DCs to allow robust CTL effector responses[4]. Vigorous T cell responses only ensue if the DCs have been properly activated by endogenous or exogenous (microbe-derived) signals and if T cells recognize and respond to a minimal display of MHC-peptide at the cell surface of the DCs. If the receptor interaction does not activate the DCs, the outcome can be CTL tolerance rather than robust effector function[5]. Ingestion of proteinaceous materials via the C-type lectin receptors does not activate the DCs, whereas uptake via the surface Toll-like receptors or Fc receptors, particularly FcRI and FcRIII, causes substantial DC activation. The precise activation status of DCs that have interacted with Hsp–protein fragment complexes through the CD91 and LOX-1 receptors remains to be defined, but clearly, in the hands of Binder and Srivastava[1], causes sufficient DC activation to allow cross-priming. An underestimated factor in the efficiency of cross-priming of viruses is likely to be the production of virus-induced interferon-α, which itself is a DC-activating factor. Thus, interferon-α is appropriately designated as endogenous danger signal, similar to the Hsps, and it strongly promotes cross-priming of CTLs[6].

Many issues concerning the Hsp escort system require confirmation and classification. For example, intact cell-derived ovalbumin protein has been identified as the source of antigen for cross-priming in cytosolic lysate fractions[7]. Binder and Srivastatava attributed the difference in outcome found in that study[7] to dose restriction of the cross-priming activity of cell lysates. However, the finding in that study[7] that antibodies to conformational determinants of soluble ovalbumin but not control antibodies completely removed the cross-priming activity from cytosolic fractions of cell lysates is difficult to reconcile with the present report[1] in which ovalbumin antibody failed to do so.

Another issue concerns the subset of DCs involved in the reported cross-priming. $CD8\alpha^+$ DCs are widely thought to be responsible for cross-presentation of cell-derived antigens[8]. This subset of DCs could be specialized in the phagosome pathway of cross-presentation. However, if the Hsp escort of the DC targeting system contributes to cross-priming by $CD8\alpha^+$ DCs, the LOX-1 and CD91 scavenger receptors should also be expressed and operational on this DC subset. This issue is unclear at present. In addition, the dogma that only $CD8\alpha^+$ DCs can efficiently cross-present cell-derived antigen to $CD8^+$ T cells needs to be examined for different types of antigens targeted to DC subsets with differences in expression of the individual receptors.

How can various other findings regarding CTL cross-presentation be explained, given these new findings? The location of an epitope in a protein sequence strongly affects the efficiency of cross-presentation[9]. In particular, CTL cross-priming is considerably more efficient for epitopes derived from mature proteins than for epitopes contained in signal sequences[9]. The new Hsp data do not immediately explain that observation, nor does the finding that proteasome substrates, rather than chaperone-bound peptides, mediate cross-priming[10]. Perhaps the latter finding reflects antigen uptake in cross-priming of particulate or aggregated material. Phagosome fusion with the endoplasmic reticulum has indeed been reported as a unique mechanism of MHC class I loading in DCs[11]. This mechanism obviously applies only to phagocytosed particulate antigen, in contrast to soluble antigen in the lysates studied here[1]. In the absence of specific receptors, phagocytic cells such as DCs are reportedly much more efficient in ingesting and processing particulate materials than soluble material[11,12]. Finally, small peptides not bound to chaperonins can apparently also traffic between adjacent cells through gap junctions[13]. Such peptide transfer causes CTL recognition of adjacent 'innocent bystander' cells and seems to constitute a unique mechanism of cross-presentation unrelated to that reported here[1].

In conclusion, the idea of cross-priming of CTLs by soluble cell-derived protein fragments coupled to Hsps has received a credibility boost by the report here from Binder and Srivastava, although independent confirmation is required. As a corollary, the hypothesis that DCs are equipped with a variety of extremely sensitive and specialized mechanisms to sample their tissue environment for processing and peptide presentation to $CD8^+$ T cells gains further momentum from this study. The precise MHC class I processing pathways in DCs involved in either phagosome-mediated uptake or specific receptor–mediated ingestion deserve further characterization to allow full understanding of cross-presentation in cancer and in infectious and autoimmune diseases.

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A smooth operator for LPS responses

Paul J Godowski

The function of CD14 in LPS signaling has remained enigmatic. Examination of the responses of cells and mice with defective CD14 shows that this molecule seems to modulate specific LPS recognition by TLR4.

