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Kill the Bacteria ... and Also Their Messengers?

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Abstract

We consider here a previously neglected aspect of recovery from infectious diseases: how animals dispose of the dead microbes in their tissues. For one of the most important disease-causing microorganisms, Gram-negative bacteria, there is now evidence that the host catabolism of a key microbial molecule is essential for full recovery. As might be expected, it is the same bacterial molecule that animals sense to detect the presence of Gram-negative bacteria in their tissues, the cell wall lipopolysaccharide (LPS). Here, we discuss current knowledge about LPS sensing with emphasis on the host enzyme that inactivates this microbial “messenger” molecule. We also consider the possibility that the rate at which stimulatory microbial molecules undergo inactivation may influence the duration and severity of diseases caused by other infectious agents.

1. LIPOPOLYSACCHARIDE (LPS) SENSING BY MD-2–TLR4

All Gram-negative bacteria living in natural environments produce LPS, a complex glycolipid that contributes to outer membrane impermeability, confers resistance to detergents and cationic antimicrobial peptides, provides cell-surface diversity, and prevents complement-mediated cell death. Animals sense the lipid A moiety of LPS via MD-2–TLR4 receptors on phagocytes and other cells, and much evidence suggests that recognizing LPS in this way is essential for detecting the presence of Gram-negative bacteria in tissues and mobilizing antibacterial defenses. The structure of lipid A is not identical in different Gram-negative bacteria, however, and not all lipid As can trigger inflammatory responses via MD-2–TLR4. Extensive structure–activity studies have shown that a bisphosphorylated, hexaacyl lipid A structure (Fig. 2.1) is most stimulatory; removal or addition of a single acyl chain can diminish potency, as can the absence of either of the backbone phosphates. Although many Gram-negative bacteria make LPSs that are poorly recognized by MD-2–TLR4, with potentially important consequences for disease pathogenesis (Munford, 2008), the aerobic commensals and pathogens that colonize the mucosae of the upper respiratory and gastrointestinal tracts generally produce LPSs that have hexaacyl lipid A moieties and are readily sensed by cells bearing MD-2–TLR4 (Munford and Varley, 2006). It is these bacteria that animals are best equipped to defeat using TLR4-based inflammatory responses, and it is the LPSs from these bacteria that are most likely to translocate into the bloodstream to produce “endotoxemia.” These are also the LPSs that can be inactivated by the unusual host lipase, acyloxyacyl hydrolase (AOAH).

2. ACYLOXYACYL HYDROLASE

AOAH was found during a search for human neutrophil enzymes that could deacylate LPS (Hall and Munford, 1983). The “bait,” a biosynthetically labeled LPS that had ¹⁴C-glucosamine and ³H-fatty acyl chains (Fig. 2.1), was opsonized with an anti-LPS antibody and fed to human neutrophils *in vitro*. The ¹⁴C dpm, which marked the carbohydrate backbone, remained chloroform-insoluble during the next 6 h, whereas the ³H in the LPS acyl chains gradually

became chloroform-soluble and could be recovered in cellular phospholipids. Further analysis showed that only the nonhydroxylated fatty acids (myristate and laurate) were released from the LPS—unexpectedly, all of the 3-hydroxymyristoyl chains remained attached to the backbone, suggesting that neutrophils may lack the ability to degrade lipid A completely. Correct interpretation of the sites of enzymatic hydrolysis became possible when the existence of acyloxyacyl linkages (Wollenweber *et al.*, 1982) in lipid A and the first accurate lipid A structure (Takayama *et al.*, 1983) were published: the myristoyl and lauroyl chains are attached to the hydroxyl functions of glucosamine-linked 3-hydroxymyristoyl residues to form acyloxyacyl linkages (Fig. 2.1, arrowheads). Denis McGarry suggested that the enzymatic activity be named “acyloxyacyl hydrolysis” and the enzyme(s) “acyloxyacyl hydrolase(s)”. A literature review revealed that enzymatic release of non-hydroxylated fatty acids from LPSs had been reported previously in slime molds (*Dictyostelium discoideum*, Nigam *et al.*, 1970; *Physarum polycephalum*, Saddler *et al.*, 1997a) and a snail (*Helix pomatia*, Saddler *et al.*, 1979b).

