Author's response to reviews

Title: Expression of a Cu,Zn superoxide dismutase typical for familial amyotrophic lateral sclerosis increases the vulnerability of neuroblastoma cells to infectious injury

Authors:

  Miriam Goos (mlotz@gwdg.de)
  Wolf-Dieter Zech (wolf.zech@web.de)
  Manoj Kumar Jaiswal (manoj.jaiswal@medizin.uni-goettingen.de)
  Saju Balakrishnan (sajbal@gmail.com)
  Sandra Ebert (sebert1@gwdg.de)
  Timothy Mitchell (T.Mitchell@bio.gla.ac.uk)
  Maria Teresa Carri (carri@uniroma2.it)
  Bernhard U. Keller (bkeller@ukps.gwdg.de)
  Roland Nau (rnau@gwdg.de)

Version: 5 Date: 2 October 2007

Author's response to reviews:

Dear Dr. Kouremenou, thank you very much for your E-mail dated 17 September 2007. We found the reviewers’ comments very helpful and revised the manuscript accordingly. Please do not hesitate to contact me, if other revisions will be necessary.

In particular, the following changes were made:

Peter Monk

Major Compulsory Revisions

In order to clarify the link between damage caused by the hemolysin pneumolysin and damage caused by activation of local immune cells in pneumococcal infections, the following sentences were inserted at the end of the Background section: “During CNS infections, Streptococcus pneumoniae primarily causes damage by the direct action of the cholesterol-binding pore-forming hemolysin pneumolysin and through microglia/monocyte activation by agonists of receptors of the innate immune system, particularly Toll-like receptor 2 (TLR2). Both mechanisms may be also of importance in patients with neurodegenerative diseases during extracerebral infections.”

1. We stated in the Conclusions: “These in vitro findings must be confirmed in animal experiments and human studies, before conclusions concerning changes in treatment can be drawn.”

2. The Discussion was shortened substantially, and both types of neuronal injury (by the action of hemolysins and of inflammatory cells) were linked closely.

Minor Essential Revisions
1. With respect to a possible endotoxin contamination of pneumolysin the following paragraph was inserted into the Methods section: "Endotoxin content of purified pneumolysin was determined using the Limulus amebocyte lysate kinetic-QCL kit (Cambrex, Nottingham, United Kingdom). The purified protein had less than 0.6 endotoxin units per mg of protein, i.e. a very low level which is unlikely to have a biological effect."

2. Details on the culture of macrophages were inserted into the Methods section: "Monocytes were allowed to adhere and were then cultivated until differentiation into macrophages for 10 to 14 days as assessed by morphologic criteria like adherence of the cells and the sprouting of ramifications and functional criteria. CD-68 staining showed 98-99% purity of the macrophage cultures."

3. Since the intracellular calcium concentration is expressed as fluorescence ratio, the unit is fluorescence ratio x hours. Principally, fluorescence ratios could serve as an estimate of the intracellular calcium concentration (unit mg/l), however, the colleagues conducting this experiments strongly discouraged us to convert fluorescence ratios into estimates of calcium concentrations. For this reason, I would suggest to leave this point as it is.

4. This spelling mistake was corrected.

Gerald Muench
No revisions requested.

Gennadij Raivich
Major Compulsory Revisions
No revisions requested.

Minor Essential Revisions
In neuroblastoma cell cultures exposed to pneumolysin and appropriate control cultures the rate of apoptotic neurons was quantified by in-situ tailing and morphology. These data were incorporated in the revised version. However, due to technical difficulties we were unable to confirm apoptosis by ordered DNA laddering. Nevertheless, we would like to mention that in our manuscript apoptosis is not only confirmed by morphology and in-situ tailing, but also by immunocytochemical detection of activated caspase 3 in injured cells.

Ericka Simpson
Major Compulsory Revisions
No revisions requested.

Minor Essential Revisions
The sentence in the Abstract beginning with "Cell viability..." was corrected.

Discretionary Revisions
1. To document the role of free radicals in neuronal death caused by infection, we included data on the neuroprotective effect of N-acetyl-cysteine into this
manuscript (new Figure 3).

2. In the Conclusion we explicitly stated: ¿These in vitro findings must be confirmed in animal experiments and human studies, before conclusions concerning changes in treatment can be drawn¿. We plan to perform appropriate animal experiments in SOD G93A transgenic mice.

Hoping that the revisions performed will find your approval, I remain sincerely yours, Roland Nau