Saudi Dental Journal (2021) 33, 979–986

King Saud University

Saudi Dental Journal

www.ksu.edu.sa
www.sciencedirect.com

ORIGINAL ARTICLE

Apical periodontitis promotes insulin resistance and alters adaptive immunity markers in rats

Renato Felipe Pereira [a], Gestter Willian Lattari Tessarin [b,c,*], Fernando Yamamoto Chiba [d], Maria Sara de Lima Coutinho Mattera [a], Amanda Gomes Pereira [e], Thais Verônica Saori Tsosura [a], Victor Gustavo Balera Brito [a], Renan Akira Fujii de Oliveira [d], Edilson Ervolino [b,f], Sandra Helena Penha de Oliveira [a], Luciano Tavares Angelo Cintra [d], Doris Hissako Matsushita [a,b]

[a] *Programa de Pós-graduação Multicêntrico em Ciências Fisiológicas, PPGMCF, SBFis, São Paulo State University (UNESP), School of Dentistry, Araçatuba, São Paulo, Brazil*
[b] *Department of Basic Sciences, São Paulo State University (UNESP), School of Dentistry, Araçatuba, SP, Brazil*
[c] *University Center North Paulista (UNORP), São José do Rio Preto, SP, Brazil*
[d] *Department of Preventive and Restorative Dentistry, São Paulo State University (UNESP), School of Dentistry, Araçatuba, SP, Brazil*
[e] *Internal Medicine Department, Botucatu Medical School, São Paulo State University (UNESP), Botucatu, Brazil*
[f] *Institute of Biosciences of Botucatu, São Paulo State University (UNESP), Botucatu, SP, Brazil*

Received 1 October 2020; revised 25 May 2021; accepted 4 July 2021
Available online 14 July 2021

KEYWORDS

Apical periodontitis;
Endodontics;
Insulin resistance;
Adaptive immunity

Abstract *Objective:* Apical periodontitis (AP) is a chronic or acute inflammatory disease usually developed from endodontic infections, predominantly due to gram-negative anaerobic bacteria invading the dental pulp. This study aimed to evaluate lymphocyte markers to assess the involvement of adaptive immunity in insulin resistance (IR) in a rat model of AP.

Design.

Forty-five male Wistar albino rats were divided into 3 groups (control, 1AP and 4AP). AP was induced in the upper right first molar (1AP), and in the first and second upper and lower right molars (4AP). The spleen was collected to evaluate the expression of transcription factors involved

* Corresponding author at: School of Dentistry - University Center North Paulista (UNORP) Ipiranga Street, 3460 São José do Rio Preto, SP ZIP CODE: 15020-040, Brazil.
E-mail address: gestter.tessarin@unesp.br (G. Willian Lattari Tessarin).

Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

https://doi.org/10.1016/j.sdentj.2021.07.005

in lymphocyte polarization, including T-bet (Th1), GATA3 (Th2), and FOXP3 (Treg). Blood samples were assessed for serum cytokine levels transcribed by the respective lymphocyte polarizations, INF-γ (Th1), IL-4 (Th2) and TGF-β (Treg). In addition, glucose and insulin levels were measured to evaluate IR by the HOMA-IR method.

Results: The results showed higher T-bet expression on AP groups, along with lower GATA3 and FOXP3 expression in the 1AP, in addition to increased GATA3 and decreased FOXP3 expression in the 4AP group compared to the CN group. There was no difference in the INF-γ levels, while IL-4 was decreased in the AP groups. Taken together, these results suggest that the adaptive immune system, with a predominance of the Th1 polarization, may be involved in the development of IR in rats with AP.

Conclusions: AP promotes increase in the expression of T-bet (4AP) and decrease of FOXP3 expressions and IL-4 levels (1AP and 4AP). However, depending on the number of lesions (1 or 4 lesions), the expression of GATA3 appears differently. Thus, innate immunity and adaptive immunity may contribute to the IR observed in rats with AP.

1. Introduction

Apical periodontitis (AP) is a chronic or acute inflammatory disease developed usually from endodontic infections, predominantly due to gram-negative anaerobic bacteria invading the dental pulp (Cachovan et al., 2014; Desai et al, 2011; Kakehashi et al., 1965; Sasaki et al., 2016). This condition initiates a local immune response and, if left untreated, the bacteria and/or its toxins can reach the periapical region and cause, in addition to the periapical immune response, an inflammatory condition that can promote bone resorption and dental loss, for example (Fouad & Acosta, 2001; Graves et al., 2011; Sasaki et al., 2016). The periapical inflammatory process involves host-derived immune factors, such as antibodies, complement system, arachidonic acid metabolites, and pro-inflammatory cytokines (Stashenko et al., 1998), and these locally synthesized mediators can reach the bloodstream (Astolphi et al., 2013; Bain et al., 2009; Zhang et al., 2011). Several studies have demonstrated the relationship between AP and health disorders (Cintra et al., 2018; Sasaki et al., 2016). Astolphi et al. (2013) and Astolphi et al. (2015), Pereira et al. (2017) and Tavares et al. (2021), using the rat model of AP observed alterations in insulin signaling, insulin sensitivity and increase in macrophage infiltration in serum and skeletal muscle that may explain the relationship between AP and IR (Astolphi et al., 2013; Astolphi et al., 2015; Pereira et al., 2017; Tavares et al., 2021). Besides that, maternal apical periodontitis and maternal AP in animal models led to insulin resistance in adult offspring (Mattera et al., 2019; Tsosura et al., 2019).