In mammals, AOA is produced by monocyte-macrophages and dendritic cells as well as neutrophils. Another prominent source, particularly in rodents, is the renal cortical epithelial (proximal tubule) cell (Feulner *et al.*, 2004). Evidence that AOA can inactivate LPS followed partial purification of the protein (Munford and Hall, 1986). Table 2.1 summarizes the bioassays used to evaluate this point. Note that AOA-treated LPS not only lacks potency in these assays but it can also inhibit the ability of untreated LPS to stimulate both human and murine cells. The species-specific inhibition reported for tetraacyl lipid A structures (such as lipid IVa) (Golenbock *et al.*, 1990; Hajjar *et al.*, 2002) does not necessarily apply to tetraacylated LPSs, such as those produced by AOA treatment, in which tetraacyl lipid A is linked to a polysaccharide chain of variable length; these tetraacyl LPSs are poor agonists for both rodent and human cells. AOA-treated LPS was the first lipid A derivative shown to inhibit LPS (Pohlman *et al.*, 1987); it and other tetraacylated lipid “A”s can compete with hexaacylated lipid A or LPS for binding LBP, CD14 (Kitchens and Munford, 1995), and, most importantly, MD-2 (Kim *et al.*, 2007).

2.1. Structure, biosynthesis

Purification of the enzyme from HL-60 human promyelocytes revealed a low abundance, glycosylated protein of Mr ~65 kDa that has two disulfide-linked subunits (Munford and Hall, 1989). The cDNA sequence indicated that the enzyme is produced as a single polypeptide chain; proteolytic cleavage is thus required to yield the two subunits (Hagen *et al.*, 1991) (Fig. 2.2). The larger subunit (50 kDa) has the GXSG consensus motif that has been found in serine-active site enzymes, and mutating the Ser to Leu inactivated the enzyme (Staab *et al.*, 1994). This subunit is now considered a GDSL or SGNH lipase (Akoh *et al.*, 2004). The smaller subunit is a member of the saposin-like protein (SAPLIP) family (Munford *et al.*, 1995). It shares six Cys residues and other features with several small proteins that act as enzymes or cofactors for glycosphingolipid catabolism (the saposins, acid sphingomyelinase), form pores in membranes (amoebophore, NK-lysin), or act at lipid–air interphases in the lung (surfactant protein B). The enzymatically active AOA large subunit is thus armed with a covalently linked “cofactor,” the saposin-like small subunit (Fig. 2.3). Without it, the enzyme did not localize in intracellular vacuoles and had lower affinity for LPS (Staab *et al.*, 1994). AOA and a closely similar *Trypanosoma brucei* inositol deacylase (Güther *et al.*, 2001) are the only known lipases that have this saposin–lipase combination, which has been very highly conserved in AOAs from *D. discoideum* to *Homo sapiens* (Munford and Varley, 2006). Whereas saposin B may participate in NK-T cell activation by transferring glycolipid antigens to CD1d (Zhou *et al.*, 2004), several attempts identify a similar role for AOA have been unsuccessful.

The human and murine AOA genes have 21 small exons on chromosome 7p14-p12 and 13, respectively. In both species, the gene extends over ~200 kb of genomic DNA. To prevent synthesis of AOA in mice, the starting ATG and the downstream 126 bp of the first exon were replaced with a neomycin resistance cassette. Mice carrying this construct do not produce AOA protein (Shao *et al.*, 2007) or have LPS-deacylating activity (Lu *et al.*, 2003; Shao *et al.*, 2007).

