

University of Massachusetts Medical School

eScholarship@UMMS

---

Open Access Articles

Open Access Publications by UMMS Authors

---

2003-10-17

## Receptor "cross talk" in innate immunity

Eicke Latz

*University of Massachusetts Medical School*

*Et al.*

Let us know how access to this document benefits you.

Follow this and additional works at: <https://escholarship.umassmed.edu/oapubs>

 Part of the Immunology and Infectious Disease Commons

---

### Repository Citation

Latz E, Golenbock DT. (2003). Receptor "cross talk" in innate immunity. Open Access Articles.  
<https://doi.org/10.1172/JCI20040>. Retrieved from <https://escholarship.umassmed.edu/oapubs/999>

This material is brought to you by eScholarship@UMMS. It has been accepted for inclusion in Open Access Articles by an authorized administrator of eScholarship@UMMS. For more information, please contact [Lisa.Palmer@umassmed.edu](mailto:Lisa.Palmer@umassmed.edu).

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.

- Jain, R.K. 2003. Molecular regulation of vessel maturation. *Nat. Med.* **9**:685–693.
- Jain, R.K. 1994. Barriers to drug delivery in solid tumors. *Sci. Am.* **271**:58–65.
- Hellstrom, M., et al. 2001. Lack of pericytes leads to endothelial hyperplasia and abnormal vascular morphogenesis. *J. Cell Biol.* **153**:543–553.
- Abramsson, A., Lindblom, P., and Betsholtz, C. 2003. Endothelial and nonendothelial sources of PDGF-B regulate pericyte recruitment and influence vascular pattern formation in tumors. *J. Clin. Invest.* **112**:1142–1151. doi:10.1172/JCI200318549.
- Lindblom, P., et al. 2003. Endothelial PDGF-B retention is required for proper investment of pericytes in the microvessel wall. *Genes Dev.* **17**:1835–1840.
- Carmeliet, P. 2003. Angiogenesis in health and disease. *Nat. Med.* **9**:653–660.
- Fukumura, D., et al. 1998. Tumor induction of VEGF promoter activity in stromal cells. *Cell.* **94**:715–725.
- Brown, E.B., et al. 2001. In vivo measurement of gene expression, angiogenesis and physiological function in tumors using multiphoton laser scanning microscopy. *Nat. Med.* **7**:864–868.
- Reinmuth, N., et al. 2001. Induction of VEGF in perivascular cells defines a potential paracrine mechanism for endothelial cell survival. *FASEB J.* **15**:1239–1241.
- Benjamin, L.E., Golijanin, D., Itin, A., Podes, D., and Keshet, E. 1999. Selective ablation of immature blood vessels in established human tumors follows vascular endothelial growth factor withdrawal. *J. Clin. Invest.* **103**:159–165.
- Bergers, G., Song, S., Meyer-Morse, N., Bergsland, E., and Hanahan, D. 2003. Benefits of targeting both pericytes and endothelial cells in the tumor vasculature with kinase inhibitors. *J. Clin. Invest.* **111**:1287–1295. doi:10.1172/JCI200317929.
- Jain, R.K. 2001. Normalizing tumor vasculature with anti-angiogenic therapy: a new paradigm for combination therapy. *Nat. Med.* **7**:987–989.
- Hurwitz, H., et al. 2003. Bevacizumab (Avastin, a monoclonal antibody to vascular endothelial growth factor) prolongs survival in first-line colorectal cancer (CRC): results of a phase III trial of bevacizumab in combination with bolus 5-FU (irinotecan, 5-fluorouracil, leucovorin) as first-line therapy in subjects with metastatic CRC. *Proceedings of the American Society of Clinical Oncology.* **22**:2107. (Abstr.)
- Hirschi, K.K., Rohovsky, S.A., and D'Amore, P.A. 1998. PDGF, TGF-beta, and heterotypic cell-cell interactions mediate endothelial cell-induced recruitment of 10T1/2 cells and their differentiation to a smooth muscle fate. *J. Cell Biol.* **141**:805–814.
- Chambers, R.C., Leoni, P., Kaminski, N., Laurent, G.J., and Heller, R.A. 2003. Global expression profiling of fibroblast responses to transforming growth factor-beta1 reveals the induction of inhibitor of differentiation-1 and provides evidence of smooth muscle cell phenotypic switching. *Am. J. Pathol.* **162**:533–546.
- Kadambi, A., et al. 2001. Vascular endothelial growth factor (VEGF)-C differentially affects tumor vascular function and leukocyte recruitment: role of VEGF-receptor 2 and host VEGF-A. *Cancer Res.* **61**:2404–2408.
- Morikawa, S., et al. 2002. Abnormalities in pericytes on blood vessels and endothelial sprouts in tumors. *Am. J. Pathol.* **160**:985–1000.
- Hobbs, S.K., et al. 1998. Regulation of transport pathways in tumor vessels: role of tumor type and microenvironment. *Proc. Natl. Acad. Sci. U. S. A.* **95**:4607–4612.