In humans, Schulze et al. (2007) showed a swift increase in serum glucose levels following the development of endodontic-periodontic lesions, suggesting a relationship between insulin resistance (IR) and endodontic infection (Schulze et al., 2007). Moreover, changes in the aortic arch, myocardium, spleen, and liver caused by increased levels of the C-reactive protein (CRP), IL-2, and IL-6 have been reported in rodents with AP (Zhang et al., 2016).

IR is defined as a subnormal biological response to normal insulin concentrations (Moller and Flier, 1991). Most studies that relate inflammation and IR have focused on the innate immune function; however, recent studies suggest an important role for the adaptive immune system mediated by T lymphocytes (Satoh and Iwabuchi, 2018; Zheng and Rudensky, 2007). These cells can differentiate into different cell phenotypes, of which the most common are Th1 and Th2. The Th1 cells are essential for intracellular pathogen control, and are involved in inflammatory reactions and late hypersensitivity (Mesquita et al., 2009). The Th2 cells are more efficient at triggering humoral immune responses, through antibody production to combat extracellular pathogens (Mesquita et al., 2009). Another type of T cell is the T regulatory cell (Treg) that acts by preventing an exacerbated inflammatory response, thus preventing tissue damage (Sakaguchi et al., 2020). These different T cells subtypes are determined by specific transcription factors (Tanriver and Diefenbach, 2014). The T-bet transcription factor promotes the differentiation of T cells into Th1 cells by INF-γ expression (Szabo et al., 2000). GATA3 expresses IL-4 which characterizes the Th2 cells (Farrar et al., 2002). Furthermore, FOXP3 regulates the expression of TGF-β, which is responsible for Treg polarization (Hall et al., 2015).

It should also be noted that the spleen plays an important role in the differentiation and proliferation of T lymphocytes (Shieh et al., 2014). Additionally, RT-PCR has been used to directly determine T-bet / GATA3 ratio to assess the balance of Th1/Th2 polarizations (Yong et al., 2011). The evaluation of transcription factors involved in lymphocyte differentiation in the spleen may provide clues about the adaptive immune response observed in AP, and the possible implications in the relationship between AP and IR.

2. Materials & methods

2.1. Animals and grouping

Forty-five male Wistar albino rats, weighing between 250 and 280 g, were obtained from the central animal house of the School of Dentistry of Araçatuba (UNESP, SP, Brazil), and kept at the Department of Basic Science (Physiology division) with a 12-hour light/dark cycle (lights on at 7:00 AM), in temperature-controlled rooms (23° ± 2 °C), access to water

and food (23% from protein, 4% fat, and 58% from carbohydrate; produced by Presence, Paulínia, São Paulo, Brazil) ad libitum, and 15 days of environmental adaptation. The animals were randomly distributed into 3 groups, with 15 animals in each group: a control group (CN), a group with 1 periapical lesion (1AP), and a group with 4 periapical lesions (4AP). All applicable international, national, and/or institutional guidelines for the care and use of animals were followed and approved by Ethics Committee on the Use of Animals (Process FOA n° 00745–2015).

2.2. Chronic apical periodontitis model and body weight measurement

Initially, the animals in the 1AP and 4AP groups (n = 10 per group) were anesthetized with an intramuscular injection of ketamine (80 mg/Kg) (Ketamina, Agener, Embu-Guaçu, Brazil) and xylazine (10 mg/Kg) (Xylazine, Dorcipec®, Monte Carlos, Brazil). Next, to induce AP, the dental pulp of the first right maxillary molar (1AP) or the first and second right maxillary and mandibular molars (4AP) were exposed to the oral environment through the occlusal surface using a surgical round bur measuring 0.1 mm in diameter (Broca LN Long Neck, Dentsply Ind e Com Ltda, Petrópolis, RJ, Brazil). During the next 30 days of apical periodontitis induction, all animals were weighed 3 times a week. In addition, their food intake was analyzed by weighing the amount of feed in the boxes. All experiments were approved by the local ethics committee according to opinion number 00745–2015 and all efforts were made to minimize animal suffering.

2.3. Glucose measurement, insulin resistance (IR), and serum concentrations of INF-γ, IL-4 and TGF-β

Thirty days after pulp exposure, the rats (n = 12 per group) were subjected to a 14-hour fast, followed by anesthetization as described above. Next, the abdominal region was disinfected using commercial iodine, and a median laparotomy was performed in 12 animals from each group (CN, 1AP and 4AP), to expose the abdominal cavity. The blood samples were collected via inferior vena cava punctures in heparinized tubes. Plasma was isolated by centrifuging the blood at 3000 × g for 15 min at 4 °C, and was stored as aliquots at −80 °C. Blood glucose was measured using the glucose oxidase method (Enzymatic glucose, Analisa Diagnóstica, Belo Horizonte, MG, Brazil) and insulinemia was evaluated by an enzyme-linked immunosorbent assay (ELISA) using a specific commercial kit (Sensitive Rat Insulin, Millipore, St. Charles, MO, USA). IR was evaluated by the homeostasis model assessment of insulin resistance (HOMA-IR) index, calculated according to the following formula (Bonora, 2000):

$$HOMA - IR = fasting\ \ glycemia\ \ (mmol/L) \times fasting\ \ insulinemia\ \ (\mu IU/mL)$$

The same blood samples used to measure glucose and IR were used to evaluate serum INF-γ, IL-4, and TGF-β by the enzyme-linked immunosorbent method (ELISA) using specific commercial kits (INF-γ, EK0374; IL-4, EK0406; TGF-β, EK0514, Boster Biological Technology), in accordance with the manufacturer's instructions.

