Author’s response to reviews

Title: Effect of Roflumilast on Inflammatory Cells in the Lungs of Cigarette Smoke-Exposed Mice

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Author’s response to reviews:

Re: Manuscript manuscript #7958378391955622 “Effect of Roflumilast on Inflammatory Cells in the Lungs of Cigarette Smoke-Exposed Mice”

Dear Editor,

The abovementioned manuscript has been corrected according to your comments and to the suggestions of the reviewers. The revised paper has been revised also by a native English speaking colleague.

Please find attached:

- a version of the Revised Manuscript
- a point-by-point response to the reviewers’ comments, organized as suggested in your cover letter

Reviewer: Joanne L. Wright

Major compulsory revisions:

“The authors should examine apoptosis and relate these data to the inflammatory cells and response to roflumilast”.

Response (R)

There is no doubt at the present time that inflammation is necessary for development of emphysema smoking mice. Unfortunately, differently to other mouse strains (such as DBA/2 mouse), which show significant apoptosis after cigarette smoke exposure, apoptosis does not characterize the lung changes induced by cigarette smoke exposure in C57 Bl/6J mice. In particular, in this strain we were not able to detect at various times after CS exposure caspase 3 positive cells, and only very few cells showed a TUNEL positive reaction.
(Bartalesi et al. ERJ, 2005). Thus, why should one examine apoptosis?

Minor essential revision:

page 7. “This effect is slightly greater than what has been previously reported”.

R. The data given are correct. In fact, in the previous study 5 mice/group were used in the macrophage groups. In the present study the number of animals in the group “smoke: macrophages” was 7; in the group “smoke+R1: macrophages” was 6 and in the group “smoke+R5: macrophages” was 7.

Discretionary revision:

“Barbera and colleagues noted that cigarette smoke induced an increase of B lymphocytes in the adventitia of vessels. It would be interesting to note if this was recapitulated in the present study and if roflumilast altered it in any way”.

R. No significant B lymphocytes infiltration was observed in the adventitia of pulmonary vessels in C57 smoking mice.

We thank the reviewer for the encouraging words about our study.

Reviewer: Guy G Brusselle

Minor essential revisions:

1) How specific is the immunodetection of dendritic cells by monoclonal antibody to fascin? Which subset of dendritic cells (DC) is detected? Myeloid DCs? Plasmacytoid DCs?

Response 1 (R.1)

Fascin is a 55-kD protein that is specifically expressed only in mature but not in immature dendritic cells (Al-Alwan et al., J Immunol, 166, 338, 2001) and required for actin polarization in DCs during immunologic synapse formation. Its expression is a prerequisite for full T-cells activation (Al-Alwan et al., J Immunol, 166, 338, 2001; Al-Alwan et al., J Immunol, 166, 1452, 2001; Geyeregger et al., Blood, 109, 4288, 2007). Since fascin is strongly related to DC cytoskeletal functions such as dendrite formation and required for DC-mediated T-cell stimulation, we analyzed its expression in order to assess the number of mature DCs with the potential ability to activate T-cell proliferation. The expression of this protein is highly restricted to DCs (Mosialos et al., Am J Pathol, 148, 593, 1996), and for this reason fascin is commonly used for detecting mature dendritic cells deriving from the different DC subsets (Bobryshev and Lord, J Histochem Cytochem, 47, 1481, 1999; Pinkus et al., Am J Clin Pathol, 118, 335, 2002; etc).

This information is now present in the revised paper (see pag. 6 paragraph #1).

Unfortunately, is not possible to distinguish the various subsets of DCs by using monoclonal antibody to fascin, a specific marker of DC maturation.

The investigation of the various DC subsets was out of the scope of this paper.
2) How specific is the immunohistochemistry (IHC) of CD4 for CD4+T-lymphocytes? CD4 is not only expressed on T-lymphocytes, but also on some monocytes/macrophages. Please comment this limitation in the discussion (or perform double staining IHC).

3) How specific is the IHC of CD8 for CD8+ T-lymphocytes? CD8 (especially CD8a) is not only expressed on T-lymphocytes, but also on some NK and DC subsets. Same advice as in comment 3.

R.2 and 3. The points are well taken. Accordingly, in the revised manuscript under “Discussion” on page …. this limitation has been discussed.

4) The discussion should be shortened; especially the last paragraph could be omitted.

R.4 The text has been modified according to the suggestion.

Minor comments:

Introduction: Page 4: first paragraph: dendritic cells are listed as adaptive immune cells, but are also important as innate immune defence; in fact, dendritic cells are linking innate and adaptive immunity.

R. The text has been modified according to the suggestion.

Methods: Page 5: second paragraph: please correct “Seven months after chronic exposure to …”, suggesting a seven month exposure-free interval, into “After chronic exposure to room air or cigarette smoke for seven months, …”

Discussion: Page 8: first sentence: please correct “Two are the main points of this study:”

R. The text has been modified according to the suggestion.

We thank the reviewer for his help in improving our manuscript.