, and the statistical tests used to analyze the results. The vast majority of manuscripts should cite prior studies using similar methods and succinctly describe the essential aspects used in the present study. Thus, the reader should still be able to understand the method used in the experimental approach and concentration of the main reagents (*e.g.*, antibodies, drugs, etc.) even when citing a previously published method. The inclusion of a "methods figure" will be rejected unless the procedure is novel and requires an illustration for comprehension. If the method is novel, then the authors should carefully describe the method and include validation experiments. If the study utilized a **commercial product**, the manuscript must state that they either followed manufacturer's protocol *or* specify any changes made to the protocol. If the study used an***in vitro*** **model** to simulate a clinical outcome, the authors must describe experiments made to validate the model, or previous literature that proved the clinical relevance of the model. Studies on **humans** must conform to the Helsinki Declaration of 1975 and state that the institutional IRB/equivalent committee(s) approved the protocol and that informed consent was obtained after the risks and benefits of participation were described to the subjects or patients recruited. Studies involving **animals** must state that the institutional animal care and use committee approved the protocol. The statistical analysis section should describe which tests were used to analyze which

dependent measures; p-values should be specified. Additional details may include randomization scheme, stratification (if any), power analysis as a basis for sample size computation, drop-outs from clinical trials, the effects of important confounding variables, and bivariate versus multivariate analysis.

5. **Results:** Only experimental results are appropriate in this section (*i.e.*, neither methods, discussion, nor conclusions should be in this section). Include only those data that are critical for the study, as defined by the aim(s). Do not include all available data without justification; any repetitive findings will be rejected from publication. All Figures, Charts and Tables should be described in their order of numbering with a brief description of the major findings. Author may consider the use of supplemental figures, tables or video clips that will be published online. Supplemental material is often used to provide additional information or control experiments that support the results section (*e.g.*, microarray data).
6. **Figures:** There are two general types of figures. The first type of figures includes photographs, radiographs or micrographs. Include only essential figures, and even if essential, the use of composite figures containing several panels of photographs is encouraged. For example, most photo-, radio- or micrographs take up one column-width, or about 185 mm wide X 185 mm tall. If instead, you construct a two columns-width figure (*i.e.*, about 175 mm wide X 125 mm high when published in the *JOE*), you would be able to place about 12 panels of photomicrographs (or radiographs, etc.) as an array of four columns across and three rows down (with each panel about 40 X 40 mm). This will require some editing to emphasize the most important feature of each photomicrograph, but it greatly increases the total number of illustrations that you can present in your paper. Remember that each panel must be clearly identified with a letter (*e.g.*, "A," "B," etc.), in order for the reader to understand each individual panel. Several nice examples of composite figures are seen in recent articles by Jeger et al (J Endod 2012;38:884–888); Olivieri et al., (J Endod 2012;38:1007 1011); Tsai et al (J Endod 2012;38:965–970). Please note that color figures may be published at no cost to the authors and authors are encouraged to use color to enhance the value of the illustration. Please note that a multipanel, composite figure only counts as one figure when considering the total number of figures in a manuscript (see section 3, below, for maximum number of allowable figures).

   The second type of figures are graphs (*i.e.*, line drawings including bar graphs) that plot a dependent measure (on the Y axis) as a function of an independent measure (usually plotted on the X axis). Examples include a graph depicting pain scores over time, etc. Graphs should be used when the overall trend of the results are more important than the exact numerical values of the results. For example, a graph is a convenient way of reporting that an ibuprofen-treated group reported less pain than a placebo group over the first 24 hours, but was the same as the placebo group for the next 96 hours. In this case, the trend of the results is the primary finding; the actual pain scores are not as critical as the relative differences between the NSAID and placebo groups.
7. **Tables:** Tables are appropriate when it is critical to present exact numerical values. However, not all results need be placed in either a table or figure. Instead, the results could simply state that there was no inhibition of growth from 0.001-0.03% NaOCl, and a 100% inhibition of growth from 0.03-3% NaOCl (N=5/group). Similarly, if the results are not significant, then it is probably not necessary to include the results in either a table or as a figure. These and many other suggestions on figure and table construction are described in additional detail in Day (1998).
8. **Discussion:** This section should be used to interpret and explain the results. Both the strengths and weaknesses of the observations should be discussed. How do these findings compare to the published literature? What are the clinical implications? Although this last section might be tentative given the nature of a particular study, the authors should realize that even preliminary clinical implications might have value for the clinical readership. Ideally, a review of the potential clinical significance is the last section of the discussion. What are the major conclusions of the study? How does the data support these conclusions
9. **Acknowledgments:** All authors must affirm that they have no financial affiliation (*e.g.*, employment, direct payment, stock holdings, retainers, consultantships, patent licensing arrangements or honoraria), or involvement with any commercial organization with direct financial interest in the subject or materials discussed in this manuscript, nor have any such arrangements existed in the past three years. Any other potential conflict of interest should be disclosed. Any author for whom this statement is not true must append a paragraph to the manuscript that fully discloses any financial or other interest that poses a conflict. Likewise the sources and correct attributions of all other grants, contracts or donations that funded the study must be disclosed