2.4. T-bet, GATA3, and FOXP-3 mRNA expression in the spleen by real-time reverse transcription-polymerase chain reaction (RT-PCR)

Total RNA was extracted from a sample (weighting around 70 mg) of the spleen (n = 5) using the Trizol reagent (Invitrogen, Thermo Fisher Scientific) according to the manufacturer's instructions. The total RNA from each sample was treated with DNAse I (DNAse I, Sigma-Aldrich) and was reverse transcribed into the complementary DNA (cDNA) using SuperScript™ II Reverse Transcriptase (SuperScript™ II Reverse Transcriptase, Invitrogen) according to the manufacturer's protocol. The relative mRNA levels were evaluated by quantitative RT-PCR using the StepOne™ Real-Time PCR System (Applied Biosystems, Life Technologies) and TaqMan probes® (Applied Biosystems, Life Technologies) for specific markers Th1 (TBX21), Th2 (GATA3) and Treg (FOXP3) using commercial probes (Thermo Fischer Scientific) (TBX21: Rn01461633_m1; GATA3: Rn00484683_m1 and FOXP3: Rn01525092_m1). Beta-actin (Rn00667869_m1) was used as an endogenous control gene to normalize the amounts of RNA in the RT-PCR.

2.5. Histologic processing of the rat's jaws

The right maxillae and the hemi-mandible (n = 5) of CN, 1AP and 4AP were dissected, fixed in 4% formaldehyde (Sigma-Aldrich, St Louis, MO, USA), diluted in 0.1 M phosphate buffered saline (PBS, pH 7.4) for 24 h, decalcified in ethylenediaminetetraacetic acid (Sigma-Aldrich, St Louis, MO, USA) (10%) diluted in PBS pH 7.4 for 30 days, processed in the conventional manner and embedded in paraffin. Four-μm-thick parasagittal histological sections of the right maxillae first molar (1AP group), or first and second molars from the maxillae and mandible (4AP group), in their longitudinal axis, were obtained using a conventional microtome (RM2155, Leica Microsystems, BD) and placed on positively charged glass slides. The sections were stained with hematoxylin-eosin, and the periapical region as well as the dental pulp were analyzed by optical microscopy (Axio Lab A1, Carl Zeiss, TH, Oberkochen) to evaluate the condition of the periapical tissues, and in case of the 1AP and 4AP groups, to verify the magnitude of the inflammatory process in the periapical lesions.

2.6. Statistical analysis

Initially, the normality and the homoscedasticity of the data were analyzed. The analysis of variance (ANOVA) was followed by the Tukey post hoc test ($P < 0.05$). Data were expressed as mean (± standard error of the mean; SEM). All analysis was performed using the GraphPad Prism version 6.0 (GraphPad Software Inc, San Diego, CA, USA).

3. Results

3.1. Histological evaluation of the dental pulp and periapical tissues

The animals in the CN group showed normal dental pulp and periapical tissues, with no sign of inflammation (Fig. 1). On the

