

Subject: [BDS] Editor Decision

Dear Caroline Andrade Maia, Gabriele Maia, José Alex da Silva, Isabella Peixoto, Warley Tavares, Bruno Guimarães, Carlos Bueno, Leopoldo Cosme-Silva, Francine Benetti:

Your submission Cyclophosphamide-induced immunomodulation as a potential modifier of apical periodontitis: a hypothesis-based study to Brazilian Dental Science, has been revised and according to reviewers' comments, there are questions to be addressed and/or points to be clarified/corrected. Please answer the reviewers considerations point-by-point in a separate document and also please make all the corrections in the text highlighted in yellow.

Deadline: 30 days

Thank you for considering Brazilian Dental Science for publishing your research. We are looking forward the revised version of you manuscript.

Sincerely,

Reviewer B:

Recommendation: Accept Submission

Questionnaire

Does the manuscript contain new and significant information to justify publication?*

Yes

Does the Abstract (Summary) clearly and accurately describe the content of the article?

Yes

Is the problem significant and concisely stated?

Yes

Are the methods or research design described comprehensively? Is the statistical analysis adequate?

Yes

Are the interpretations and conclusions justified by the results?

Yes

Is adequate reference made to other work in the field?

Yes

Is the language acceptable?

Yes

Manuscript Structure

Length of article is:*

Adequate

Number of tables is:

Adequate

Number of figures is:

Adequate

Please state any conflict(s) of interest that you have in relation to the review of this paper (state “none” if this is not applicable).

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Rating

Interest*

Good

Quality

Excellet

Originality

Excellent

Overall

Excellent

Recommendation

Accept

Would you be willing to review a revision of this manuscript?

Yes

Comments

Comments to the Author

Reviewer C:

Recommendation: Revisions Required

Questionnaire

Does the manuscript contain new and significant information to justify publication?*

Yes

Does the Abstract (Summary) clearly and accurately describe the content of the article?

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Are the methods or research design described comprehensively? Is the statistical analysis adequate?

No

Are the interpretations and conclusions justified by the results?

No

Is adequate reference made to other work in the field?

Yes

Is the language acceptable?

Yes

Manuscript Structure

Length of article is:*

Too Short

Number of tables is:

Too Short

Number of figures is:

Too Short

Please state any conflict(s) of interest that you have in relation to the review of this paper (state “none” if this is not applicable).

None

Rating

Interest*

Good

Quality

Good

Originality

Good

Overall

Good

Recommendation

Major Revision

Would you be willing to review a revision of this manuscript?

Yes

Comments

Comments to the Author

The manuscript proposes a plausible and clinically relevant hypothesis at the interface of cancer immunopharmacology and endodontics: that cyclophosphamide (CPD)-induced modulation of T-lymphocyte subsets alters the immunoinflammatory response in apical periodontitis (AP). The topic is timely, the writing is generally clear, and the reference base is reasonable. However, the central argument suffers from an unresolved internal contradiction regarding the direction of the proposed effect, the hypothesis as stated is too broad to be falsifiable, and several claims and references require correction. I would categorize this as requiring major revision before it can be considered further.

1- The manuscript does not reconcile the two opposing pharmacological actions of CPD, and this undermines the core hypothesis. The text correctly notes that low-dose CPD is immunostimulatory (depleting regulatory T cells and shifting responses toward a Th1/Th17 pro-inflammatory phenotype), whereas high doses produce broad lymphodepletion and immunosuppression. These two regimens predict opposite outcomes in AP: a Treg-depleting, Th17-enhancing effect should exacerbate inflammatory bone resorption, whereas generalized immunosuppression should reduce inflammatory expression. The paper invokes both mechanisms but treats them as if they support a single hypothesis. The authors should explicitly state which dose regimen and which net effect they are proposing, and either commit to one directional prediction or formally present a dose-dependent, biphasic model with separate predictions for low- versus high-dose exposure.

2 - As currently worded, the hypothesis is not falsifiable. The statement that CPD

"may influence lesion development, progression, radiographic expression, and periapical healing, potentially masking disease severity or modifying treatment outcomes" accommodates an increase, a decrease, or no change in essentially any parameter. A hypothesis paper should advance a specific, directional, and testable prediction. I recommend the authors reformulate the hypothesis into one or two concrete predictions (for example, "low-dose CPD increases periapical bone resorption via Treg depletion and Th17 expansion" and/or "high-dose CPD reduces the radiographic size of periapical lesions despite ongoing tissue-level inflammation") and state what experimental or clinical observation would refute each.

3 - The "masking" claim leans too heavily on references 28–30, which involve mixed immunomodulators (not CPD) and show inconsistent results. Soften this, note opposing findings, and distinguish reduced radiographic detectability from reduced disease activity.