2.2. Enzymatic activity

As noted, AOA removes secondary (piggyback, acyloxyacyl-linked) chains from different positions on the diglucosamine lipid A backbone without attacking any of the primary glucosamine-linked chains (Erwin and Munford, 1990). Immunoadsorption of AOA from leukocyte lysates removed all enzymatic activity toward LPS, indicating that AOA was the only LPS-deacylating enzyme in these cells, and recombinant AOA can remove both of the secondary acyl chains from the LPS backbone. It can also be a phospholipase A₁/B, diglyceride lipase, and acyl transferase *in vitro*, and it has a preference for cleaving saturated (or mid-length) acyl chains from both phospholipid and LPS substrates (Munford and Hunter, 1992). AOA can remove acyl chains that are attached to different positions on the diglucosamine and glycerol backbones of LPS and glycerolipids, respectively (Erwin and Munford, 1990; Munford and Hunter, 1992). It thus has specificity for acyl chain character, not backbone position. Transfected fibroblasts secrete the precursor, which can be taken up by the same or different cells and proteolytically processed into the mature enzyme (Feulner *et al.*, 2004). The AOA precursor peptide has enzymatic activity toward both LPS and phosphatidylcholine (PC), yet cleaving the precursor to form the mature enzyme increased its activity toward LPS more than 10-fold without altering its ability to act on PC (Staab *et al.*, 1994).

3. LPS DEACYLATION *IN VIVO*

The double-radiolabeled LPS substrate can also be used to quantitate the rate and extent of LPS deacylation *in vivo*. Whether the LPS is injected subcutaneously (footpad, skin site), intraperitoneally or intravenously, deacylation occurs over many hours (Lu *et al.*, 2005; Shao *et al.*, 2007). Despite this seemingly sluggish performance, AOA-mediated deacylation completely inactivated almost 80% of a subcutaneous dose of LPS before the LPS could travel to draining lymph nodes (Lu *et al.*, 2005). No loss of the LPS secondary acyl chains was detected in *Aoah*^{-/-} mice, and in neither *Aoah*^{-/-} nor *Aoah*^{+/+} animals was there loss of primary (3-hydroxymyristoyl) acyl chains from the backbone. LPS deacylation *in vivo* is thus remarkably selective and limited. Of the various LPS-catabolizing enzymes produced by *D. discoideum* (Verret *et al.*, 1982a,b), which eat bacteria as a foodstuff, only AOA has been conserved during animal evolution.

One important unresolved issue is the extent to which LPS deacylation occurs inside and outside host cells. An intracellular site was suggested by the enzyme's acid pH optimum, its location in an intracellular vacuole (Staab *et al.*, 1994), and the apparent colocalization of AOA and deacylated LPS in neutrophils (Luchi and Munford, 1993). Both rabbit macrophages and murine dendritic cells deacylate the LPS in phagocytosed *Escherichia coli* in an AOA-dependent fashion (Katz *et al.*, 1999; Lu *et al.*, 2003). Moreover, AOA does not act on LPS in buffered salt solutions in the absence of a nonionic detergent such as Triton X-100, suggesting that it may require an intracellular environment or factor(s) to do its job. The enzyme can be secreted by rabbit neutrophils and monocytes (Erwin and Munford, 1991), however, and extracellular deacylation has been demonstrated in rabbit peritoneal exudate fluid (Katz *et al.*, 1999), a rich mixture of extravasated plasma, leukocyte products, and other molecules. The AOA secreted into the urine by renal cortical epithelial cells can also deacylate LPS (Feulner *et al.*, 2004). Gioannini *et al.* recently reported that CD14 and LBP can bind LPS in a way that allows AOA to deacylate its lipid A moiety (Gioannini *et*

al., 2007), providing an attractive mechanism for extracellular LPS deacylation (Weinrauch *et al.*, 1999). The relative contributions of intra- and extracellular deacylation to LPS inactivation *in vivo* remain uncertain.

