Reviewer's report

Title: Contribution of different pneumococcal virulence factors to experimental meningitis in mice

Version: 1 Date: 26 June 2013

Reviewer: Denis Grandgirard

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'Contribution of different pneumococcal virulence factors to experimental meningitis in mice'

Susanna Ricci, Alice Gerlini, Andrea Pammolli, Damiana Chiavolini, Velia Braione, Sergio A Tripodi, Bruna Colombari, Elisabetta Blasi, Marco R Oggioni, Samuele Peppoloni and Gianni Pozzi BMC Infectious Diseases Research article

In the present manuscript, the authors proposed to determine the influence of PspA and PspC membrane proteins, as well as the capsule in the virulence of Streptococcus pneumoniae in meningitis, once bacteria have already reached the subarachnoid space. To this aim, they used a characterized model of intracranial inoculation of serotype 4 (TIGR) pneumococci in adult MF1 mice. A special focus was also put on the interaction between the different strains and microglia in in vitro experiments, using BV-1 murine microglia.

Experiments were well designed and appropriate to answer the proposed hypotheses.

- Major Compulsory Revisions : none

- Minor Essential Revisions.

1. In vitro assays with exposure of microglia to S.pneumoniae: it has been reported that S.pneumoniae was able to induce apoptosis in microglia cells. Has the viability of the microglia been tested over the duration of the different assays?

2. Phagolysosome acidification assay (Materials and Methods, p.7). Is it unclear how is the percentages determined? % of the total number of phagosome ? % of the total number of phagosomes which contain bacteria?

3. Mice model : what is the rationale for using 10e4 CFU for the assessment of PM by histology (p9), while bacterial titers in the brains were determined with an inoculum of 10e2?

4. Results: mouse survival, p.11. Indicate in the subheading that survival was tested over 10 days.

5. Results: histological analysis: the authors didn’t show the results for FP28 and FP262 (p14). Does it mean that there was no difference in comparison to the wild type TIGR4? In the case of a difference, these data need to be shown. Otherwise state clearly that there is no difference.

6. Discussion, p15 second paragraph: Be more precise : “Mice infected with
PspA-deficient strain FP262 showed increased survival and prolonged time-to-death FOR ALL INOCULI as well as lower viable counts in both blood and brain (24 post-infection) compared to TIGR4 FOR THE TWO LOWER DOSES OF INOCULUM”.

7. Discussion, p15, 3-5 lanes from the end: the authors stated that “the reduced adaptation of FP262 in the CNS environement at early PM stages did not affect its capability of inducing inflammation and brain damage according to histological data”. The histological data concerning FP262 are mentioned as “not shown” (page 14, see also comment 5). Furthermore, histology was performed on animals infected with a CFU of 10e4 and titers determined on animals infected with a CFU of 10e2. A dosis effect is to be considered here. Please modify the sentence accordingly.

8. Tables: for all tables (Table 1, Tables S1-4) : please indicate sample size (n) for all data

9. Legend of figure 2 : p29. There is an error concerning inoculum size for FP23, which should be 10e5 and not 10e2.

Level of interest: An article of importance in its field

Quality of written English: Acceptable

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests:

'I declare that I have no competing interests'