Cyclic or linear conformations of sequences binding lipid A: does it really matter?

The response of Liddington and Hoess to my recent article in *TIM*[1] contains some inaccuracies. The synthetic peptide corresponding to amino acids 41–51 of *Limulus* anti-lipopolysaccharide (anti-LPS) factor (LALF)[2], which they quote as being cyclic, is actually linear[3]. However, this peptide synthesized in a cyclic conformation and tested against the linear one shows comparable: (1) binding affinity for lipid A, (2) selectivity[4] of binding when competing with polymyxin B, and (3) inhibition of LPS toxicity *in vitro* and *in vivo*.

By contrast, the synthetic peptide corresponding to the sequence 33–41 of natural LALF (half of the loop amino acids 32–53), which does not conform to the requirements of the $(AB)_n$ model (where A is a cationic amino acid and B a hydrophobic amino acid), does not show either binding activity for lipid A or inhibition of LPS activity *in vitro* or *in vivo*. Notably, Asn37 in the natural sequence is replaced by lysine in the recombinant LALF reported by Liddington and Hoess. This point mutation allows amino acids 32–40 of recombinant LALF to meet the features reported in my article to be required for binding lipid A.

Therefore, direct evidence supports amino acids 41–51 in the loop of natural LALF being the region responsible for binding and detoxification of lipid A, with the cyclic rearrangement not being critical. This peptide sequence and those selected from the amino acid sequences of CD14 (amino acids 67–75 and 68–78), LPS-binding protein (amino acids 92–100 and 376–384) and bactericidal/permeability-increasing protein (amino acids 27–34 and 90–99) are predicted to bind lipid A by the $(AB)_n$ model, considering a reasonable degree of reordering of the amino acids, and these peptides experimentally inhibit the lipid-A-mediated toxicity *in vitro* and *in vivo*[3].

Finally, the significant role of the cyclic conformation for short peptides binding lipid A and mimicking the structure of polymyxin B [which corresponds to the cyclic $(AB)_n/(ABC)_n$ model, where C is an aliphatic, hydrophobic amino acid] has been established previously[4], although this may not generalize to natural proteins. As in the case of the peptide corresponding to amino acids 41–51 of natural LALF, one should proceed in the popperian style: to disprove a theory one must first run the crucial experiment!

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References

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