10. **References:** The reference style follows Index Medicus and can be easily learned from reading past issues of the *JOE*. The *JOE* uses the Vancouver reference style, which can be found in most citation management software products. Citations are placed in parentheses at the end of a sentence or at the end of a clause that requires a literature citation. Do not use superscript for references. Original reports are limited to 35 references. There are no limits in the number of references for review articles.

3. **Manuscripts Category Classifications and Requirements**

Manuscripts submitted to the *JOE* must fall into one of the following categories. The abstracts for all these categories would have a maximum word count of 250 words:

1. CONSORT Randomized Clinical Trial-Manuscripts in this category must strictly adhere to the Consolidated Standards of Reporting Trials-CONSORT- minimum guidelines for the publication of randomized clinical trials. These guidelines can be found at *www.consort-statement.org/*. These manuscripts have a limit of 3,500 words, [including abstract, introduction, materials and methods, results, discussion and acknowledgments; excluding figure legends and references]. In addition, there is a limit of a total of 4 figures and 4 tables*.
2. Review Article-Manuscripts in this category are either narrative articles, or systematic reviews/meta-analyses. Case report/Clinical Technique articles even when followed by extensive review of the literature will should be categorized as "Case Report/Clinical Technique". These manuscripts have a limit of 3,500 words, [including abstract, introduction, discussion and acknowledgments; excluding figure legends and references]. In addition, there is a limit of a total of 4 figures and 4 tables*.
3. Clinical Research (*e.g.*, prospective or retrospective studies on patients or patient records, or research on biopsies, excluding the use of human teeth for technique studies). These manuscripts have a limit of 3,500 words [including abstract, introduction, materials and methods, results, discussion and acknowledgments; excluding figure legends and references]. In addition, there is a limit of a total of 4 figures and 4 tables*.
4. Basic Research Biology (animal or culture studies on biological research on physiology, development, stem cell differentiation, inflammation or pathology). Manuscripts that have a primary focus on biology should be submitted in this category while manuscripts that have a primary focus on materials should be submitted in the Basic Research Technology category. For example, a study on cytotoxicity of a material should be submitted in the Basic Research Technology category, even if it was performed in animals with histological analyses. These manuscripts have a limit of 2,500 words [including abstract, introduction, materials and methods, results, discussion and acknowledgments; excluding figure legends and references]. In addition, there is a limit of a total of 4 figures or 4 tables*.
5. Basic Research Technology (Manuscripts submitted in this category focus primarily on research related to techniques and materials used, or with potential clinical use, in endodontics). These manuscripts have a limit of 2,500 words [including abstract, introduction, materials and methods, results, discussion and acknowledgments; excluding figure legends and references]. In addition, there is a limit of a total of 3 figures and tables *.
6. Case Report/Clinical Technique (*e.g.*, report of an unusual clinical case or the use of cutting-edge technology in a clinical case). These manuscripts have a limit of 2,500 words [including abstract, introduction, materials and methods, results, discussion and acknowledgments; excluding figure legends and references]. In addition, there is a limit of a total of 4 figures or tables*.

