Reviewer's report

Title: Intracellular expression of toll-like receptor 4 in neuroblastoma cells and their unresponsiveness to lipopolysaccharide

Version: 3 Date: 11 October 2006

Reviewer: Holger Heine

Reviewer's report:

General

In the manuscript from Hassan et al. “Intracellular expression of toll-like receptor 4 in neuroblastoma cells and their unresponsiveness to lipopolysaccharide”, the authors accredit the failure of two neuroblastoma cell lines to respond LPS to the intracellular localization of TLR4 or the lack of expression of IRF-3. This conclusion is drawn from experiments that appear to show the lack of NF-kB activation, phosphorylation of IRF-3 and phosphorylation of MAP kinases such as p38 and JNK after stimulation with LPS. However, there are some concerns regarding these experiments that should be properly addressed before the manuscript can be accepted.

Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)

Fig. 2:
- as control that the anti-TLR4 antibody actually detects cell surface TLR4, U937 cells should be used as positive control
- since mRNA expression does not necessarily correlate with protein expression at least the expression of CD14 can be easily investigated in the same experiment

Fig. 3:
- negative controls (i.e. lanes with everything but cDNA) should be included in the figure

Fig. 4 and 5:
- it is clearly seen that the LPS preparation used in this experiment activates the cells in terms of I-kB-? degradation. Is the activation stronger when higher LPS concentration (i.e. 1µg/ml) are used? Since these cell lines may not express cell surface CD14, the sensitivity towards LPS could be greatly reduced as compared to monocytes. In addition, it is well-known that these commercial LPS preparations not only contain LPS but also other TLR ligands such as lipopeptides. Thus, in order to exclude that this weak activation is induced by other ligands, purified LPS preparations should be used.
- the luciferase assays should serve as indication that NF-kB is not activated by LPS in these cells. However, the sensitivity of these particular assay appear to be very weak. Even the positive control IL-1ß is only able to induce about 3.5fold or 2fold activation of the NF-kB reporter, respectively. In other papers, activation in the range of 10 to 100fold is not unusual. Thus, the overall sensitivity may be too low to detect LPS-induced activation.

Fig. 7:
- given the fact that NB-1 cells express only intracellular TLR4, activation of MAP kinases may take considerably longer than in U937 cells. Therefore, a kinetic up to 2hours should be performed. Does activation of ERK1/2 not occur as well?

Discussion
- the clear lack of IRF-3 expression might be responsible for the inability to activate the MyD88-independent pathway. However, since MyD88 is expressed at least at mRNA level, this should be sufficient to induce cytokine production. In order to really exclude activation of these cells by LPS, the lack of cytokine induction (i.e. IL-8) should be demonstrated as well.

Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author
can be trusted to correct)

page 2, lane 22-23: Collectively, NB-1 cells are capable to avoid their response …
p4, l15: … confirm the lack of TLR-4 expression in neuroblstoma cells.
p8, l19: commonly?
p8, l20: Intracellular but not cell surface expression of TLR4 in NB-1 cells (suggestion)
p9, l21: … that mRNAs of the three molecules are expressed in …
p11, l6: … that LPS induces the phosphorylation of IRF-3 in U937 but not in NB-1 cells.
p11, l7: IRF-3

Discretionary Revisions (which the author can choose to ignore)

What next?: Unable to decide on acceptance or rejection until the authors have responded to the major compulsory revisions

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Needs some language corrections before being published

Statistical review: No

Declaration of competing interests:

I declare that I have no competing interests