Very little is known about how AOA_H activity is regulated *in vivo*. In part this reflects the enzyme's low abundance, which has hindered quantitative detection of both AOA_H protein and mRNA. In addition, it has not been possible to detect AOA_H activity or protein in human plasma or serum. Since the enzyme is easily measured in rodent and rabbit serum, most studies of AOA_H regulation have been performed in these animals. In rabbits, plasma AOA_H levels rise dramatically within a few minutes of an intravenous injection of LPS and remain elevated for many hours (Erwin and Munford, 1991). The increase in AOA_H activity was significantly less in animals that had been given methchloroethamine to induce leukopenia, suggesting that much of the extracellular enzyme is produced and released by neutrophils or monocytes. Indeed, rabbit leukocytes released AOA_H in response to stimulation by LPS *ex vivo* (Erwin and Munford, 1991). In studies performed in mice, Cody *et al.* found that AOA_H mRNA and activity in liver and lung increased several-fold following intraperitoneal treatment with LPS (Cody *et al.*, 1997). In addition, low concentrations of LPS and interferon- γ induced greater than 10-fold increases in AOA_H mRNA in cultured murine macrophages. Neither IL-10 nor dexamethasone prevented AOA_H mRNA accumulation in response to LPS, in keeping with the discovery, many years later, that AOA_H participates in the anti-inflammatory (recovery) phase of local infection. Indeed, Mages *et al.* (2008) found an approximately sixfold increase in AOA_H mRNA abundance in LPS-primed (tolerant) murine macrophages relative to unstimulated controls. Immature murine dendritic cells also produce AOA_H; cytokine-induced maturation was associated with diminished LPS-deacylating ability, whereas exposure to LPS, CpG oligonucleotides, or staphylococci was stimulatory (Lu *et al.*, 2003). Unfortunately, it is uncertain that mice are useful models for human AOA_H regulation, since the tissue-specific expression of the enzyme differs substantially (mice produce much more AOA_H in the kidney than do humans, and less in myeloid cells). DeLeo and colleagues found that AOA_H mRNA abundance decreased approximately twofold in human neutrophils during the 6 h following phagocytosis of latex beads, a time when many of the neutrophils were undergoing apoptosis (Kobayashi *et al.*, 2003). Further study of AOA_H regulation in human phagocytes is needed.

4. INACTIVATING LPS *IN VIVO*

Animals have several mechanisms for inactivating LPS (Munford, 2005), including lipid A-neutralizing proteins (bactericidal permeability-increasing protein, lactoferrin, lysozyme, collectins, etc. (Chaby, 2004)), specific and cross-reactive anti-LPS antibodies, and sequestration of the lipid A moiety within lipoprotein micelles. Although intestinal alkaline phosphatase can inactivate LPS in zebrafish (Bates *et al.*, 2007), a role for endogenous alkaline phosphatase in LPS inactivation in mammals has not been established. At present, AOA_H is the only endogenous enzyme known to inactivate LPS in tissues.

Early expectations that AOA_H would protect animals from LPS-induced inflammation met with disappointment when it was learned that *Aoah*^{-/-} and *Aoah*^{+/+} mice had similar acute inflammatory responses to LPS and indistinguishable survival outcomes following LPS or Gram-negative bacterial challenge (Fig. 2.4A). In addition, macrophages and dendritic cells from wild-type and AOA_H-deficient animals had similar responses to LPS *in vitro*. On the other hand, the *Aoah*^{+/+} mice that survived a *Neisseria meningitidis* challenge recovered more rapidly than did the surviving *Aoah*^{-/-} mice (Fig. 2.4B). Suspecting that the enzyme's role might be discernable only when late responses to LPS and Gram-negative bacteria were studied, we began to look for long-term abnormalities in LPS-treated mice. Three AOA_H-dependent phenotypes have now been identified, each of which reflects the ability of persistently active (fully acylated) LPS to stimulate cells *in vivo* for long periods of time. It

seems that the enzyme's low abundance and slow deacylation rate are useful for a host defense that responds rapidly and vigorously to LPS but then needs to inactivate this microbial "messenger" so as to avoid prolonged cell activation and possible immunosuppression.

