Author's response to reviews

Title: Cytokine and Immunoglobulin Production by PWM-stimulated Peripheral and Tumor-Infiltrating Lymphocytes of Undifferentiated Nasopharyngeal Carcinoma (NPC) Patients

Authors:

Lilia Fliss-Jaber (lilia.fliss@laposte.net)
Radhia Houissa-Kastally (radhia.kastally@rns.tn)
Kamel Bouzouita (kamel.bouzouita@rns.tn)
Naceur Khediri (lilia.fliss@laposte.net)
Ridha Khelifa (khelifa.ridha@rns.tn)

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RESPONSE TO REVIEWERS' COMMENTS

RESPONSE TO REVIEWER 1 (L. Young)

GENERAL

We have now modified the text on page 4, 4th paragraph in "Background" section, to remove the apparent confusion "between the ability to successfully isolate EBV-specific cytotoxic T lymphocytes (CTLs) from NPC patients and the activity of these cells in cytotoxicity assays".

MAJOR COMPULSORY REVISIONS

We recognize "the inevitable variability in the lymphocyte populations used in these studies..." as stated by Reviewer 1. Indeed, we have considered this aspect during our work but could not demonstrate any significant correlations between individual differences in the proportions of the various cell subsets present in the lymphocyte cultures and cytokine or immunoglobulin production following PWM stimulation in vitro. We have now addressed this question in the manuscript by adding our data on phenotypic analysis of PBL and TIL in a new paragraph entitled "Immunophenotyping of lymphocyte preparations" in the "Results" section (2nd paragraph) and discussed the possible effects of individual lymphocyte subset variations on our results (Discussion, page 15). We also indicated the method used for immunophenotyping at the end of the section on "Lymphocyte preparations" in "Methods" (page 7).

RESPONSE TO REVIEWER 2 (G. Niedobitek)

MAJOR COMPULSORY REVISIONS

1. We have now improved the description of the lymphocyte preparations and unified this description for PBL and TIL under a unique heading. We also added one reference (# 28) for lymphocyte separation over Ficoll.

2. As we explained in our response to the major comments of Reviewer 1, we recognize the importance of the composition of our lymphocyte preparations. However, we did not include lymphocyte immunophenotyping data in the first version of our manuscript because we could not find any significant correlations between these data and the differences in cytokine and immunoglobulin production observed. We have now inserted pertinent immunophenotyping data in a new paragraph entitled "Immunophenotyping of lymphocyte preparations" in the "Results" section, 2nd paragraph. We also discussed these data in relation to the observed differences in cytokine and immunoglobulin production at the end of the Discussion section, page 15. In addition, we indicated the method used for immunophenotyping at the end of "Lymphocyte preparations" in the Methods section.

3. We have chosen to use PWM in our work because in humans, this mitogen is able to stimulate both T and B lymphocytes. Its ability to induce cytokine synthesis by T lymphocytes is similar to that of PHA.
The use of PWM allowed us to study the responses of both T and B lymphocytes simultaneously in each culture. We have now addressed this point in "Discussion" on top of page 14.

4. We have now updated the references quoted in "Discussion" as suggested by Reviewer 2 and discussed the possible role of regulatory T cells in the increase of IL-10 in TIL, and the possible role of LMP1 in inducing regulatory T cells.

MINOR ESSENTIAL REVISIONS

1. Reference 20 (Lassoued et al.) is now removed as suggested by Reviewer 2.

2. Serological data of patients and controls are now included in the first paragraph in "Results", page 10. We also indicated the serological method used at the end of the 2nd paragraph in "Methods".

3. We have now clarified the number of biopsy samples and indicated that biopsies and peripheral blood were collected on the same day from each of the 17 patients (at the beginning of the "Lymphocyte preparations" section).

4. Dilution figures for PWM have now been removed (section on "Stimulation by PWM"; page 8).

5. The controversial aspect of IL-10 expression by NPC tumor cells has now been included, and the corresponding reference quoted accordingly (in "Background", page 5, 2nd paragraph and "Discussion", page 12, 2nd paragraph).

DISCRETIONARY REVISIONS

We have now corrected the ambiguous statement about EBNA3 in the 4th paragraph on page four. The word "usually" has been removed.

RESPONSE TO REVIEWER 3 (KW Lo)

MINOR ESSENTIAL REVISIONS

We have now improved the figures by correcting the indications on the horizontal axes and in the legends. However, we did not group the figures together since, according to the Instructions for BMC Cancer Authors, each figure should be on a separate page.

DISCRETIONARY REVISIONS

We have now included EBV serology data in the manuscript (Results, 1st paragraph, page 10) and indicated the serological method used in "Methods" at the end of "Patients and Controls" section. All 17 patients showed a typical EBV antibody pattern characteristic of NPC. All patients were histopathologically confirmed undifferentiated NPC, as now indicated in the beginning of "Patients and Controls" section (page 6).

Concerning the EBV status of the tumor samples used, we believe that given the typical serological profile of the patients, we can safely assume all these tumors to be EBV positive. In addition, we are not aware of any case of undifferentiated NPC tumor ever found negative for EBV in Tunisia. These are the main reasons why we did not look for EBER by in situ hybridization in the tumor biopsy samples.