CD14 has long been considered to have an important yet nonessential function in Toll-like receptor 4 (TLR4) signaling, acting to concentrate lipopolysaccharide (LPS) and thereby increase the sensitivity of the receptor complex to its ligands. In this issue of *Nature Immunology*, Jiang *et al.* provide a new perspective on the functions of CD14 and the complex of TLR4 and its coreceptor MD2 (ref. 1). They show that CD14 is essential for activation of the TLR4-MD2 complex by the smooth but not the rough form of LPS. However, CD14 can switch the 'downstream' signaling pathways triggered by rough LPS. Thus, the TLR4-MD2 complex may operate in distinct modes depending on the specific binding and presentation of its ligand.

LPS, a structural component of outer membranes of Gram-negative bacteria, is also a potent activator of the innate immune

Paul J. Godowski is in the Departments of Immunology and Molecular Biology, Genentech, South San Francisco, California 94080, USA.
e-mail: ski@gene.com

response that stimulates host defenses to pathogens. However, unregulated responses to LPS can lead to septic shock syndrome, a pathological condition with manifestations such as hypotension, acute respiratory distress syndrome, disseminated intravascular coagulation and multiple organ failure. Cellular responses to LPS are mediated by TLR4 in cooperation with MD2 (ref. 2). CD14, a glycosylphosphatidylinositol-anchored protein that also exists in a soluble form, can also bind LPS.

The composition of LPS varies between different species of bacteria, and some bacteria modulate the structure (or 'chemotype') of LPS to evade the innate immune system and maintain outer membrane integrity[3]. *Escherichia coli* LPS consists of lipid A; a polysaccharide component with an inner and outer core; and the highly variable O-antigen portion composed of oligosaccharide subunits. The biosynthesis of LPS is sequential. The inner core sugars are added to lipid A, followed by the addition of oligosaccharide subunits to the outer core to form the O side chain. Bacterial mutants that fail to add the inner core or the O-specific chain are said to produce 'rough LPS' because of the morphology of the colonies they form. Wild-type strains produce 'smooth LPS' and grow as smooth colonies. Lipid A, the minimal structure of LPS with TLR4-stimulatory activity, consists of a variable number of C_{12-14} fatty acids linked to a phosphorylated *N*-acetylglucosamine dimer. The composition and number and these acyl side chains are important in the activation of TLR4-MD2, as different lipid A structures vary considerably in potency.

At least two distinct intracellular signaling pathways are activated by TLR4-MD2 (ref. 4). One pathway, called the 'MyD88-dependent' pathway, requires the Toll–interleukin 1 receptor domain adaptors MyD88 and Mal and leads to early activation of the transcription factor NF-κB and the production of proinflammatory cytokines, including tumor necrosis factor (TNF). A second set of Toll–interleukin 1 receptor domain adaptors, TRIF and TRAM, are required for LPS-induced phosphorylation and dimerization of the transcription factor IRF-3, leading to the synthesis of interferon-β (IFN-β). This 'MyD88-independent' pathway can also activate NF-κB signaling at late times.

Jiang *et al.* have used a forward genetic screen in mice to elucidate the function of genes involved in the LPS signaling pathway. Peritoneal macrophages isolated from one of the mutant strains (called Heedless) are hyporesponsive for TNF production when challenged with smooth LPS but produce normal amounts of TNF when challenged with rough LPS or lipid A. Moreover, Heedless mice injected with rough LPS, but not those injected with smooth LPS, produce high serum concentrations of TNF. The Heedless phenotype was shown to be due to a nonsense mutation resulting in a truncated form of CD14. Soluble CD14 can 'rescue' the response of Heedless macrophages to smooth LPS, and CD14-deficient macrophages show responses to LPS chemotypes similar to those of macrophages from Heedless mice. These data indicate that TLR4-MD2 distinguishes between smooth and rough LPS, requiring CD14 for activation of MyD88-dependent signaling by the former but not the latter.

Although Heedless mice have normal concentrations of serum TNF after injection of rough LPS, they are resistant to LPS-induced mortality. Because production of IFN-β is essential for LPS-induced endotoxic shock in mice[5], Jiang *et al.* hypothesized that induction of the MyD88-independent pathway leading to IFN-β expression is defective in Heedless macrophages. Indeed, CD14 is required for activation of the TRIF-TRAM pathway and production of type I interferon in both isolated macrophages and serum from mice injected with rough or smooth LPS. Thus, TLR4-MD2 can bind rough but not smooth LPS in the absence of CD14 and activate MyD88-dependent signaling, but activation of MyD88-independent signaling by either LPS chemotype requires CD14.