* Figures, if submitted as multipanel figures must not exceed 1 page length. Manuscripts submitted with more than the allowed number of figures or tables will require approval of the *JOE* Editor or associate editors. If you are not sure whether your manuscript falls within one of the categories above, or would like to request preapproval for submission of additional figures please contact the Editor by email at*jendodontics@uthscsa.edu*.

Importantly, adhering to the general writing methods described in these guidelines (and in the resources listed below) will help to reduce the size of the manuscript while maintaining its focus and significance. Authors are encouraged to focus on only the essential aspects of the study and to avoid inclusion of extraneous text and figures. The Editor may reject manuscripts that exceed these limitations.

Available Resources:

Strunk W, White EB. The Elements of Style. Allyn & Bacon, 4th ed, 2000, ISBN 020530902X.
Day R. How to Write and Publish a Scientific Paper. Oryx Press, 5th ed. 1998. ISBN 1-57356-164-9.
Woods G. English Grammar for Dummies. Hungry Minds:NY, 2001 (an entertaining review of grammar).
Alley M. The Craft of Scientific Writing. Springer, 3rd edition 1996 SBN 0-387-94766-3.
Alley M. The Craft of Editing. Springer, 2000 SBN 0-387-98964-1.

Apêndice 6

INSTRUCTIONS TO AUTHORS

Mission, scope, and submission policy

Brazilian Oral Research - BOR (online version ISSN 1807-3107) is the official publication of the *Sociedade Brasileira de Pesquisa Odontológica* - SBPqO (the Brazilian division of the International Association for Dental Research - IADR). The journal has an Impact Factor™ of 0.937 (Institute for Scientific Information - ISI), is peer-reviewed (double-blind system), and its mission is to disseminate and promote an information interchange concerning the several fields in dentistry research and/or related areas with gold open access.

BOR invites the submission of original and review manuscripts and papers in the following typology: Original Research (complete manuscript or Short Communication), Critical Review of Literature, Systematic Review (and Meta-Analysis) and Letters to the Editor. All submissions must be exclusive to.

Manuscripts and all corresponding documentation should be exclusively submitted through ScholarOne Manuscripts™ via the online submission link (http://mc04.manuscriptcentral.com/bor-scielo).

The evaluation process of manuscript's scientific content will only be initiated after meeting of all the requirements described in the present Instructions for Authors. Any manuscript that does not meet these requirements will be returned to the corresponding author for adaptations.

Important: Once having been accepted on their scientific merit, all manuscripts will be submitted for grammar and style revision as per the English language. Contact BOR by bor@sbpqo.org.br to get information about the recommended translation companies.The authors should forward the revised text with the enclosed revision certificate provided by the chosen editing company. **Linguistic revisions performed by companies that do not provide the mentioned certificate will not be accepted.** As an exception, this rule does not apply when one of the authors is a native English speaker.

Presentation of the manuscript

The manuscript text should be written in English and provided in a digital file compatible with "Microsoft Word" (in DOC, DOCX, or RTF format).

All figures (including those in layouts/combinations) must be provided in individual and separate files, according to recommendations described under the specific topic.
Photographs, micrographs, and radiographs should be provided in TIFF format, according to the recommendations described under the specific topic.

Charts, drawings, layouts, and other vector illustrations must be provided in a PDF format individually in separate files, according to the recommendations described under the specific topic.

Video files may be submitted as per the specifications, including the author's anonymity (for purposes of evaluation) and respect for the patient's rights.

Important: ScholarOne™ allows upload of a set of files up to 10 MB. In case the video file exceeds this size, it is possible to leave information about the link to access the video. The use of patients' initials, names, and/or registry numbers is prohibited in the reproduction of clinical documentation. The identification of patients is prohibited. An informed consent statement, signed by the patient, concerning the use of his/her image should be provided by the author(s) when requested by **BOR**. The Copyright legislation in force must be respected and the source cited when the manuscript reproduces any previously published material (including texts, charts, tables, figures, or any other materials).

Title page (compulsory data)

- This must indicate the specialty* or research field focused on in the manuscript.