## Receptor “cross talk” in innate immunity

Eicke Latz and Douglas T. Golenbock

Division of Infectious Diseases and Immunology, University of Massachusetts Medical School, Worcester, Massachusetts, USA

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.

*J. Clin. Invest.* **112**:1136–1137 (2003). doi:10.1172/JCI200320040.

**Address correspondence to:** Douglas T. Golenbock, Division of Infectious Diseases and Immunology, University of Massachusetts Medical School, Lazare Research Building 308, 364 Plantation Street, Worcester, Massachusetts 01605, USA. Phone: (508) 856-5982; Fax: (508) 864-5463; E-mail: douglas.golenbock@umassmed.edu.

**Conflict of interest:** The authors have declared that no conflict of interest exists.

**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. Polymor-

phonuclear 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 *gp91<sup>phox</sup>*, 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.

1. Fan, J., Frey, R.S., and Malik, A.B. 2003. TLR4 signaling induces TLR2 expression in endothelial cells via neutrophil NADPH oxidase. *J. Clin. Invest.* **112**:1234–1243. doi:10.1172/JCI200318696.
2. Faure, E., et al. 2001. Bacterial lipopolysaccharide and IFN-gamma induce Toll-like receptor 2 and Toll-like receptor 4 expression in human endothelial cells: role of NF-kappa B activation. *J. Immunol.* **166**:2018–2024.
3. Visintin, A., et al. 2001. Regulation of Toll-like receptors in human monocytes and dendritic cells. *J. Immunol.* **166**:249–255.
4. Poltorak, A., et al. 1998. Defective LPS signaling in C3H/HeJ and C57BL/10ScCr mice: mutations in Tlr4 gene. *Science*. **282**:2085–2088.
5. Heine, H., et al. 1999. Cutting edge: cells that carry a null allele for toll-like receptor 2 are capable of responding to endotoxin. *J. Immunol.* **162**:6971–6975.
6. Lien, E., et al. 1999. Toll-like receptor 2 functions as a pattern recognition receptor for diverse bacterial products. *J. Biol. Chem.* **274**:33419–33425.
7. Takeuchi, O., et al. 1999. Differential roles of TLR2 and TLR4 in recognition of gram-negative and gram-positive bacterial cell wall components. *Immunity*. **11**:443–451.
8. Takeuchi, O., Hoshino, K., and Akira, S. 2000. Cutting edge: TLR2-deficient and MyD88-deficient mice are highly susceptible to *Staphylococcus aureus* infection. *J. Immunol.* **165**:5392–5396.
9. Yoshimura, A., et al. 1999. Cutting edge: recognition of Gram-positive bacterial cell wall components by the innate immune system occurs via Toll-like receptor 2. *J. Immunol.* **163**:1–5.
10. Dobrovolskaia, M.A., and Vogel, S.N. 2002. Toll receptors, CD14, and macrophage activation and deactivation by LPS. *Microbes Infect.* **4**:903–914.
11. Compton, T., et al. 2003. Human cytomegalovirus activates inflammatory cytokine responses via CD14 and Toll-like receptor 2. *J. Virol.* **77**:4588–4596.
12. Lund, J., Sato, A., Akira, S., Medzhitov, R., and Iwasaki, A. 2003. Toll-like receptor 9-mediated recognition of Herpes simplex virus-2 by plasmacytoid dendritic cells. *J. Exp. Med.* **198**:513–520.