Because type I interferons are important in antiviral defenses, Jiang *et al.* examined the responses of Heedless macrophages to vesicular stomatitis virus (VSV) and found that these macrophages or those from mice with a defect in TLR4, are hypersusceptible to VSV infection. Presumably TLR4-MD2 is required in macrophages for defense against VSV via induction of the MyD88-independent pathway, because IRF-3 but not NF-κB is activated after infection of wild-type macrophages. Although the nature of the TLR4 ligand produced by VSV infection remains to be identified, these results are also unique, as it had previously been believed that TLR7 signals responses to VSV[6]. The results of Jiang *et al.* are not necessarily in conflict with those presented earlier, as TLR4 may be the main receptor by which macrophages detect VSV, while TLR7 may be more important in other cell types. The authors also report that VSV infection of macrophages results in TLR4-dependent production of IFN-α. Although the participation of TRIF or TRAM was not demonstrated here, these adaptors have been

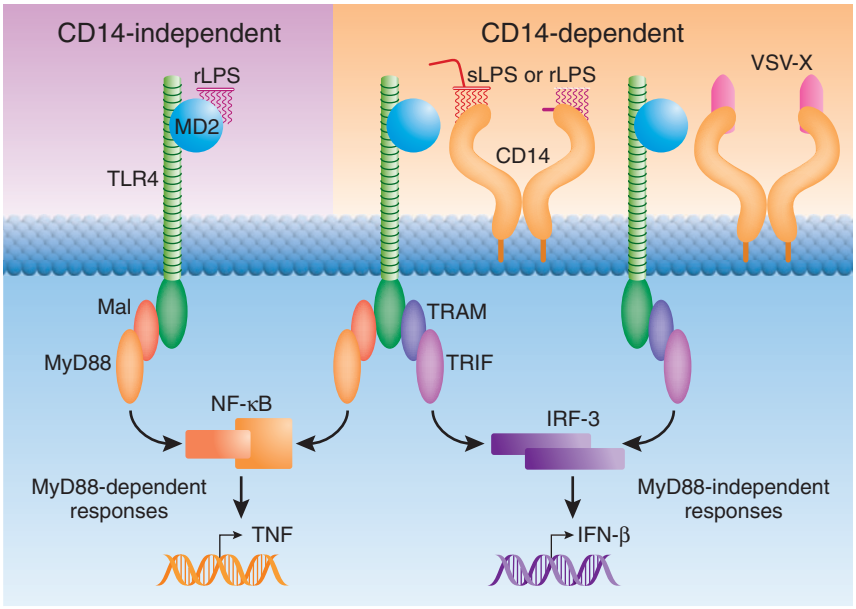

Figure 1 CD14-independent and CD14-dependent signaling by TLR4-MD2. TLR4-MD2 can bind rough but not smooth LPS without a requirement for CD14. Signaling by this complex is limited to the MyD88-dependent pathway, using the adaptors Mal and MyD88 to activate NF–κB, resulting in transcription of TNF. TLR4-MD2 can bind both rough LPS (rLPS) and smooth LPS (sLPS) in a CD14-dependent process. In addition to MyD88-dependent signals, these complexes also signal MyD88-independent responses via TRAM and TRIF, leading to IRF-3 activation and IFN-β transcription. A third mode of TLR4-MD2 signaling is possible with an as-yet-unidentified product of VSV infection (VSV-X) that activates IRF-3 but not NF-κB in a process that requires both CD14 and TLR4. Although the participation of known TLR4-MD2 adaptors was not examined, this response is consistent with a mode that is restricted to MyD88-independent signaling.

shown to activate IRF7 in response to LPS, linking the MyD88-independent pathway to IFN-α expression[7].

Based on these data, Jiang *et al.* propose two modes of TLR4-MD2 signaling (**Fig. 1**). The first mode is induced by rough LPS but not by smooth LPS, which engages the TLR4-MD2 complex in the absence of CD14 and initiates solely MyD88-dependent responses. In the second mode, which is CD14 dependent, either smooth or rough LPS bound to TLR4-MD2 initiates both MyD88-dependent and MyD88-independent responses. The observation that VSV can induce TLR4-MD2 in a CD14-dependent way to activate IRF-3 but not NF-κB suggests a third mode of receptor signaling. In this mode, TLR4-MD2 activates only MyD88-independent responses. At this point, the details of this mode remain speculative, because the VSV product that activates TLR4 is still a mystery and the requirement for TRIF or TRAM has yet to be formally tested.

