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DISCUSSIONS

# Reply to the Letter of G. Matera, A. Quirino, A. G. Lamberti, A. Focà, and M. C. Liberto

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Dear authors of Discussion Letter "*Bartonella*: stealthy pathogens or novel drug factories", Giovanni Matera, Angela Quirino, Angelo Giuseppe Lamberti, Alfredo Focà, and Maria Carla Liberto.

We are kindly thankful to you for your attention and comments to our review "Structural analysis of lipopolysaccharides from Gram-negative bacteria" published in *Biochemistry (Moscow)*. We apologize profoundly for our mistake in quoting data of Prof. Dr. Giovanni Matera et al. [1] and Prof. Dr. Ulrich Zähringer et al. [2] on review p. 390: "In experiments with human whole blood, LPS from *B. henselae* whose pentaacyl lipid A contains an acyloxyacyl residue 16:0[3-O-(28:0(27-OH))] also did not induce the release of TNF-α [105]" [3]. In fact, these experiments were performed with LPS from *Bartonella quintana* rather than from *Bartonella henselae* as we reported [1-3].

At the time of publishing of the review, the structure of lipid A from *B. quintana* endotoxin, whose biological activity is described in the above-mentioned sentence, was not characterized. Only structures of lipid A from endotoxins of *B. henselae*, a bacterium related to *B. quintana* were known [2]. Structural variants of lipid A from *B. henselae* may include rare acyloxyacyl residues of different composition: 12:0[3-O(26:0(25-OH))], 12:0[3-O(28:0(27-OH))], and 16:0[3-O(26:0(25-OH))] [2]. The authors of this work revealed that the endotoxins from *B. henselae* were 1000 times less active than those from *Salmonella enterica* sv. Friedenau in inducing IL-8 synthesis by human embryonic kidney 293 cells (HEK 293) transfected with CD14 and Toll-like receptors [2]. In the early study by the group of M. C. Liberto and A. Focà using human whole blood it was determined that the endotoxins from *B. quintana* did not induce TNF-α synthesis [1]. Antagonistic activity of LPS from *B. quintana* was elucidated in the work of C. Popa with coworkers [4].

LPS molecules, which exhibit low endotoxic activity but retain the ability to interact with agonist-specific receptors blocking agonist-mediated cellular responses, have been referred as endotoxin antagonists. For instance, Re-LPS from *B. quintana* do not cause the stimulation of human mononuclear cells to production of TNF-α, IL-1β, or IL-6 up to tested concentration 1 μg/ml. In addition, the application of tenfold excess of antagonists, Re-LPS from *B. quintana*, was sufficient for prevention of agonistically highly active S-LPS *Escherichia coli*-driven mRNA synthesis of the mentioned cytokines [4]. Moreover, TNF-α and IL-6 release from human peripheral blood mononuclear cells activated by SR-LPS from *Veillonella parvula* ATCC 10790 were significantly inhibited by preliminary exposure of the cells to Re-LPS from *B. quintana* [5]. LPS with the similar antagonistic properties are produced by phototrophic bacteria such as *Rhodobacter sphaeroides* and *Rhodobacter capsulatus* [6] as well as by marine bacterium *Marinomonas communis* ATCC 27118$^T$ [7]. Unlike S-LPS from *E. coli* O111:B4 triggering already TNF-α production in human whole blood at their concentration 10 ng/ml, LPS from *Rb. sphaeroides* even at higher concentrations (up to 10 μg/ml) do not elicit such effect [6]. The lipid A from *Rb. sphaeroides* upon simultaneously addition with agonistically active lipid A from *Salmonella minnesota* R595 (1 ng/ml) or with LPS from *Helicobacter pylori* (100 ng/ml) inhibits IL-8 production by human monocytes at antagonist concentrations 100 ng/ml or 10 μg/ml, respectively [8]. The main structural features of lipids A from *Rb. sphaeroides* and *Rb. capsulatus* are the presence in their fatty acid residues of rare 3-oxo and unsaturated groups (table). By using lipid A synthetic analogs, the role of *cis* or *trans* configurations of double bond in the establishment of peculiar activity and antag-

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onistic property of *Rb. sphaeroides* lipid A was elucidated [6]. It was shown that the capacity of the synthetic analogs to block TNF-α release in response to S-LPS *E. coli* O111:B4 (10 ng/ml) decreased in the series: 2′-*trans*-lipid A > LPS *Rb. sphaeroides* ≥ 2′-*cis*-lipid A > 2-*cis*-lipid A. Thus, the lipid A analog from *Rb. sphaeroides* with unsaturated bond in *trans* configuration possessed the most pronounced antagonistic activity [6]. Further, the reduction of 3-oxo and unsaturated groups in fatty acid residues (14:0(3-oxo) and 14:0[3-O(cisΔ$^7$14:1)]) of natural lipid A *Rb. sphaeroides* did not increase its endotoxic activity [12].