*Anatomy; Basic Implantodontology and Biomaterials; Behavioral Sciences; Biochemistry; Cariology; Community Dental Health; Craniofacial Biology; Dental Materials; Dentistry; Endodontic Therapy; Forensic Dentistry; Geriatric Dentistry; Imaginology; Immunology; Implantodontology – Prosthetics; Implantodontology – Surgical; Infection Control; Microbiology; Mouth and Jaw Surgery; Occlusion; Oral Pathology; Orthodontics; Orthopedics; Pediatric Dentistry; Periodontics; Pharmacology; Physiology; Prosthesis; Pulp Biology; Social/Community Dentistry; Stomatology; Temporomandibular Joint Dysfunction.

- Informative and concise title, limited to a maximum of 110 characters, including spaces.
- Names of all authors written out in full, including respective telephone numbers and email addresses for correspondence. We recommend that authors collate the names present in the Cover Letter with the profile created in ScholarOne™, to avoid discrepancies.
- The participation of each author must be justified on a separate page, which should meet the authorship and co-authorship criteria

adopted by the International Committee of Medical Journal Editors, available at http://www.icmje.org/recommendations/browse/roles-and-responsibilities/defining-the-role-of-authors-and-contributors.html

- Data of institutional/professional affiliation of all authors, including university (or other institution), college/program, department, city, state, and country, presented according to internal citation norms established by each author's institution. Verify that such affiliations are correctly entered in ScholarOne™.

Abstract: This should be presented as a single structured paragraph (but with no subdivisions into sections) containing the objective of the work, methodology, results, and conclusions. In the System if applicable, use the Special characters tool for special characters.

Keywords: Ranging from 3 (three) to 5 (five) main descriptors should be provided, chosen from the keywords registered at http://decs.bvs.br/ or http://www.nlm.nih.gov/mesh/MBrowser.html (no synonyms will be accepted).

Main Text

Introduction: This should present the relevance of the study, and its connection with other published works in the same line of research or field, identifying its limitations and possible biases. The objective of the study should be concisely presented at the end of this section.

Methodology: All the features of the material pertinent to the research subject should be provided (*e.g.*, tissue samples or research subjects). The experimental, analytical, and statistical methods should be described in a concise manner, although in detail, sufficient to allow others to recreate the work. Data from manufacturers or suppliers of products, equipment, or software must be explicit when first mentioned in this section, as follows: manufacturer's name, city, and country. The computer programs and statistical methods must also be specified. Unless the objective of the work is to compare products or specific systems, the trade names of techniques, as well as products, or scientific and clinical equipment should only be cited in the "Methodology" and "Acknowledgments" sections, according to each case. Generic names should be used in the remainder of the manuscript, including the title. Manuscripts containing radiographs, microradiographs, or SEM images, the following information must be included: radiation source, filters, and kV levels used. Manuscripts reporting studies on humans should include proof that the research was ethically conducted according to the Helsinki Declaration (*World Medical Association,* http://www.wma.net/en/30publications/10policies/b3/). The approval protocol number issued by an Institutional Ethics Committee must be cited. Observational studies should follow the STROBE guidelines (http://strobe-statement.org/), and the check list must be submitted. Clinical Trials must be reported according to the CONSORT Statement standard protocol (http://www.consort-statement.org/); systematic reviews and meta-analysis must follow the PRISMA (http://www.prisma-statement.org/), or Cochrane protocol (http://www.cochrane.org/).

Clinical Trials

Clinical Trials according to the CONSORT guidelines, available at www.consort-statement.org. The clinical trial registration number and the research registration name will be published along with the article.

Manuscripts reporting studies performed on animals must also include proof that the research was conducted in an ethical manner, and the approval protocol number issued by an Institutional Ethics Committee should be cited. In case the research contains a gene registration, before submission, the new gene sequences must be included in a public database, and the access number should be provided to BOR. The authors may use the following databases:

- GenBank: http://www.ncbi.nlm.nih.gov/Genbank/submit
- EMBL: http://www.ebi.ac.uk/embl/Submission/index.html
- DDBJ: http://www.ddbj.nig.ac.jp

Manuscript submissions including microarray data must include the information recommended by the MIAME guidelines (Minimum Information About a Microarray Experiment: http://www.mged.org/index.html) and/or itemize how the experimental details were submitted to a publicly available database, such as:

- ArrayExpress: http://www.ebi.ac.uk/arrayexpress/
- GEO: http://www.ncbi.nlm.nih.gov/geo/

Results: These should be presented in the same order as the experiment was performed, as described under the "Methodology" section. The most significant results should be described. Text, tables, and figures should not be repetitive. Statistically relevant results should be presented with enclosed corresponding p values.