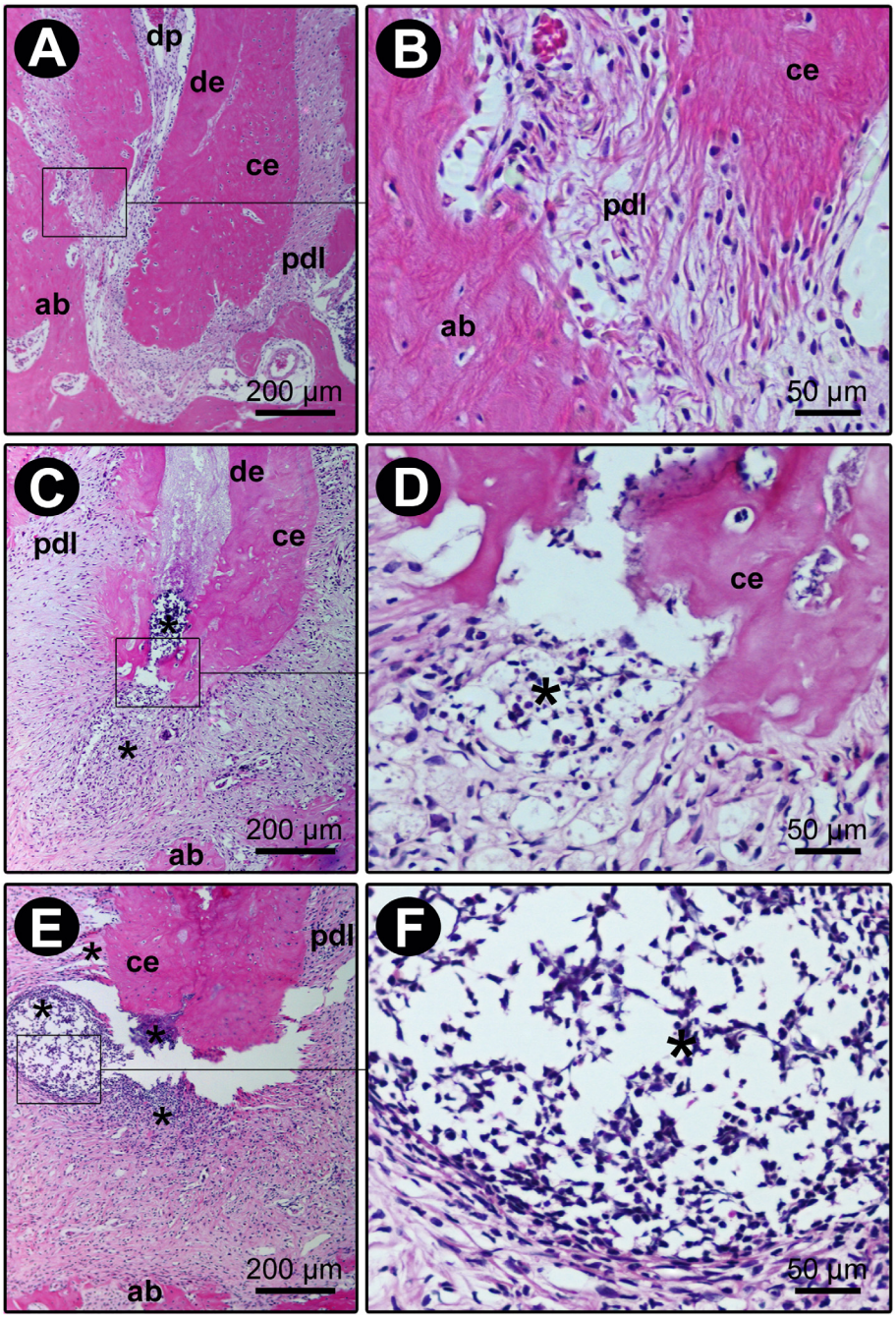

Fig. 1 Histological aspect of periapical tissues in different experimental groups. Photomicrographs showing histological characteristics of mandibular molar periapical tissues in CN (A – B), 1AP (C – D) and 4AP (E – F) groups. Note in 1AP and 4AP groups total necrosis of the dental pulp, the presence of inflammatory infiltrate extending through the apex of the tooth and alveolar bone resorption in the periapical region. Abbreviations and symbols: asterisks, inflammatory infiltrate; ab, alveolar bone; ce, cement; de, dentine, dp, dental pulp; pdl, periodontal ligament. Staining: hematoxylin and eosin (HE). Scale bars: A, C and E, 200 µm; B, D and F, 50 µm. Original magnification: A, C and E, 50x; B, D and F, 400x.

other hand, in the 1AP and 4AP groups, total necrosis of the entire dental pulp was observed. In both groups, the presence of periapical lesions was associated with the roots of the teeth whose pulp was exposed to the oral environment. Such lesions were characterized by an intense inflammatory infiltrate, composed predominantly of lymphocytes and small amounts of neutrophils (Fig. 1).

3.2. Glycemia, insulinemia and HOMA-IR index

There were no differences observed in glycemia and insulinemia among the groups (Table 1). However, the HOMA-IR index showed that the 1LP and 4LP groups presented higher IR when compared to the CN group (Table 1). No difference

Table 1 Glycemia, insulinemia and HOMA-IR index in the control (CN), 1 apical periodontitis (1AP) and 4 apical periodontitis (4AP) groups.

Parameters	CN	1PL	4PL
Glucose (mmol/L)	6,94 ± 0,33	6,74 ± 0,14	6,77 ± 0,21
Insulin (μIU/mL)	19,14 ± 6,35	29,21 ± 4,21	31,80 ± 3,12
HOMA-IR	5,42 ± 1,86	10,18 ± 0,77*	10,31 ± 0,97*

Values are presented as the mean ± SEM (n = 10 animals per group). *p < 0.05 CN vs. 1AP and 4AP.

Table 2 Serum IFN-γ, IL-4, and TGF-β concentrations in the control (CN), 1 apical periodontitis (1AP) and 4 apical periodontitis (4AP) groups.

Parameters	CN	1PL	4PL
IFN-γ	38,9 ± 2,33	40,47 ± 4,64	39,94 ± 4,06
IL-4	25,9 ± 0,39	22,73 ± 0,82*	23,08 ± 0,81*
TGF-β	121,6 ± 15,83	175,5 ± 13,38	166 ± 17,69

Values are presented as the mean ± SEM (n = 12 animals per group). *p < 0.01 CN vs. 4PL.

in the HOMA-IR index between the 1AP and 4AP groups was observed (Table 1).

3.3. Assessment of gene expression of lymphocyte markers in spleen

Lymphocyte populations (Th1, Th2 and Treg) were evaluated in the spleen samples by assessing the expression of transcription factors responsible for the differentiation of lymphocytes using the RT-PCR method. Fig. 2a shows an increase in T-bet gene expression (Th1 subpopulation inducer) in the 4AP group when compared to the CN and 1AP groups; however, no difference was observed between CN and 1AP.

Fig. 2b shows that the 4AP group had a higher expression of the GATA3 gene (Th2 subpopulation inducer) when compared to the CN and 1AP group. Interestingly, lower GATA3 expression values were observed in the 1AP group when compared to the CN group.