Synthetic compounds Rhiz 1/1, Rhiz 1/2 and Rhiz 1/3—analogs of lipid A from *Rhizobium* sin-1—provide additional examples of lipid A antagonists [11]. They differ from each other by acyloxyacyl residue at N-2′ position of a glucosamine (GlcN) residue (table). The exposure of differentiated Mono Mac 6 cells to these compounds up to their concentration 100 μg/ml (10% serum) led to nearly undetectable TNF-α production. The ability of *Rhizobium* sin-1 lipid A analogs to inhibit TNF-α release induced by S-LPS *E. coli* O55:B5 (10 ng/ml) was assessed and decrease in the sequence: LPS *Rhizobium* sin-1 > Rhiz 1/2 ≥ Rhiz 1/1 > Rhiz 1/3 [11, 13]. These

Synthetic analogs of natural lipids A

| Synthetic analog | GlcN II | | | GlcN I | | | References |
|---|---|---|---|---|---|---|---|
| | O-4' | O-3' | N-2' | O-3 | N-2 | O-1 | |
| Compound 2′-*trans* lipid A analog from *Rb. sphaeroides* | P | 10:0(3-OH) | 14:0[3-O(transΔ$^7$14:1)] | 10:0(3-OH) | 14:0(3-oxo) | P | [6] |
| Compound 2′-*cis* lipid A analog from *Rb. sphaeroides* | P | 10:0(3-OH) | 14:0[3-O(cisΔ$^7$14:1)] | 10:0(3-OH) | 14:0(3-oxo) | P | [6] |
| Compound 2-*cis* lipid A analog from *Rb. sphaeroides* | P | 10:0(3-OH) | 14:0(3-OH) | 10:0(3-OH) | 14:0[3-O(cisΔ$^7$14:1)] | P | [6] |
| Compound No. 5 | P | 10:0(3-OH) | 14:0(3-OH) | 10:0(3-OH) | 14:0(3-OH) | P | [9] |
| Compound No. 6 | P | 10:0(3-OH) | 10:0(3-OH) | 10:0(3-OH) | 10:0(3-OH) | P | [9] |
| Compound No. 3 – lipid A analog from *P. gingivalis* | H | H | i17:0[3-O(16:0)] | 16:0(3-OH) | i17:0(3-OH) | P | [10] |
| Compound No. 4 – lipid A analog from *P. gingivalis* | H | i15:0(3-OH) | i17:0[3-O(16:0)] | H | i17:0(3-OH) | P | [10] |
| | GlcN II | | | 2-aminogluconolactone | | | |
| | O-4' | O-3' | N-2' | O-3 | N-2 | | |
| Rhiz 1/1 – lipid A analog from *Rhizobium* sin-1 | H | 16:0(3-OH) | 16:0[3-O(28:0(27-OH))] | 16:0(3-OH) | 16:0(3-OH) | | [11] |
| Rhiz 1/2 – lipid A analog from *Rhizobium* sin-1 | H | 16:0(3-OH) | 16:0[3-O(28:0)] | 16:0(3-OH) | 16:0(3-OH) | | |
| Rhiz 1/3 – lipid A analog from *Rhizobium* sin-1 | H | 16:0(3-OH) | 16:0[3-O(14:0)] | 16:0(3-OH) | 16:0(3-OH) | | |

Note: GlcN, glucosamine; i, iso-fatty acids.

data indicate that the antagonistic activity of lipid A *Rhizobium* sin-1 is favored by the presence of long chain 27-hydroxyoctacosanoic fatty acid residue. This conclusion is supported by the experiments showing that the substitution of 27-hydroxyoctacosanoic fatty acid residue by the relative shorter tetradecanoic acid residue (C14) devoid of hydroxyl group such in compound Rhiz 1/3 causes significant reduction of antagonistic activity of the studied synthetic analog [11, 13, 14].

The importance of fatty acid residue extension in the maintenance of lipids A antagonistic activity was also reported [9]. Thus, lipid A synthetic analog—tetraacylated compound No. 6 comprised of 3-hydroxydecanoic fatty acid residues only—does not prevent endotoxin-induced IL-6 production. However, another tetraacylated synthetic compound No. 5, differing from compound No. 6 by substitution of two 3-hydroxydecanoic fatty acid residues at N-2′ and N-2 positions of GlcN II and GlcN I by 3-hydroxytetradecanoic acid residues (table), has pronounced antagonistic activity [9].

The role of fatty acid distribution between two glucosamine residues in the manifestation of antagonistic activity by lipids A had been evaluated using tetraacylated synthetic analogs of lipid A from *Porphyromonas gingivalis*—compound No. 3 and compound No. 4 (table). The latter compound is characterized by asymmetrical [3 + 1] fatty acid residue distribution, whereas the former by a pseudo-symmetrical one [2 + 2]. Comparing the ability of these compounds to inhibit TNF-α release in response to S-LPS *E. coli* O55:B5 from differentiated Mono Mac 6 cells revealed that compound No. 3 was the most potent antagonist in comparison to compound No. 4 [10].

LPS from *Francisella tularensis* possessing tetraacylated lipid A with three 3-hydroxyoctadecanoic (C18) fatty acid residues in pseudo-symmetrical distribution have no either agonistic or antagonistic activities [15].

Taking these data into consideration, it can be concluded that the tendency of lipid A to exhibit antagonistic activity is dependent on its composition, with the most pronounced impact of the length and distribution of lipid A fatty acids between two glucosamine residues [10].

In conclusion, we would like to express our gratitude to colleagues of Dr. Giovanni Matera from Catanzaro University (Italy) for broadening the data regarding opportunistic infections caused by *B. quintana* and biological activity of LPS from this bacterium as well as for the possibility to discuss the dependence of lipid A antagonistic activity on its composition.

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