Tables: These must be numbered and cited consecutively in the main text, in Arabic numerals. Tables must be submitted separately from the text in DOC, DOCX, or RTF format.

Discussion: This must discuss the study results in relation to the work hypothesis and relevant literature. It should describe the similarities and differences of the study in relation to similar studies found in literature, and provide explanations for the possible differences found. It must also identify the study's limitations and make suggestions for future research.

Conclusions: These must be presented in a concise manner and be strictly based on the results obtained in the research. Detailing of results, including numerical values, etc., must not be repeated.

Acknowledgments: Contributions by colleagues (technical assistance, critical comments, etc.) must be given, and any bond between authors and companies must be revealed. This section must describe the research funding source(s), including the corresponding process numbers.

Plagiarism

BOR employs a plagiarism detection system. When you send your manuscript to the journal it may be analyzed-not merely for the repetition of names/affiliations, but rather the sentences or texts used.

References: Only publications from peer-reviewed journals will be accepted as references. Unfinished manuscripts, dissertations, theses, or abstracts presented in congresses will not be accepted as references. References to books should be avoided.

Reference citations must be identified in the text with superscript Arabic numerals. The complete reference list must be presented after the "Acknowledgments" section, and the references must be numbered and presented in Vancouver Style in compliance with the guidelines provided by the International Committee of Medical Journal Editors, as presented in Uniform Requirements for Manuscripts Submitted to Biomedical Journals (http://www.ncbi.nlm.nih.gov/books/NBK7256/). The journal titles should be abbreviated according to the List of Journals Indexed in Index Medicus (http://www.ncbi.nlm.nih.gov/nlmcatalog/journals). The authors shall bear full responsibility for the accuracy of their references.

Spelling of scientific terms: When first mentioned in the main text, scientific names (binomials of microbiological, zoological, and botanical nomenclature) must be written out in full, as well as the names of chemical compounds and elements.

Units of measurement: These must be presented according to the International System of Units (http://www.bipm.org or http://www.inmetro.gov.br/consumidor/unidLegaisMed.asp).

Footnotes on the main text: These must be indicated by asterisks and restricted to the bare minimum.

Figures: Photographs, microradiographs, and radiographs must be at least 10 cm wide, have at least 500 dpi of resolution, and be provided in TIFF format. Charts, drawings, layouts, and other vector illustrations must be provided in a PDF format. All the figures must be submitted individually in separate files (not inserted into the text file). Figures must be numbered and consecutively cited in the main text in Arabic numerals. Figure legends should be inserted together at the end of the text, after the references.

Characteristics and layouts of types of manuscripts

Original Research

Limited to 30,000 characters including spaces (considering the introduction, methodology, results, discussion, conclusion, acknowledgments, tables, references, and figure legends). A maximum of 8 (eight) figures and 40 (forty) references will be accepted. The abstract can contain a maximum of 250 words.

Layout - Text Files

- Title Page
- Main text (30,000 characters including spaces)
- Abstract: a maximum of 250 words
- Keywords: 3 (three)-5 (five) main descriptors
- Introduction
- Methodology
- Results
- Discussion
- Conclusion
- Acknowledgments
- Tables
- References: maximum of 40 references
- Figure legends

Layout - Graphic Files

- Figures: a maximum of 8 (eight) figures, as described above.

Short Communication

Limited to 10,000 characters including spaces (considering the introduction, methodology, results, discussion, conclusion, acknowledgments, tables, references, and figure legends). A maximum of 2 (two) figures and 12 (twelve) references will be allowed. The abstract can contain a maximum of 100 words.

Layout - Text Files

- Title page
- Main text (10,000 characters including spaces)
- Abstract: a maximum of 100 words
- Descriptors: 3 (three)-5 (five) main descriptors
- Introduction
- Methodology
- Results
- Discussion
- Conclusion
- Acknowledgments
- Tables
- References: a maximum of 12 references
- Figure legends

Layout- Graphic Files

- Figures: a maximum of 2 (two) figures, as described above.