4.1. AOA-dependent phenotypes

4.1.1. Prolonged elevations in polyclonal plasma IgM and IgG₃ antibodies—LPS is a B cell mitogen in mice, which express TLR4 on B cells. B cell proliferation and polyclonal antibody production are thus quantitative indices of LPS stimulation. Subcutaneous LPS inoculation elicits much greater IgM and IgG₃ responses in *Aoah*^{-/-} mice than in wild-type mice, suggesting that AOA normally exerts a braking influence on B cell stimulation by LPS *in vivo* (Lu *et al.*, 2005) (Fig. 2.5A). Indeed, the presence of the enzyme also prevents impressive, prolonged enlargement of the lymph nodes that drain the site of inoculation. *Tlr4*^{-/-}, *Aoah*^{-/-} mice developed neither lymphadenopathy nor elevated antibody titers, in keeping with TLR4's essential role in LPS signaling.

4.1.2. Persistent hepatomegaly—Much of the LPS that enters the bloodstream from the gastrointestinal tract travels via the portal venous system to the liver. Hepatic macrophages (Kupffer cells), which take up a large fraction of this LPS, are also the major AOA-producing cells in the liver (Shao *et al.*, 2007). Small intravenous doses of LPS induce prolonged, possibly irreversible, hepatomegaly in *Aoah*^{-/-} mice. This phenomenon was first noticed during experiments performed to define the time-course of LPS deacylation in the liver. In wild-type mice, the liver weight/body weight ratio increased transiently, peaking 3 days after i.v. injection and returning to baseline by day 7. In contrast, in *Aoah*^{-/-} mice the liver continued to enlarge, reaching 30–50% above baseline within 1 week of i.v. injection and remaining enlarged for at least 3 weeks (Shao *et al.*, 2007). Although the basis for this striking phenomenon remains uncertain, it is associated with the retention of fully acylated LPS by Kupffer cells and sinusoidal engorgement with blood that contains neutrophils, B cells, CD4 and CD8 T cells, and monocytes ((Shao *et al.*, 2007); B.M. Shao, unpublished results). LPS also induces impressive splenomegaly in *Aoah*^{-/-} mice but this response is transient, resolving within 2 weeks (Shao *et al.*, 2007).

4.1.3. Prolonged tolerance and immunosuppression—Another remarkable consequence of AOA deficiency is the development of prolonged endotoxin tolerance and immunosuppression following exposure to very small amounts of LPS. In this context, tolerance refers to the ability of a small priming dose of LPS to induce a state of cellular reprogramming in which responses to subsequent, larger doses of LPS and several other microbial agonists are altered. Endotoxin tolerance is known to occur in many animals, including humans; it is usually considered an adaptation to prevent excessive inflammatory reactions to invading microbes—as others have suggested, it may prevent "friendly fire" while animals recover from infection (Cross, 2002; Medvedev *et al.*, 2006). The duration of the tolerant period is influenced by several factors, including LPS dose, the route of administration, and the animal's ability to deacylate the LPS. Whereas *Aoah*^{+/+} mice recover from the tolerant state within 5–10 days after intraperitoneal exposure to a small dose of LPS or *E. coli*, *Aoah*^{-/-} mice remain tolerant for at least 4 months! Moreover, LPS-primed *Aoah*^{-/-} mice were exquisitely sensitive to challenge with a virulent *E. coli* strain; susceptibility was associated with delayed production of TNF and IL-6 and massive bacterial growth during the first 24 h after inoculation (Lu *et al.*, 2008). Initial analyses of mRNA expression by LPS-primed *Aoah*^{+/+} and *Aoah*^{-/-} peritoneal macrophages suggest that LPS exposure induces low-grade, persistent activation in AOA-deficient animals, largely in keeping with the reprogramming phenomenon observed previously in macrophages induced to develop tolerance *in vitro* (Foster *et al.*, 2007; Mages *et al.*, 2008) but differing from those studies in many of the individual mRNAs that are up- and downregulated during the tolerant period (A. Varley, M. Lu,

unpublished results). As with the other two phenotypes, prolonged tolerance in *Aoah*^{-/-} mice is associated with the presence of fully acylated LPS in cells for long periods of time (Lu *et al.*, 2008).

