Reviewer's report

Title: Increase of Telomere Length in Lymphocytes T of Adults of different ages, stimulated with Concanavalin A.

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Reviewer: Mariani Erminia

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The manuscript “Increase of Telomere Length in Lymphocytes T of Adults of different ages, stimulated with Concanavalin A. “ by Blanca Murillo-Ortiz et al. reports the data concerning the comparison of proliferative potential of lymphocytes (unstimulated and Con A stimulated) obtained from young and old subjects and the modifications, induced by the two culture conditions, on telomere length.

Major Compulsory Revisions

1. In general, the data are obtained on PBMC but presented (title, abstract, aim, results) and discussed as if they were obtained on T cells.

2. Abstract
   a. Background: is missing
   b. Results: “old adults samples showed higher loss of telomeric DNA (P = 0.04)” does not correspond to the results in Table 1 that report p=0.03.
   “Viability cell counts” are not reported in the paper.
   “O PBMC had shorter telomeres than group Y (P = 0.04)” does not correspond to Table 1 in which this value of p is related to the comparison before/after stimulation of the young group.

3. Introduction
   Last sentence: the aim concerns T lymphocytes, but the study was conducted on PBMC.

4. Material and methods
   a. T cell isolation and culture: T cells were neither isolated nor used in culture. Total PBMC were used.
   b. Flow cytometry analysis: is not clear the labeling with CD25 monoclonal antibody, because no data concerning this marker are reported in the paper.
   c. Telomeric measurement: sentence 2, “DNA samples were extracted from white blood cells” this is in contrast with the previous description of cell preparation.
   d. Statistics: if medians are used, they match with ranges or percentiles, while the indication of the SE is correct if the data are reported as means.
5. Results

a. Telomeric length in lymphocytes before and after stimulation with Con A:
   sentence 3 “Consequently, …… (Group O)…..telomeric DNA (p=0.04)” in the
   table 1 this p value is related to the comparison before/after stimulation in Group
   Y.

b. Sentence 4 is contradictory, the Group Y is reported to have “an increased
telomere length” after ConA , “no changes in telomeric lenght… with Con A
stimulus ” and again “capable to increase the telomere length”. In Table 1, a
significant increase (p=0.04) is reported in the Y Group following Con A
stimulation.

c. Lymphocyte proliferation and replicative potential: last line p=0.04 is related to
Table 1 and not to Figure 1 and in any case it represents the p value of the
comparison between culture conditions within the Y Group and non between O
and Y Groups.

6. Discussion

a. Paragraph 5 (“The loss of genome…”) sentence “Here, we reported that
un-stimulated T lymphocytes from group O have shorter telomeres than group
Y”. Even if the amount of T lymphocytes is prevalent among PBMC, telomere
analysis was not performed on purified T lymphocytes.

b. Following sentence: “….a 5 kb increase in telomeric length was found in T
lymphocytes from group after In vitro Con A stimulation”. For T lymphocyte , see
the previous comment. It is not clear from which group. As concerning the
increase, in Table 1, a stable difference of about 6 Kb is reported between O and
Y groups and neither O nor Y telomeres extended of 5Kb after stimulation.

7. Figure 1 and Table 1 legends: data are presented as medians and SE: see the
comment in material and methods-statistic. However, supposing that the figure 1
reports means instead of medians, the SE are not reported.

Minor Essential Revisions

1. References: some publication years and pages are wrong or missing,
   reference n.31 is in press from 2002.

Level of interest: An article whose findings are important to those with closely
related research interests

Quality of written English: Needs some language corrections before being
published

Statistical review: Yes, and I have assessed the statistics in my report.

Declaration of competing interests:
I declare that I have no competing interests