Critical Review of Literature

The submission of this type of manuscript will be performed only by invitation of the BOR Publishing Commission. All manuscripts will be submitted to peer-

review. This type of manuscript must have a descriptive and discursive content, focusing on a comprehensive presentation and discussion of important and innovative scientific issues, with a limit of 30,000 characters including spaces (considering the introduction, methodology, results, discussion, conclusion, acknowledgments, tables, references, and figure legends). It must include a clear presentation of the scientific object, logical argumentation, a methodological and theoretical critical analysis of the studies, and a summarized conclusion. A maximum of 6 (six) figures and 50 (fifty) references is permitted. The abstract must contain a maximum of 250 words.

Layout- Text Files

- Title page
- Main text (30,000 characters including spaces)
- Abstract: a maximum of 250 words
- Keywords: 3 (three)-5 (five) main descriptors
- Introduction
- Methodology
- Results
- Discussion
- Conclusion
- Acknowledgments
- Tables
- References: maximum of 50 references
- Figure legends

Layout - Graphic Files

- Figures: a maximum of 6 (six) figures, as described above.

Systematic Review and Meta-Analysis

While summarizing the results of original studies, quantitative or qualitative, this type of manuscript should answer a specific question, with a limit of 30,000 characters, including spaces, and follow the Cochrane format and style (www.cochrane.org). The manuscript must report, in detail, the process of the search and retrieval of the original works, the selection criteria of the studies included in the review, and provide an abstract of the results obtained in the reviewed studies (with or without a meta-analysis approach). There is no limit to the number of references or figures. Tables and figures, if included, must present the features of the reviewed studies, the compared interventions, and the corresponding results, as well as those studies excluded from the review. Other tables and figures relevant to the review must be presented as previously described. The abstract can contain a maximum of 250 words.

Layout - Text Files

- Title page
- Main text (30,000 characters including spaces)
- Abstract: a maximum of 250 words
- Question formulation
- Location of the studies
- Critical Evaluation and Data Collection
- Data analysis and presentation
- Improvement
- Review update
- References: no limit on the number of references
- Tables

Layout - Graphic Files

- Figures: no limit on the number of figures

Letter to the Editor

Letters must include evidence to support an opinion of the author(s) about the scientific or editorial content of the BOR, and must be limited to 500 words. No figures or tables are permitted.

Copyright transfer agreement and responsibility statements

The manuscript submitted for publication must include the Copyright Transfer Agreement and the Responsibility Statements, available in the online system and mandatory.

CHECKLIST FOR INITIAL SUBMISSION

- Title Page file (in DOC, DOCX, or RTF format).
- Main text file (Main Document, manuscript), in DOC, DOCX, or RTF format.
- Tables, in DOC, DOCX, or RTF format.
- Declaration of interests and funding, submitted in a separate document and in a PDF format. (if applicable)
- Justification for participation of each author, provided in a separate document and in a PDF format.
- Photographs, microradiographs, and radiographs (10 cm minimum width, 500 dpi minimum resolution) in TIFF format. (http://www.ncbi.nlm.nih.gov/pmc/pub/filespec-images/)
- Charts, drawings, layouts, and other vector illustrations in a PDF format.

- Each figure should be submitted individually in separate files (not inserted in the text file).

Publication fees

Authors are not required to pay for the submission or review of articles.

EXAMPLES OF REFERENCES

Journals

Goracci C, Tavares AU, Fabianelli A, Monticelli F, Raffaelli O, Cardoso PC, et al. The adhesion between fiber posts and root canal walls: comparison between microtensile and push-out bond strength measurements. Eur J Oral Sci. 2004 Aug;112(4):353-61.

Bhutta ZA, Darmstadt GL, Hasan BS, Haws RA. Community-based interventions for improving perinatal and neonatal health outcomes in developing countries: a review of the evidence. Pediatrics. 2005;115(2 Suppl):519-617. doi:10.1542/peds.2004-1441.