4.1.4. Do AOA-dependent phenotypes require exposure to LPS?—If these AOA-dependent phenotypes result from the enzyme's ability to deacylate LPS, they should not occur in *Aoah*^{-/-} animals when the MD-2-TLR4 signaling pathway is activated by a non-LPS agonist. We tested this hypothesis using UT12, an agonistic monoclonal antibody to MD-2-TLR4 that was developed by Shoichiro Ohta and colleagues (Ohta *et al.*, 2006). For each of the phenotypes discussed above, *Aoah*^{-/-} and *Aoah*^{+/+} mice had indistinguishable responses to UT12; an example is shown in Fig. 2.5B, which should be compared with Fig. 2.5A. AOA's ability to act on LPS, and not other potential substrates, is thus likely to account for the prolonged LPS-induced responses observed in *Aoah*^{-/-} mice.

4.2. AOA-dependent immunomodulation: Only *in vivo*?

Whereas LPS-injected *Aoah*^{+/+} mice produce significantly less antibody than do *Aoah*^{-/-} mice, naïve splenocytes from *Aoah*^{-/-} and *Aoah*^{+/+} animals proliferate and produce antibody to the same extent when they are exposed to LPS *in vitro* (Lu *et al.*, 2005). AOA thus has a strikingly different influence on the *in vivo* and *in vitro* responses of splenocytes to LPS. Similarly, whereas LPS-primed *Aoah*^{-/-} peritoneal macrophages retain their tolerant (reprogrammed) phenotype when they are removed from the peritoneal cavity and grown for several days *ex vivo*, naïve *Aoah*^{-/-} and *Aoah*^{+/+} macrophages recover from tolerance at the same rate when they are first exposed to LPS *in vitro*. Again, the *in vivo* phenotype cannot be modeled *in vitro*. It should be interesting to define the properties of the *in vivo* environment that allow LPS inactivation to have such an important impact on the duration and nature of host responses to LPS.

4.3. Providing AOA prevents prolonged responses to LPS *in vivo*

The three AOA-dependent phenotypes have been observed on two widely different murine strain backgrounds, C3H/HeN and C57Bl/6, indicating that these responses to LPS are not strain specific. Another way to show that the observations in *Aoah*^{-/-} animals are truly the result of AOA deficiency is to prevent or ameliorate them by providing AOA. We found that intravenous doses of recombinant human AOA, given prior to LPS injection, can prevent LPS-induced hepatomegaly (Shao *et al.*, 2007) and that producing AOA *in vivo* using a gutted adenoviral vector can prevent prolonged LPS-induced tolerance (Lu *et al.*, 2008). Recombinant adenoviruses produce their cargo proteins largely in hepatocytes, whereas AOA is made by phagocytic cells and renal cortical epithelial cells. To study the effects of overproducing AOA in cells that normally make it, we also engineered mice that produce large amounts of AOA in macrophages and dendritic cells. These mice express AOA from the human CD68 promoter in a cassette developed by David Greaves at the University of Oxford (Gough *et al.*, 2001). The transgenic mice recovered from LPS challenge more rapidly than did wildtype mice and they were protected from LPS-induced hepatosplenomegaly (N. Ojogun *et al.*, in press). The transgenic animals were also less likely to succumb to an *E. coli* challenge, confirming the important role that LPS plays in Gram-negative bacterial toxicity *in vivo* and raising the possibility that increasing AOA levels might ameliorate harmful responses to Gram-negatives in other animals, including humans (Munford, 2008). For example, early studies suggested that AOA might play a protective role in bovine coliform mastitis (Dosogne *et al.*, 1998; McDermott *et al.*, 1991). Engineering transgenic cattle to overproduce AOA in myeloid cells or milk might thus be advantageous for the dairy industry.