The expression of FOXP3 (Treg subpopulation inducer) was reduced in the 1AP and 4AP groups when compared to the CN group, however, no difference was observed between the 1AP and 4AP groups (Fig. 2c).

3.4. Serum INF-γ, IL-4, and TGF-β concentrations

Table 2 demonstrates that the serum INF-γ concentration was no difference in the groups. In case of the IL-4 levels, a reduction in the 1AP and 4AP groups was observed. In addition, no difference was observed among the groups regarding serum TGF-β concentration.

4. Discussion

The present study demonstrated that rats with 1 and 4 periapical lesioned teeth presented an altered expression of transcription factors involved in the differentiation of lymphocytes in the spleen. These splenic alterations were correlated with changes in the plasma cytokines, which may be related to a systemic modulation of the Th1 and Th2 response. These conditions may be involved with innate and adaptive immunity and probably can contribute with insulin resistance (IR) and other situations that caused alterations in health.

There is a growing body of evidence linking T lymphocyte activity to IR and diabetes (Xia et al., 2017). Our findings showed that, in AP-induced IR (1AP or 4AP), the Th1 polarization is increased.

A study with T-bet gene knockout mice observed that the animals were protected from high-fat-diet induced IR (Stolarczyk et al., 2014). The authors attributed the absence of IR to a reduced production of INF-γ. These results are consistent with those of studies that reported that INF-γ deficiency may improve glycemic homeostasis in insulin-resistant animals (O'Rourke et al., 2012; Rocha et al., 2008). Additionally, the treatment of adipocytes (3 T3-L1) with INF-γ impairs the insulin signaling and glucose uptake, supporting the hypothesis that Th1 polarization impairs insulin signaling (Wada et al., 2011).

On the other hand, the results demonstrated higher T-bet expression in the spleen, and there was no difference in serum INF-γ levels. This finding can be explained by epigenetic mechanisms, such as DNA methylation, which can control gene expression. This particular epigenetic modification is associated with gene silencing (Bobetsis et al., 2007).

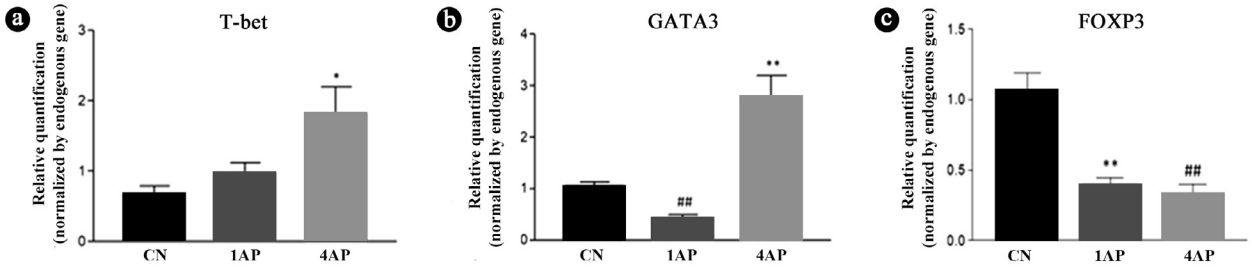

Fig. 2 Assessment of gene expression of lymphocyte markers in spleen of the control (CN), 1 periapical lesion (1AP) and 4 periapical lesions (4AP) groups obtained by RT-PCR method. In A, T-bet; in B, GATA-3; in C, FOXP3. The results were presented as mean (±SEM) (n = 5). * p < 0.05; ** p < 0.01; ## p < 0.001.

The inability of methylated transcription factors to bind to DNA has been proposed as a possible mechanism for gene silencing and may provide a possible explanation for the negative regulation of gene transcription (Bobetsis et al., 2007; Campos et al., 2013). In this context, Campos et al. (2013), when investigating methylation, together with the gene and protein expression of the IFNG gene, which encodes INF-γ, in periapical cells, observed that this gene presents partial or total DNA methylation (Campos et al., 2013). In addition, it suggests that epigenetic alteration is a common alteration in periapical inflammatory lesions. This study may explain that, although our results show an increase in the expression of T-bet (transcription factor), there was no increase in the plasma concentrations of INF-γ, because the IFNG may present a hypermethylation of DNA. This result is in agreement with Cintra et al. (2016) who also found no difference in serum INF-γ levels (Cintra et al., 2016). Furthermore, the knockout animals for INF-γ, IL-10, ICAM-1 and CCR5 showed periapical lesions greater than those of control animals (De Rossi et al., 2008).

The T-bet functions are antagonized by the GATA3 transcription factor, of which IL-4 is one of the main products. Our findings demonstrated a reduction in IL-4 levels in both groups with AP. Chang et al. (2012) reported that IL-4 treatment in mice improves insulin sensitivity, glucose tolerance, and reduces body weight. These results suggest that IL-4 has favorable effects on glycemic homeostasis (Chang et al., 2011). Additionally, with respect to Th2 polarization, a decrease in GATA3 expression in the 1AP and an increase in the 4AP were observed. Although it was unable to provide an explanation for this result, a study by Wright et al. (2018) using an inflammatory model of esophagitis also demonstrated an increased T-bet and GATA3 expression similar to that observed in our study (Wright et al., 2018).

