Evaluation report of bachelor thesis: Lipopolysaccharide contamination of recombinant proteins and its significance for immunological studies

The thesis written by Barbora Svobodova deals with purification of recombinant proteins from lipopolysaccharide contamination. Subject of this work is important as presence of LPS in protein solution could potentially induce immune response and thus interfere with the effect of studied recombinant protein. The method of Triton X-114 extraction was used to get rid of LPS from solution and remaining LPS was quantified by Limulus amoebocyte assay. Student had reached 94% efficiency in LPS removal from protein solution. Second part of thesis is devoted to determination of minimal concentration of LPS to be able to elicit immune response by suspension of splenocytes measured by production of TNF-alpha.

Formally is thesis composed of several chapters: Abstract (1/2 page), Introduction (1/2 page), Theoretical background (6 pages), Materials and Methods (8 pages), Conclusion (1/2 page), and References (4 pages). However there are missing separate chapters Results and Discussion, instead those are part of Materials and Methods. Thesis is written by decent English, in most cases clearly with just few typing errors and rare strange expressions like e.g. 'undesirable contamination'. Theoretical background is covering all necessary information.

Comments:

1. References are ordered by numbers however first reference starts with number 2, than from ref. 10 skips to ref. 26 and so on…Thus one could trace how paragraphs had been moved during writing.
2. Sentence on page 8 'Binding them they mediate a mechanism of their elimination' does not make sense.
3. In methods the concentration of cells per milliliter which was used in experiment is provided however without volume so we do not know how many cells were used.
4. On page 14 you state that 'endotoxin unit/ml=ng/ml'. On the next page in table 2 you provide concentration of LPS in your protein solution in endotoxin unit/ml and ng/ml. From the numbers one could make deduction that 1EU=0.1ng. Clarify this discrepancy.
5. Table 3 shows data for calibration curve for TNF-alpha. However curve itself is missing. As it is obvious from term 'curve' data should be presented in a form of a curve.
6. Descriptions for tables are not much informative (e.g. table 4 'Absorbance data after ELISA for all the other solutions'). It should be always clearly and exactly stated what is in the table.
7. Data from table 4 show three measurements of absorbance. I guess those are triplicates of one experiment. There is no analysis of those data; triplicates should be averaged and plotted on graph against concentration of TNF-alpha.

Questions:

1. Can you discuss what could be theoretically the reason for the non linear correlation between concentration of LPS used for stimulation and amount of TNF-alpha produced by splenocytes?
2. Some detergent may interfere with the measurement of absorbance. Does the presence of Triton X-114 affect absorbance?

The thesis meets requirements for bachelor thesis and provides valuable information about purification process of recombinant proteins from LPS contamination. I recommend thesis of Barbora Svobodova to the defence.

Mgr. Jaroslava Lieskovska, Ph.D.

In Ceske Budejovice, June 14, 2010.