Exactly how specific LPS chemotypes and CD14 dictate the intracellular responses of TLR4-MD2 signaling is unclear. TLR4-MD2 makes a distinction between rough and smooth LPS, because only the former is bound in the absence of CD14. But this does not explain why rough LPS bound by TLR4-MD2 with or without the aid of CD14 initiates different intracellular signaling pathways. Ligand-dependent TLR4-MD2 formation of oligomers is a key step in receptor signaling[8], but CD14 has not been detected in those complexes. LPS-stimulated TLR4-MD2 has been reported to associate in complexes that include CD11b-CD18, CD55, CD81, hsp70, hsp90, GDF5 and CXCR4 (ref. 9). Moreover, LPS analogs that are competitive antagonists induce receptor complexes of a composition different from those induced by agonists. Although the function of CD14 was not examined in these studies[9], the data suggest that the 'shape' of the lipid A portion of LPS can influence the nature of the signaling complex. Are the details of the interaction between rough LPS and TLR4-MD2 influenced by CD14? Crystallographic studies have shown that the leucine-rich array of CD14 folds into a horseshoe-shaped structure with a deep hydrophobic pocket near its N terminus[10]. The top of the pocket is surrounded by a hydrophilic rim and many deep grooves cascade off the lip of the rim and down the outside of the pocket. CD14 probably forms a dimer, and LPS may be presented to TLR4-MD2 with the lipid portion bound within the hydrophobic cup, with the long carbohydrate chains that distinguish smooth from rough LPS wrapped over the rim and into the external grooves. Modeling studies predict an immunoglobulin domain fold for MD2 that, analogous to the binding by CD14, may bind LPS with a least some of the acyl side chains of lipid A buried inside a hydrophobic pocket. Two models have been proposed to explain how LPS activates the TLR4-MD2 formation of oligomers[11]. In one model, the lipid A acyl side chains are shared by two MD2 proteins, thereby linking neighboring TLR4-MD2 complexes. A second model proposes that LPS induces a conformational change in TLR4-MD2, leading to the recruitment of a second receptor complex. Detailed structural studies of TLR4-MD2 bound to lipid A with or without CD14 may shed light on how different modes of signaling can be established.

The data presented here have additional implications for the nature of host responses to LPS. Although TLR4 is widely expressed, CD14 expression is limited to myeloid cells. This suggests that rough LPS will activate a broader range of cells than will smooth LPS. Furthermore, the nature of the LPS response of the nonmyeloid cells may change depending on the availability of soluble CD14 released from myeloid cells, which at high concentrations can substitute for membrane-bound CD14. Finally, the structure and proportions of smooth and rough LPS vary among different species of Gram-negative bacteria. Thus, cellular responses to these bacteria or to isolated LPS preparations may vary depending on the proportion of smooth and rough LPS chemotypes. The demonstration TLR4-MD2 can discriminate between LPS chemotypes and that the nature of the biological response can be dictated by CD14 provides an additional layer of complexity regarding the innate response to pathogens.

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Trimming the fat: a *Brucella abortus* survival strategy

Craig R Roy

Some microbes can circumvent phagocytosis by interfering with membrane transport systems. Brucella do this by making cyclic glucan molecules that interfere with lipid raft–mediated vacuole maturation.

Many pathogenic microbes have the ability to survive in macrophages. A common strategy used by bacterial pathogens to avoid being killed by macrophages is to short-circuit the membrane transport process that leads to fusion of the phagosome with lysosomes[1,2]. In this issue of *Nature Immunology*, Arellano-Reynoso and colleagues have identified a mechanism by which the bacterial pathogen *Brucella abortus* is able to disrupt phagosome transport to avoid fusion with lysosomes[3]. They show that cyclic β-1,2 glucans (CβG) produced by *B. abortus* has a biochemical activity that can disturb the formation of lipid rafts and that CβG production is necessary for the *B. abortus*-containing vacuole (BCV) to avoid fusion with lysosomes. These data provide new insight into a tactic often used for bacterial immune evasion.

A fundamental aspect of innate immunity is the extraordinary ability of macrophages to ingest microbes and rapidly transport them to lysosomes, where they are usually destroyed. 'Phagocytosis' is the term often used to describe the process whereby macrophages ingest invading microbes, and the resulting

Craig R. Roy is with the Section of Microbial Pathogenesis, Yale University School of Medicine, Boyer Center for Molecular Medicine, New Haven, Connecticut 06536, USA.
e-mail: craig.roy@yale.edu