The role of the Th2 cells in insulin sensitivity was discussed by Ricardo-Gonzales et al. (2010) (Ricardo-Gonzalez et al., 2010). In this study, a model of Th2 inactivation was developed through the inhibition of the STAT-6 protein, and it was observed that animals with Th2 deficiency were more prone to IR. Therefore, it could be postulated that the Th1 increase and the Th2 decrease reported in the present study could be involved in the IR observed in the AP rats.

In type 2 diabetes, Treg cells may inhibit the inflammatory response in many ways, such as through the suppression of cytokine release, the modulation of the microenvironment, and the altering of the expression of surface receptors (Guzmán-Flores and Portales-Pérez, 2013). Two studies have demonstrated that the number of Treg cells decreased in patients with T2DM (Jagannathan-Bogdan et al., 2011; Zeng et al., 2012). Eller et al. (2011) evaluated the role of Treg cells in IR (Eller et al., 2011) Using a genetic model of obesity associated with a pharmacological treatment to deplete the Treg cells, observing that animals with depleted Treg cells were less sensitive to insulin than the control group. The authors identified increased Th1 phenotype markers (INF-γ, TNF-α) in the animals that did not express Treg. Furthermore, it was noted that the transfer of Treg cells from healthy animals resulted in an improvement in insulin sensitivity. These data indicate the importance of these cells in the maintenance of insulin sensitivity, and are consistent with the findings of the present study, which reported a reduced expression of Treg cells in the spleens of insulin resistant rats (1AP and 4AP groups).

No difference was observed in the TGF-β blood levels between the groups. While the role of TGF-β in IR has not been extensively studied, reports have associated this cytokine with the worsening of the metabolic profile (Yadav et al., 2011). Collectively, these findings suggest that, in rats, AP stimulates Th1 polarization, which is supported in literature as a triggering factor for the development of IR. Moreover, the present study also noted a reduction of Th2 and Treg polarizations, which may demonstrate their anti-inflammatory effects in the IR.

Our results complement previously published studies which reported an increase in TNF-α and impairments in the insulin signal transduction in the muscle and adipose tissue of rats with PL (Astolphi et al., 2015). In addition, other studies from our research group identified an increase in the macrophage level as well as an increase in pro-inflammatory agents such as LPS, and HSP70 in muscle tissue of adult rats (Pereira et al., 2017) that can support other findings related to IR.

5. Conclusion

The present study demonstrated that AP promotes: an increase in the expression of T-bet; a decrease in FOXP3 expression and IL-4 levels. However, depending on the intensity of the lesion (1 or 4 lesions), the expression of GATA3 appears differently. Therefore, both the innate immunity and adaptive immunity may contribute to the IR observed in rats with AP. Some of the possible mechanisms that could be responsible for these developments include the recruitment of macrophages, the activation of inflammatory pathways in muscle tissue and a modulation of lymphocyte gene expression in the spleen. These results could help elucidate the mechanisms involved in the development of IR from localized inflammation, such as AP.

Funding

This work was supported by The São Paulo Research Foundation (FAPESP) [grants numbers #2014/17619–6; #2014/26517–2; #2016/24829–2; #2018/17795–0].

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

Astolphi, R.D., Curbete, M.M., Chiba, F.Y., Cintra, L.T., Ervolino, E., da Mota, M.S., Sumida, D.H., 2015. Periapical Lesions Decrease Insulin Signaling in Rat Skeletal Muscle. J. Endod. 41 (8), 1305–1310.

Astolphi, R.D., Curbete, M.M., Colombo, N.H., Shirakashi, D.J., Chiba, F.Y., Prieto, A.K., Sumida, D.H., 2013. Periapical lesions decrease insulin signal and cause insulin resistance. J. Endod. 39 (5), 648–652.

Bain, J.L., Lester, S.R., Henry, W.D., Naftel, J.P., Johnson, R.B., 2009. Effects of induced periapical abscesses on rat pregnancy outcomes. Arch. Oral. Biol. 54 (2), 162–171.

Bobetsis, Y.A., Barros, S.P., Lin, D.M., Weidman, J.R., Dolinoy, D. C., Jirtle, R.L., Offenbacher, S., 2007. Bacterial infection promotes DNA hypermethylation. J. Dent. Res. 86 (2), 169–174.

Bonora, E., Targher, G., Alberiche, M., Bonadonna, R.C., Saggiani, F., Zenere, M.B., Muggeo, M., 2000. Homeostasis model assessment closely mirrors the glucose clamp technique in the assessment of insulin sensitivity: studies in subjects with various degrees of glucose tolerance and insulin sensitivity. Diabetes Care 23 (1), 57–63.

Cachovan, G., Blessmann, M., Schön, G., Rother, U., Heiland, M., Stürenburg, E., Sobottka, I., 2014. Radiography-based score indicative for the pathogenicity of bacteria in odontogenic infections. Acta Odontol. Scand. 72 (7), 530–536.

Campos, K., Gomes, C.C., de Fátima Correia-Silva, J., Farias, L.C., Fonseca-Silva, T., Bernardes, V.F., Gomez, R.S., 2013. Methylation pattern of IFNG in periapical granulomas and radicular cysts. J. Endod. 39 (4), 493–496.

Chang, Y.H., Ho, K.T., Lu, S.H., Huang, C.N., Shiau, M.-Y., 2011. Regulation of glucose/lipid metabolism and insulin sensitivity by interleukin-4. International Journal of Obesity 36 (7), 993–998.