4 - Address confounding. CPD patients also receive corticosteroids, other chemotherapeutics, and antibiotics, and have altered oral health, so AP changes can't be attributed to CPD alone. Acknowledge this and indicate how proposed studies would control for it.

5 - Make "Future directions" concrete. Given the group's existing induced-AP mouse model (ref. 21), propose a specific design, dose, timing, and readouts (micro-CT, Treg/Th17, RANKL/OPG, key cytokines) that would directly test the hypothesis.

6 - Duplicate references: 7 and 26 are identical; 6 and 14 are the same source with different years. Consolidate and renumber.

7 - Standardize terminology: capitalize "T lymphocytes"/"T cells" throughout; make English and Portuguese keyword capitalization consistent.

8 - Figure 1 is cited but has no legend, and I couldn't evaluate it. Include the figure and a self-explanatory legend.

Reviewer E:

Recommendation: Revisions Required

Questionnaire

Does the manuscript contain new and significant information to justify publication?*

Yes

Does the Abstract (Summary) clearly and accurately describe the content of the article?

Yes

Is the problem significant and concisely stated?

Yes

Are the methods or research design described comprehensively? Is the statistical analysis adequate?

No

Are the interpretations and conclusions justified by the results?

No

Is adequate reference made to other work in the field?

Yes

Is the language acceptable?

Yes

Manuscript Structure

Length of article is:*

Too Short

Number of tables is:

Too Short

Number of figures is:

Too Short

Please state any conflict(s) of interest that you have in relation to the review of this paper (state “none” if this is not applicable).

None

Rating

Interest*

Good

Quality

Good

Originality

Good

Overall

Good

Recommendation

Major Revision

Would you be willing to review a revision of this manuscript?

Yes

Comments*Comments to the Author*

The manuscript addresses an interesting and clinically relevant topic by proposing a potential interaction between cyclophosphamide-induced immunomodulation and apical periodontitis. The biological rationale is generally plausible and the manuscript is well organized. However, several conceptual and methodological issues limit the strength of the proposed hypothesis. I recommend major revision before further consideration.

Major comments

1. The manuscript overemphasizes T-cell modulation while underrepresenting the complexity of apical periodontitis pathogenesis.

Although T lymphocytes are important regulators of periapical inflammation, AP is a multifactorial disease involving extensive participation of innate immune cells, macrophages, neutrophils, dendritic cells, osteoclasts, and numerous cytokine networks. The manuscript sometimes gives the impression that T-cell modulation alone could explain major changes in lesion behavior. The authors should better contextualize the relative contribution of T cells within the broader osteoimmune response involved in AP.

2. The proposed mechanism linking CPD-induced Treg depletion to AP progression remains speculative and lacks direct supporting evidence.

The manuscript extrapolates findings from oncology and immunotherapy literature to the periapical environment without presenting evidence that the same immunological shifts occur within periapical lesions. The authors should explicitly acknowledge that no direct experimental evidence currently demonstrates CPD-induced modulation of the Treg/Th17 balance in AP lesions and discuss this

limitation more clearly.

3. Greater attention should be given to the distinction between systemic and local immune responses.

Several sections assume that systemic immunomodulation induced by CPD will necessarily translate into comparable alterations in the local periapical microenvironment. However, local immune regulation within AP lesions is strongly influenced by persistent microbial stimulation and tissue-specific signaling pathways. The authors should discuss potential discrepancies between systemic immune alterations and local periapical immune responses.

4. The hypothesis would benefit from inclusion of alternative biological scenarios.

The manuscript predominantly discusses mechanisms that could aggravate inflammatory bone destruction. However, CPD-induced lymphodepletion could theoretically reduce immune-mediated tissue destruction in some contexts. A balanced discussion of competing mechanisms would strengthen the scientific rigor of the hypothesis.

5. The section on radiographic masking requires additional mechanistic justification.

The hypothesis that CPD may mask radiographic expression of AP is intriguing but currently insufficiently developed. Radiographic lesion size is largely determined by mineralized tissue changes rather than immune-cell activity alone. The authors should provide a clearer biological pathway linking CPD-mediated immune alterations to reduced radiographic detectability.

Minor comments

6. Terminology should be standardized throughout the manuscript.

Terms such as “T lymphocytes”, “T cells”, “regulatory T cells”, “Tregs”, and “T-cell subsets” should be used consistently.

7. Some statements require additional references.

For example, the assertion that CPD-induced immune modulation may alter radiographic expression and diagnostic detectability of AP appears insufficiently supported by the currently cited literature.

8. Figure 1 is cited in the Introduction; however, it was not available in the version of the manuscript provided for review. Therefore, it was not possible to evaluate its scientific content, quality, or relevance to the proposed hypothesis. Please ensure that the figure and its corresponding legend are included in the revised submission.
