functional significance of MC coverage and EC-MC association in tumors? Mere physical proximity does not guarantee proper molecular interaction or microstructural integration between MCs and ECs. For example, despite their tight EC-MC association (4), T241 fibrosarcomas have high vascular permeability (16). Furthermore, improved MC coverage does not guarantee normal vascular function. For example, 80% of the vascular surface area of the murine mammary carcinoma MCaIV is covered with MCs (17), yet these tumor vessels are profoundly leaky (18). Is it possible that the tumor vessel–associated MCs *increase* the vessel permeability by expressing VEGF? In addition to controlling vessel function and integrity, do these perivascular cells lead endothelial sprouts during angiogenesis, as suggested by both intravital microscopy and immunohistochemistry (8, 17)? The present study is an important step in answering these urgent questions.


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# Receptor "cross talk" in innate immunity


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**Toll-like receptors (TLRs) recognize microbial molecular signatures and can initiate innate immune responses against invading pathogens. A new study (see the related article beginning on page 1234) reports how TLR2 expression by endothelia is locally upregulated by the action of activated polymorphonuclear neutrophils via an unprecedented mechanism involving cell-cell interaction and NAD(P)H oxidase. The report reveals yet another way in which the primordial innate immune system is remarkably complex.**



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**Nonstandard abbreviations used:** Toll-like receptor (TLR); polymorphonuclear neutrophil (PMN).

In this issue of the *JCI*, Fan et al. report on receptor "cross talk" between members of the Toll-like receptor (TLR) family (1). This elegant study confirms previous observations that inflammation via TLR4 results in the enhanced expression of TLR2 (2, 3). However, Fan et al. elucidate a new mechanism of enhancement of endothelial TLR2 expression that may have important physiological consequences. Polymorphonuclear neutrophils (PMNs) that have been activated by endotoxin (LPS) can instruct endothelia to upregulate TLR2 and thus sensitize endothelia to TLR2 ligands. This message is sent to endothelial cells by the release of free oxygen radicals as the result of a CD18-dependent cell-cell interaction. TLR2 expression in endothelium, for example, was dramatically enhanced when endothelium was co-incubated with activated PMN from normal mice but not mice with a targeted lesion in $gp^{91phox}$, a member of the NAD(P)H oxidase complex. The enhanced TLR2 expression was demonstrated to result in subsequently enhanced responses to peptidoglycan, a TLR2 ligand. The ability of LPS to sensitize endothelial cells for TLR2 stimuli via LPS-induced activation of PMNs represents a previously unsuspected positive feedback loop. What distinguishes this paper by Fan et al. from a large number of reports that purport to demonstrate receptor cross talk" in the innate immune system is that the authors go on to demonstrate that the relationship among TLR4, TLR2, and the oxidative machinery has functional consequences. Dur-

ing the course of experimental Gram-negative infection, the absence of this enhanced TLR2 expression led to a blunted response to bacterial challenge: the influx of PMNs into infected lung was reduced by nearly 60% in TLR2 knockout mice.

In the last few years, it has become commonplace to refer to the complex physiological loops in immunity using phrases such as "immunological cross talk" and "immunological synapse," as if we are surprised by their existence. Indeed, when TLRs were first discovered — and it became clear that TLR4 was a receptor for LPS while TLR2 recognized purified peptidoglycan, lipoteichoic acids, and other Gram-positive bacterial products — the simplistic notion that TLR2 was a Gram-positive receptor and TLR4 a Gram-negative receptor evolved into dogma (4–9). This dogma (to which we, unfortunately, contributed) has now been shattered by the observation by Fan et al. (1) that the TLR2 KO mouse has a clear phenotype with respect to Gram-negative infections. It is time to shake ourselves free of the concept that the TLR family is individually devoted to single classes of microorganisms. Rather, TLRs are devoted to definable ligands and were designed through the nearly perfect hand of evolution to work in a concerted effort to protect the host from infection.

## A mechanism to enhance local inflammatory responses

Perhaps the most instructive aspect of the study by Fan et al. (1) is not simply the upregulation of TLR2 in response to LPS, nor the fact that this process is mediated by a product of the oxidative burst (functioning like a cytokine), but the implication that these processes are so perfectly coordinated temporally. When one considers the interactions of the innate immune system as microbes are first encountered, the value of such temporal organization is great. Consider the numerous studies over the years that have demonstrated how the interaction of whole Gram-negative bacteria with immune effector cells is similar to the interaction of LPS with these cells. These studies are instructive but fall far short of teaching us how the host responds to real infection. Unlike LPS, Gram-negative bacteria persist in tissues and — if not immediately killed through the actions of PMNs, complement, and other antimicrobial factors — may invade the host. Survival depends upon the innate immune system, which must be able to monitor and respond to pathogens over a prolonged period of time.

Because of the need for a prolonged response to bacterial infection, it has always seemed somewhat surprising that responses to LPS have a finite time limit. This time-limited response of the innate immune system to the presence of Gram-negative bacterial endotoxin, known as tolerance, means that within hours after exposure to LPS, innate immune cells are incapable of responding again to re-challenge (10). But it is now clear that as LPS sensitivity wanes, the immune system has at its disposal the capability of marshaling responses via oxidative metabolites and their ability to upregulate other TLRs. The subsequent means of responding to bacteria depend upon the ability of the innate immune system to destroy microbes and enhance the release of alternative immune stimuli. The TLRs that are utilized are the ones that bind the constituents of degrading bacteria, such as lipopeptides, peptidoglycan, heat shock proteins, and 2′-deoxyribo(cytidine-phosphate-guanosine)–rich (CpG-rich) DNA. It seems plausible that activated PMNs may even alter the phenomenon of LPS tolerance, at least in a localized context, by setting into action a positive feedback loop at sites to which PMNs are chemotactically attracted (normally the site of infectious challenge). This would locally enhance inflammatory responses needed to fight infection while reducing the general responsiveness of endothelia in noninfected areas of the host. Finally, this complex process of temporally regulated responses to microbes via different TLRs is unlikely to be restricted to bacteria. Recent studies, for example, have documented the recognition of herpes viruses by TLR2 (11). As these viruses uncoat and replicate, it is likely that their DNA also encounters TLR9, as recently described for herpes simplex virus 2 (12). The general theme is that, as pathogens march through their target cells, a highly coordinated and intricate immune response program is likely initiated.

The message, quite simply, is that innate immunity is not simple. What began as the study of fruit flies and caterpillars has become the basis of our hopes for new cures for diseases as lethal as sepsis and systemic lupus erythematosus. While most investigators choose to simplify their in vitro models of disease, it is the complex experiments, such as those of Fan et al. (1), that best reveal how biology functions outside of the test tube.


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