Cintra, L.T., Samuel, R.O., Azuma, M.M., de Queiróz, A.O., Ervolino, E., Sumida, D.H., Gomes-Filho, J.E., 2016. Multiple Apical Periodontitis Influences Serum Levels of Cytokines and Nitric Oxide. J. Endod. 42 (5), 747–751.

Cintra, L.T.A., Estrela, C., Azuma, M.M., Queiroz, Í., Kawai, T., Gomes-Filho, J.E., 2018. Endodontic medicine: interrelationships among apical periodontitis, systemic disorders, and tissue responses of dental materials. Braz Oral Res. 32, (suppl 1) e68.

De Rossi, A., Rocha, L.B., Rossi, M.A., 2008. Interferon-gamma, interleukin-10, Intercellular adhesion molecule-1, and chemokine receptor 5, but not interleukin-4, attenuate the development of periapical lesions. J. Endod. 34 (1), 31–38.

Desai, S.V., Love, R.M., Rich, A.M., Seymour, G.J., 2011. Toll-like receptor 2 expression in refractory periapical lesions. Int. Endod. J. 44 (10), 907–916.

Eller, K., Kirsch, A., Wolf, A.M., Sopper, S., Tagwerker, A., Stanzl, U., Eller, P., 2011. Potential role of regulatory T cells in reversing obesity-linked insulin resistance and diabetic nephropathy. Diabetes 60 (11), 2954–2962.

Farrar, J.D., Asnagli, H., Murphy, K.M., 2002. T helper subset development: roles of instruction, selection, and transcription. J. Clin. Invest. 109 (4), 431–435.

Fouad, A.F., Acosta, A.W., 2001. Periapical lesion progression and cytokine expression in an LPS hyporesponsive model. Int Endod J. 34 (7), 506–513.

Graves, D.T., Oates, T., Garlet, G.P., 2011. Review of osteoimmunology and the host response in endodontic and periodontal lesions. J. Oral Microbiol. 3.

Guzmán-Flores, J.M., Portales-Pérez, D.P., 2013. Mechanisms of suppression of regulatory T-cells (Treg). Gac. Med. Mex 149 (6), 630–638.

Hall, B.M., Tran, G.T., Robinson, C.M., Hodgkinson, S.J., 2015. Induction of antigen specific CD4(+)CD25(+)Foxp3(+)T regulatory cells from naïve natural thymic derived T regulatory cells. Int. Immunopharmacol. 28 (2), 875–886.

Jagannathan-Bogdan, M., McDonnell, M.E., Shin, H., Rehman, Q., Hasturk, H., Apovian, C.M., Nikolajczyk, B.S., 2011. Elevated proinflammatory cytokine production by a skewed T cell compartment requires monocytes and promotes inflammation in type 2 diabetes. J. Immunol. 186 (2), 1162–1172.

Kakehashi, S., Stanley, H.R., Fitzgerald, R.J., 1965. The effects of surgical exposures of dental pulps in germ-free and conventional laboratory rats. Oral Surg. Oral Med. Oral Pathol. 20, 340–349.

Mattera, M.S.L.C., Chiba, F.Y., Lopes, F.L., Tsosura, T.V.S., Peres, M.A., Brito, V.G.B., de Oliveira, S.H.P., Pereira, R.F., Marani, F., Dos Santos, R.M., Belardi, B.E., Tessarin, G.W.L., Benites, M.L., Ervolino, E., Heimann, J.C., Sumida, D.H., 2019. Effect of maternal periodontitis on GLUT4 and inflammatory pathway in adult offspring. J Periodontol. 90 (8), 884–893.

Mesquita, D., Cruvinel, W.M., Câmara, N.O., Kállas, E.G., Andrade, L.E., 2009. Autoimmune diseases in the TH17 era. Braz J. Med. Biol. Res. 42 (6), 476–486.

Moller, D.E., Flier, J.S., 1991. Insulin resistance–mechanisms, syndromes, and implications. N. Engl. J. Med. 325 (13), 938–948.

O'Rourke, R.W., White, A.E., Metcalf, M.D., Winters, B.R., Diggs, B.S., Zhu, X., Marks, D.L., 2012. Systemic inflammation and insulin sensitivity in obese IFN-γ knockout mice. Metabolism 61 (8), 1152–1161.

Pereira, R.F., Cintra, L.T.A., Tessarin, G.W.L., Chiba, F.Y., de Lima Coutinho Mattera, M.S., Scaramele, N.F., Sumida, D. H., 2017. Periapical Lesions Increase Macrophage Infiltration and Inflammatory Signaling in Muscle Tissue of Rats. J. Endod. 43 (6), 982-988.

Ricardo-Gonzalez, R.R., Red Eagle, A., Odegaard, J.I., Jouihan, H., Morel, C.R., Heredia, J.E., Chawla, A., 2010. IL-4/STAT6 immune axis regulates peripheral nutrient metabolism and insulin sensitivity. Proc. Natl. Acad. Sci. USA 107 (52), 22617–22622.

Rocha, V.Z., Folco, E.J., Sukhova, G., Shimizu, K., Gotsman, I., Vernon, A.H., Libby, P., 2008. Interferon-gamma, a Th1 cytokine, regulates fat inflammation: a role for adaptive immunity in obesity. Circ. Res. 103 (5), 467–476.