Usunoff KG, Itzev DE, Rolfs A, Schmitt O, Wree A. Nitric oxide synthase-containing neurons in the amygdaloid nuclear complex of the rat. Anat Embryol (Berl). 2006 Oct 27. Epub ahead of print. doi: 10.1007/s00429-006-0134-9

Walsh B, Steiner A, Pickering RM, Ward-Basu J. Economic evaluation of nurse led intermediate care versus standard care for post-acute medical patients: cost minimisation analysis of data from a randomised controlled trial. BMJ. 2005 Mar 26;330(7493):699. Epub 2005 Mar 9.

Papers with Title and Text in Languages Other Than English

Li YJ, He X, Liu LN, Lan YY, Wang AM, Wang YL. [Studies on chemical constituents in herb of Polygonum orientale]. Zhongguo Ahong Yao Za Zhi. 2005 Mar;30(6):444-6. Chinese.

Supplements or Special Editions

Pucca Junior GA, Lucena EHG, Cawahisa PT. Financing national policy on oral health in Brazil in the context of the Unified Health System. Braz Oral Res. 2010 Aug;24 Spec Iss 1:26-32.

Online Journals

Barata RB, Ribeiro MCSA, De Sordi M. Desigualdades sociais e homicídios na cidade de São Paulo, 1998. Rev Bras Epidemiol. 2008;11(1):3-13 [cited 2008 Feb 23]. Available from: http://www.scielosp.org/pdf/rbepid/v11n1/01.pdf.

Books

Stedman TL. Stedman's medical dictionary: a vocabulary of medicine and its allied sciences, with pronunciations and derivations. 20th ed. Baltimore: Williams & Wilkins; 1961. 259 p.

Books Online

Foley KM, Gelband H, editors. Improving palliative care for cancer [monograph on the Internet]. Washington: National Academy Press; 2001 [cited 2002 Jul 9]. Available from: http://www.nap.edu/books/0309074029/html/.

Websites

Cancer-Pain.org [homepage on the Internet]. New York: Association of Cancer Online Resources, Inc.; c2000 [cited 2002 Jul 9]. Available from: http://www.cancer-pain.org/.

Instituto Brasileiro de Geografia e Estatística [homepage]. Brasília (DF): Instituto Brasileiro de Geografia e Estatística; 2010 [cited 2010 Nov 27]. Available from: http://www.ibge.gov.br/home/default.php.

World Health Organization [homepage]. Geneva: World Health Organization; 2011 [cited 2011 Jan 17]. Available from: http://www.who.int/en/

Produção Científica no Doutorado

Participação de citocinas relacionadas às respostas Th17/Treg em lesões periapicais inflamatórias crônicas. Projeto de pesquisa apresentado e aprovado pelo Colegiado de Pós-Graduação em Odontologia (CPGO) e pelo Comitê de Ética e Pesquisa (COEP) da Universidade Federal de Minas Gerais (UFMG).

Imbalance of Treg/Th17 cells in the development of chronic inflammatory periapical lesions. A systematic review. Projeto de pesquisa apresentado ao pelo Colegiado de Pós-Graduação em Odontologia (CPGO) da Universidade Federal de Minas Gerais (UFMG). Artigo científico submetido a revista Brazilian Oral Research.

Participação dos linfócitos Th17/Treg em lesões periapicais inflamatórias crônicas. Projeto de pesquisa contendo resultados de análises imunohistoquícas de IL-17 e FoxP3 aplicada a amostras do arquivo do laboratório de Patologia da faculdade de Odontologia da Universidade Federal de Minas Gerais.

Comparação e análise da eficácia de um exaguante bucal contendo 5% de própolis e da clorexidina para o controle da placa e gengivite. Coordenador / Vagner Santos. Projeto de pesquisa apresentado ao colegiado de pós-graduação em odontologia (CPGO) da Universidade Federal de Minas Gerais. Aprovado na Plataforma Brasil CAAE: 149.23013.6.0000.5149.

Avaliação da expressão de VEGF no complexo Dentina-Polpa após ação de

diferentes agentes de cimentação. Trabalho apresentado na 31° Reunião Anual da Sociedade Brasileira de Pesquisa Odontológica (SBPqO), realizada em Águas de Lindóia, Brasil, no período de 03 a 06 de novembro de 2014.