5. POTENTIAL CLINICAL CONNECTIONS

5.1. AOA deficiency

Large-scale screens for AOA-deficient humans have not been performed. One group has reported an association between a particular AOA haplotype and risk of asthma (Barnes *et al.*, 2006); whether or not this haplotype is associated with altered AOA production or activity is not known. Screening for AOA deficiency might also be fruitful in individuals with viral, alcoholic or nonalcoholic steatohepatitis, for which gut-derived LPS may be a contributing factor (Tilg and Diehl, 2000); severe sepsis induced by Gram-negative bacteria that make hexaacyl LPS (see Section 1); prolonged recovery from Gram-negative bacterial diseases; autoimmune diseases in which a role for LPS has been suggested (such as Guillain–Barre syndrome following exposure to *Campylobacter jejuni* (Ang *et al.*, 2002)); or patients with xanthogranulomatous pyelonephritis or malakoplakia, rare complications of bacterial infection in which macrophages accumulate lipid and polysaccharides (Gregg *et al.*, 1999). A role for AOA-mediated LPS inactivation might also be sought in patients with HIV/AIDS, in whom immune dysfunction has been related to the absorption of bacterial molecules, including LPS, from the gastrointestinal tract into portal blood (Brenchley *et al.*, 2006; Jiang *et al.*, 2009).

5.2. Host inactivation of microbial agonists other than LPS

Greater understanding of how animals inactivate other microbial molecules could also have important consequences for patient care. For example, some individuals who become infected with *Borrelia burgdorferi* remain symptomatic despite having received effective antibiotic therapy; it has not been possible to grow *B. burgdorferi* or detect their DNA in inflamed tissues from such patients (Marques, 2008). Perhaps their persistent symptoms are related to an inability to inactivate stimulatory bacterial lipoproteins, which activate host cells via TLR2, or too-rapid deacylation of the *Borrelia* glycolipid that stimulates protective NK-T cells (Tupin *et al.*, 2008). The same question might be raised regarding recovery from staphylococcal and streptococcal diseases, which can be very prolonged despite negative cultures; lipoproteins are the most potent known immunostimulatory staphylococcal molecules. Recovery from certain viral infections may also take a long time (Didierlaurent *et al.*, 2008), raising the possibility that hosts might differ in their ability to degrade stimulatory viral nucleic acids or proteins. If they shorten the time to full recovery, measures that enhance the host's ability to inactivate stimulatory microbial molecules would be a useful adjunct to antimicrobial chemotherapy. Since recovery from infectious diseases can take many weeks, with increased risk of another infection during that period (Yende *et al.*, 2008), further investigation of this possibility is clearly warranted.

6. CONCLUSION

Studies of LPS inactivation by an unusual host lipase have revealed that killing Gram-negative bacteria does not prepare an animal to confront another microbial invader: the major microbial “messenger” molecule must also be inactivated. Although it seems likely that persistence of microbial agonists other than LPS could also have long-term consequences, much more work is required to test this notion and to identify the important catabolic pathways. If the LPS-primed *Aoah*^{-/-} mouse is a fruitful model, eliminating bioactive microbial molecules should hasten recovery from infection-induced immunosuppression (tolerance) and possibly prevent other lingering symptoms and signs of disease.

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FIGURE 2.1.

The structure of *Escherichia coli* lipid A. Arrows indicate the acyloxyacyl-linkages. The secondary acyl chains are shown as dashed lines.

FIGURE 2.2.

