Author’s response to reviews

Title: Lower CD28+ T cell proportions were associated with CMV-seropositivity in patients with Hashimoto’s thyroiditis

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Author’s response to reviews: see over
Dear editor,

thank you for the possibility to send a revised version of the manuscript MS 1582222207880279 “Lower CD28+ T cell proportions were associated with CMV-seropositivity in patients with Hashimoto’s thyroiditis”.

All comments were addressed in the revised version of the manuscript. All questions raised by the reviewers could be answered, accordingly. Changes within the manuscript are colored in yellow. The responses to the reviewers are given below.

All co-authors read the manuscript and agreed to its content.

Thank you in advance.

Best regards,

Martina Prelog
Lower CD28+ T cell proportions were associated with CMV-seropositivity in patients with Hashimoto’s thyroiditis

Comments to reviewers:

Reviewer: Roman Junik

Minor essential revisions:

Methods:

Study population:

We agree with the reviewer that the term “healthy according to WHO definition” is misleading. Immunological healthy controls were recruited at the outpatient clinic and consisted of patients who were scheduled for elective surgery (plastic surgery, hernia etc.). We changed the sentence to “Immunological HC scheduled for elective surgery (e.g. hernia or plastic surgery)...” (see page 5, revised manuscript).

The spelling of “fluoresceinisothiocyanat” was changed accordingly (see page 6, revised manuscript).

According to the suggestion of the reviewer, we transferred the paragraph on description of molecules/cells and of TRECs to the Background section (see page 4 and 5, revised manuscript).

Results:

We thank the reviewer for raising this important point. We agree that the wide age range in the subjects may limit comparison.

To get a better age-matching, we adjusted the mean and median age of HT by excluding 8 older HT patients and by including 6 recently diagnosed (disease duration <6 months) HT patients (see table 1, revised manuscript). Consequently, all parameters investigated in the HT group were recalculated and a new statistical analysis was performed. With this approach, for many parameters results became clearer. The recently diagnosed HT group was a very interesting subgroup of patients, worth to be described separately in the revised manuscript.

Table 1: Six HT patients had TSH concentrations above the age range (see page 12, revised manuscript). We could not find any significant difference regarding lymphocyte proportions between hypo- and euthyroid HT patients. This information was added to the revised version of the manuscript (see page 13).
Additionally: L-thyroxine means levothyroxine. This was changed in the revised version of the manuscript (page 7). Fourteen patients were treated with levothyroxine.
Reviewer: Kouki Mori

1. Patients

To get a better age-matching, we adjusted the mean and median age of HT by excluding 8 older HT patients and by including 6 recently diagnosed (disease duration <6 months) HT patients (see table 1, revised manuscript). Consequently, all parameters investigated in the HT group were recalculated and a new statistical analysis was performed. With this approach, for many parameters results became clearer. The recently diagnosed HT group was a very interesting subgroup of patients, worth to be described separately in the revised manuscript.

HT patients with other autoimmune diseases were not included into the study (see page 6, revised manuscript).

2. Methods

According to the suggestions of the reviewer, methods for identification of TREC's and relative telomere lengths were described in detail in the revised manuscript (see pages 7-9, revised manuscript). Negative controls were included into PCR and immunohistochemistry (see pages 7-10 and figures 4 and 5, revised manuscript).

3. Discussions

CMV seropositivity is not different in HT patients compared to other autoimmune diseases. This information was added to the revised version of the manuscript (see table 1 and page 12).

The significance of the alterations was discussed in relation to the pathogenesis of HT (see page 15, revised manuscript).

Studies were presented which had demonstrated that HT may result from local autoimmune mechanisms in the thyroid gland (see page 15, revised manuscript).
Reviewer: Elisabetta Caselli

Major Compulsory Revisions

1. We thank for this criticism and re-wrote this sentence in the first line of the discussion (see page 15, revised manuscript), which was also suggested by another reviewer. We agree that the association between CMV and immune dysregulation is well known particularly in elderly persons. Our work corroborates that virus infection is related to an altered proportion of peripheral lymphocytes. However, we think that CMV rather accelerates previously existing changes of T cell proportions caused by autoimmunity and is not primarily involved in development of thyroid autoimmunity. These aspects were also addressed in further experiments (CMV-specific lymphocytes in ELISPOT assay, CMV in situ hybridization), as suggested by the reviewer, and discussed in the revised version of the manuscript (see page 16).

2. We thank for raising this important point. We do not think that CMV infection is associated with the development of Hashimoto’s disease, but the study demonstrated that patients with HT show alterations of the peripheral T cell proportions also characteristically found in other autoimmune diseases. These changes seem to be accelerated by CMV seropositivity, as seen in other autoimmune diseases. We tried to make this clearer in the discussion section of the revised version of the manuscript (see page 16).

3. This is absolutely true and was stated in the discussion section of the revised version of the manuscript (see page 16).

4. We thank for this suggestion and included results from CMV in situ hybridization into the revised manuscript (see page 14, figure 5).

5. As suggested by the reviewer, we performed ELISPOT analysis in the 5 CMV-seropositive HT patients and 5 HC. A normal response was found for all HT patients and HC (see page 14, figure 3). As discussed, we suggest that CMV infection is not the primarily cause of HT, but that the alterations which were associated with CMV may be an epiphenomenon of autoimmunity (see page 16).

Discretionary Revisions:

1. To get a better age-matching, we adjusted the mean and median age of HT by excluding 8 older HT patients and by including 6 recently diagnosed (disease duration <6 months) HT patients (see table 1, revised manuscript). Consequently, all parameters investigated in the HT group were recalculated and a new statistical analysis was performed. With this approach, for many parameters results became clearer. The recently diagnosed HT group was a very interesting subgroup of patients, worth to be described separately in the revised manuscript.
2. There was no difference in CMV-seropositivity between HT patients (positive: 27.8%) and HC (positive: 30.0%) (see table 1 and page 12, revised manuscript).

3. IgM responses were included into the revised version of the manuscript (see page 12). However, only one HT patient and 3 HC were positive for IgM.

Minor essential revisions:

The abstract and the background were re-written, accordingly.