Sakaguchi, S., Mikami, N., Wing, J.B., Tanaka, A., Ichiyama, K., Ohkura, N., 2020. Regulatory T Cells and Human Disease. Annu. Rev. Immunol. 38, 541–566.

Sasaki, H., Hirai, K., Martins, C.M., Furusho, H., Battaglino, R., Hashimoto, K., 2016. Interrelationship Between Periapical Lesion and Systemic Metabolic Disorders. Curr. Pharm. Des. 22 (15), 2204–2215.

Satoh, M., Iwabuchi, K., 2018. Role of Natural Killer T Cells in the Development of Obesity and Insulin Resistance: Insights From Recent Progress. Front Immunol. 9, 1314.

Schulze, A., Schönauer, M., Busse, M., 2007. Sudden improvement of insulin sensitivity related to an endodontic treatment. J. Periodontol. 78 (12), 2380–2384.

Shieh, S.J., Varkey, P., Chen, P.Y., Chang, S.Y., Huang, L.L., 2014. Counting CD4(+) and CD8(+) T cells in the spleen: a novel in vivo method for assessing biomaterial immunotoxicity. Regen. Biomater 1 (1), 11–16.

Stashenko, P., Teles, R., D'Souza, R., 1998. Periapical inflammatory responses and their modulation. Crit. Rev. Oral Biol. Med. 9 (4), 498–521.

Stolarczyk, E., Lord, G.M., Howard, J.K., 2014. The immune cell transcription factor T-bet: A novel metabolic regulator. Adipocyte 3 (1), 58–62.

Szabo, S.J., Kim, S.T., Costa, G.L., Zhang, X., Fathman, C.G., Glimcher, L.H., 2000. A novel transcription factor, T-bet, directs Th1 lineage commitment. Cell 100 (6), 655–669.

Tanriver, Y., Diefenbach, A., 2014. Transcription factors controlling development and function of innate lymphoid cells. Int. Immunol. 26 (3), 119–128.

Tavares BS, Tsosura T, Mattera M, Santelli JO, Belardi BE, Chiba FY, Cintra L, Cantiga-Silva C, Matsushita DH. 2021 Effects of melatonin on insulin signaling and inflammatory pathways of rats with apical periodontitis. Int Endod J. doi: 10.1111/iej.13474. Epub ahead of print. PMID: 33411973.

Tsosura, T.V.S., Chiba, F.Y., Mattera, M.S.L.C., Pereira, R.F., Cintra, L.T.A., Conti, L.C., Santos, R.M.D., Mateus, J.H.P., Garbin, C.A.S., Sumida, D.H., 2019. Maternal apical periodontitis is associated with insulin resistance in adult offspring. Int Endod J. 52 (7), 1040–1050.

Wada, T., Hoshino, M., Kimura, Y., Ojima, M., Nakano, T., Koya, D., Sasaoka, T., 2011. Both type I and II IFN induce insulin resistance by inducing different isoforms of SOCS expression in 3T3-L1 adipocytes. Am. J. Physiol. Endocrinol. Metab. 300 (6), E1112–E1123.

Wright, B.L., Nguyen, N., Shim, K.P., Masterson, J.C., Jacobsen, E. A., Ochkur, S.I., Furuta, G.T., 2018. Increased GATA-3 and T-bet expression in eosinophilic esophagitis versus gastroesophageal reflux disease. J. Allergy Clin. Immunol. 141 (5), 1919–1921. e1915.

Xia, C., Rao, X., Zhong, J., 2017. Role of T Lymphocytes in Type 2 Diabetes and Diabetes-Associated Inflammation. J. Diabetes Res. 2017, 6494795.

Yadav, H., Quijano, C., Kamaraju, A.K., Gavrilova, O., Malek, R., Chen, W., Rane, S.G., 2011. Protection from obesity and diabetes by blockade of TGF-β/Smad3 signaling. Cell Metab. 14 (1), 67–79.

Yong, J., Chen, G.Q., Huang, B., Wu, S., 2011. Correlation between the ratio of T-bet/GATA-3 and the levels of IL-4 and IFN-γ in patients with allergic asthma. Mol. Med. Rep. 4 (4), 663–666.

Zeng, L., Lu, H., Deng, H., Mu, P., Li, X., Wang, M., 2012. Noninferiority effects on glycemic control and β-cell function improvement in newly diagnosed type 2 diabetes patients: basal insulin monotherapy versus continuous subcutaneous insulin infusion treatment. Diabetes Technol. Ther. 14 (1), 35–42.

Zhang, H., Bain, J.L., Caskey, C.P., Sandifer, L.C., Johnson, R.B., 2011. Effects of gender on serum biomarkers of systemic inflammation coincident to experimentally-induced periapical lesions. Arch. Oral Biol. 56 (2), 168–176.

Zhang, J., Huang, X., Lu, B., Zhang, C., Cai, Z., 2016. Can apical periodontitis affect serum levels of CRP, IL-2, and IL-6 as well as induce pathological changes in remote organs? Clin. Oral Investig. 20 (7), 1617–1624.

Zheng, Y., Rudensky, A.Y., 2007. Foxp3 in control of the regulatory T cell lineage. Nat. Immunol. 8 (5), 457–462.