Reviewer's report

Title: Prevalence of Epstein-Barr virus DNA and Porphyromonas gingivalis in Japanese periimplantitis patients

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Reviewer: Jannet Katz

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Title: Prevalence of Epstein-Barr virus DNA and Porphyromonas gingivalis in Japanese periimplantitis patients

Main concerns:

1. The authors showed in 2013, (PLoS ONE 8(8): e71990) the presence of EBV and P. gingivalis DNA in deep periodontal pockets of patients with chronic periodontitis (CP). In the manuscript under review, the authors showed the presence of EBV and P. gingivalis DNA in deep periodontal pocket of peri-implantitis (PI) patients. Thus, it is unclear what new information is being provided in the current manuscript. Assessing differences in the cytokines that may be present in the deep periodontal pockets of the different groups of people evaluated in the study under review, would have provide novelty. Furthermore, it would have been intriguing to determine if vIL-10 is present in the deep periodontal pockets evaluated, since EBV is known to encode this immunomodulatory cytokine.

Furthermore, the association between herpes viruses and periodontal pathogens like P. gingivalis has been shown in previous publications (appropriately cited by the authors), hence making it more difficult to determine the novelty of the study under review. Moreover, while the authors suggest an association between EBV and P. gingivalis that likely contributes to the etiology and pathogenesis of periodontal disease, they do not show or discuss how EBV promotes the etiology and development of periodontal disease.

2. EBV is transmitted through saliva and studies have shown that gingival epithelial cells are infected with EBV and may serve as an oral reservoir of latent EBV-infected cells. Moreover, over 90% of the population is infected with EBV and infection of EBV is widespread and not limited to the periodontal foci. Thus, it would be interesting to determine the presence and quantity of EBV in the saliva of healthy periodontal patients, healthy PI patients and PI patients. Would there be a difference in the levels of EBV in these groups? Would a higher viral load occur in PI patients?
3. Based on a paper published by one of the authors in 2012 (Biochimie 94, 839-846), the authors suggest in the manuscript under review (Discussion section), that the presence of P. gingivalis reactivated the latent EBV due to the butyric acid produced by P. gingivalis. Butyric acid was shown to be an inhibitor of HDAC, consequently increasing histone acetylation and the transcriptional activity of the BLZF1 gene, which is a marker of active EBV. While activation of EBV by sodium butyrate has been used for many, many years, the novelty of their paper rested on the interesting observation that P. gingivalis via the butyric acid it produces in the supernatant could drive EBV to induce lytic replication determined by the transcriptional activity of the BLZF1 gene. However, all the studies in this published work were done in vitro. In the manuscript under review, the amount of butyric acid in the deep periodontal pockets was not assessed, thus, the suggested reactivation of EBV by P. gingivalis is at best speculative. Assessing the levels of butyric acid in the deep periodontal pockets may be a very difficult task, but the authors could estimate the number of P. gingivalis from the real PCR data obtained in the deep periodontal pockets of the different patients and set up the correspondent P. gingivalis cultures to determine the amount of butyric acid present in the supernatants. The supernatants could then be used to determine if they activate latent EBV in a Daudi (or AKATA) cell line by assessing the transcriptional activity of BLZF, RTA, or gp350 expression.

Minor concerns:

1. The authors should obtain assistance from a fluent English speaking person to edit their manuscript as there are some unclear statements.

2. The authors mentioned that PI provokes bone destruction with suppuration (SUP), through peri-implant mucositis (PM), but in the Methods section, they stated that PM "was defined as bleeding on gentle probing" and PM is never used again in the manuscript. Therefore, the term peri-implant mucositis (PM) should be altogether eliminated.

3. It is puzzling why "healthy sites of PPD" (healthy sites do not have deep periodontal pockets) or better said healthy gingival sulcus was to be less than 3 mm among the healthy controls, but the healthy implant sites were accepted to be less than 4 mm.

4. While in general there is room for speculation based on results obtained and published literature, in the manuscript under review, the Discussion is highly speculative without foundation as the only thing that was assessed was the DNA of EBV and P. gingivalis in
PI patients and the copy numbers of these were compared with that found in healthy periodontal patients and in healthy PI patients.

5. Tables lack footnotes. I assume that ND=Not detected? Table 1 indicates that 45, instead of 5 healthy control males were used.

Are the methods appropriate and well described?
If not, please specify what is required in your comments to the authors.

Yes

Does the work include the necessary controls?
If not, please specify which controls are required in your comments to the authors.

Yes

Are the conclusions drawn adequately supported by the data shown?
If not, please explain in your comments to the authors.

Yes

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I am able to assess the statistics

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