AOAH structure. The precursor (top) undergoes proteolytic processing to yield the mature protein (bottom). SAPLIP, sphingolipid activator protein-like peptide.

FIGURE 2.3.

AOAH (left), with its covalently linked saposin-like subunit (SAPLIP), acts on the bacterial glycolipid, LPS, whereas glucosylceramidase (right), requires saposin C to cleave its eukaryotic glycolipid substrate, glucosylceramide.

FIGURE 2.4.

(A) *Aoah*^{+/+} and *Aoah*^{-/-} C3H/HeN mice had similar survival time-courses following intraperitoneal challenge with *Neisseria meningitidis* (5×10^7 colony-forming units), yet (B) the surviving *Aoah*^{+/+} mice recovered more rapidly than did the *Aoah*^{-/-} mice. Recovery was measured using a clinical scoring system that included piloerection, diarrhea, ocular exudate, immobility, and body weight.

FIGURE 2.5.

(A) Antibody responses of *Aoah*^{+/+} (closed boxes) and *Aoah*^{-/-} (open circles) mice to a single subcutaneous dose of 10^7 cfu live *Neisseria meningitidis*. (B) Antibody responses to a single injection of UT12 agonistic antibody to MD-2—TLR4. Note that the responses of *Aoah*^{+/+} and *Aoah*^{-/-} mice were different only when LPS was used to activate MD-2—TLR4. $N=3-5$ mice/group. The data in (A) were published in Lu *et al.* (2005).

TABLE 2.1

AOAH inactivates LPS

Animal host	Assay	LPS used	Reduction in activity after AOAH treatment	References
A. Evidence that AOAH-treated LPS has decreased bioactivity <i>in vivo</i>				
Rabbit	Dermal Shwartzman reaction	<i>Salmonella typhimurium</i>	~100-fold	Munford and Hall (1986)
Rabbit	CSF leukocytosis	<i>Haemophilus influenzae</i> type b	>10-fold	Syrogiannopoulos et al. (1988)
Mouse	Hepatomegaly	<i>Escherichia coli</i>	>10-fold	Shao et al. (2007)
Mouse	Polyclonal antibody production	<i>E. coli</i> , <i>Neisseria meningitidis</i>	~100-fold	Lu et al. (2005)
Cell source	Cell type	Assay	Reduction in activity after AOAH treatment	References
B. Evidence that AOAH-treated LPS has decreased bioactivity <i>in vitro</i>				
Human	Umbilical vein endothelial cells	Leukocyte adhesion	~90%	Pohlman et al. (1987)
Human	Umbilical vein endothelial cells	Production of prostanooids, plasminogen activator inhibitor-1	~90%	Riedo et al. (1990)
Human	Neutrophils	Adherence, superoxide production, secondary granule protein release, CD11b expression	>95%	Dal Nogare and Yarbrough (1990)
Human	Monocyte (THP-1 cell)	IL-1 production, NF- κ B activation	>95%	Kitchens et al. (1992)
Mouse	Splenocytes	Proliferation	10–500-fold	Erwin et al. (1991), Lu et al. (2005), Munford and Hall (1986)
Mouse	Macrophages, dendritic cells	Cytokine production	50-fold or greater	Lu et al. (2003, 2005)
Cell source	Cell type	Assay	Inhibition by equimolar concentration of dLPS (%) <i>in vitro</i>	References
C. Evidence that AOAH-treated LPS can inhibit LPS-induced cell responses				
Human	Endothelial cells	Neutrophil adhesion	~30%	Kovach et al. (1990), Pohlman et al. (1987)
Human	Endothelial cells	Prostanoid, plasminogen activator inhibitor-1 production	~60%	Riedo et al. (1990)
Human	Monocytes (THP-1 cell line)	NF- κ B activation	66%	Kitchens and Munford (1995), Kitchens et al. (1992)
Mouse	Splenocytes	Proliferation, antibody production	20–40%	Erwin et al. (1991), Lu et al.