that places them at increased risk for clinical disease (**Fig. 1**).

The authors note that the genotypes associated with blunted $CD4^+$ depletion were the same as those associated with better $CD4^+$ recovery[1,2]. Whether these relationships result from increases in the rate of $CD4^+$ T cell production, decreases in the rate of $CD4^+$ T cell destruction or alterations in the distribution of $CD4^+$ T cells in the tissues and peripheral blood remains to be determined.

Regardless of the mechanism, the findings suggest a direct physiologic role of the CCR5-CCL3L1 receptor axis in $CD4^+$ T cell homeostasis. The chemokine system has a major role in determining the trafficking of white blood cells, including lymphocytes. Accordingly, changes in the distribution of $CD4^+$ T cells between peripheral blood, lymphoid organs and target tissues as a consequence of variations in the CCR5 chemokine system could help to explain the differences in peripheral blood $CD4^+$ T cell numbers seen in this study. Indeed, data from randomized clinical trials suggest that individuals receiving the CCR5 inhibitor maraviroc develop higher $CD4^+$ T cell counts than individuals not receiving this agent.[4]

The clinical significance of the higher $CD4^+$ T cell counts achieved with maraviroc is unclear. However, the fact that the increases in $CD4^+$ T cell count associated with the polymorphisms seen by Ahuja *et al.*[1] are most pronounced in the later years after the initiation of HAART suggest that these $CD4^+$ T cell count increases are more likely related to increases in $CD4^+$ T cell production or decreases in $CD4^+$ T cell death instead of merely differences in trafficking (which would be more likely to result in immediate changes in cell counts).

The question of when to begin HAART has been discussed in the HIV field for years. These new findings suggest that there may not be a single answer and add support to the idea that the optimal time to initiate HAART may depend on an individuals' genetic makeup.

Current treatment guidelines for HIV infection recommend initiating HAART when AIDS develops or the $CD4^+$ T cell count declines below 350 cells/mm^3 (ref. 3). There are two reasons for deferring HAART until this time. First, data from a large cohort collaboration indicate that the predicted risk of dying or developing an AIDS-defining opportunistic disease if HAART is started at higher counts is not substantially smaller than it is when initiation is deferred until a $CD4^+$ T cell count of 350 cells/mm^3 (ref. 5). Second, there are concerns about possible long-term toxicities of HAART that might negate its benefits. If groups of participants with high $CD4^+$ T cell counts and more favorable benefit-to-risk ratios could be reliably identified, they could be targeted for early HAART.

It is possible that any increased risk associated with poorer recovery of $CD4^+$ T cell count among those in the moderate-high GRG may not be confined to AIDS-defining opportunistic diseases. Findings from cohort studies and a large clinical trial of HAART interruption indicate that untreated HIV infection may increase the risk both of AIDS and of serious non-AIDS conditions such as hepatic failure, renal disease, cardiovascular disease and non–AIDS-defining malignancies. The relationship between $CD4^+$ T cell count and risk of these non-AIDS diseases is graded—the higher the $CD4^+$ T cell count, the lower the risk[6–9]. Thus, predictions of absolute risk reduction resulting from earlier use of HAART that consider only AIDS and death, and not morbidity from other diseases, may underestimate the potential benefit of HAART, overall and in high-risk subgroups.

Such concerns have generated interest in conducting a large trial on the timing of HAART[10]. For instance, trials of early HAART comparing initiation at $CD4^+$ T cell counts greater than 500 cells/mm^3 with initiation at the counts currently recommended by guidelines should provide opportunities to more carefully assess whether the CCL3L1-CCR5 risk stratification described by Ahuja *et al.*[1] can be replicated and whether the resulting $CD4^+$ T cell count differences are clinically important.

The new findings add to the list of genetic variants that may affect the course of HIV infection and the response to therapy. For instance, testing for the HLA B5701 allele to minimize the risk of hypersensitivity reactions to the nucleoside analog abacavir is now recommended practice in the United States[4]. A recent genome-wide analysis of people with HIV identified two sets of polymorphisms linked to control of HIV replication and one set related to disease progression[11].

There is yet more work to do in identifying the features of the host responsible for control of HIV infection. Such research, including the findings of Ahuja *et al.*[1], could lead to innovative approaches to therapy and prevention.

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Bacteria fight back against Toll-like receptors

Luke A J O'Neill

A strain of *Escherichia coli* that causes urinary tract infections seems to take hold in the body by interfering with signaling through Toll-like receptors (TLRs). The mechanism involves a secreted bacterial protein that is taken up by cells and clogs up the TLR signaling mechanism (pages 399–406).

The innate immune response to infection is a rapid and highly potent process[1] that often involves TLRs. TLRs are an important family of innate sensors, recognizing diverse microbial products and launching signaling pathways that ultimately lead to the clearance of the pathogen from the host and the establishment of a memory response in anticipation of any subsequent attack[1]. Multiple mechanisms have been found in microbes, notably viruses, that avoid or subvert TLR activation[2]. How bacteria achieve this is less well understood.

Cirl *et al.*[3] begin to fill in the gaps, introducing an intriguing mechanism used by certain bacteria to prevent TLR signaling. They find that strains of *E. coli* that infect the kidney, and several strains of *Brucella*, secrete pro-

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teins that are internalized into macrophages and disable TLR signaling by targeting MyD88, the key TLR signaling adaptor. This strategy emphasizes the importance of TLRs for innate immunity in humans and provides insights into bacterial virulence and infectious diseases that might point to new therapies.

The inspiration for the study of Cirl *et al.*[3] was the previous observation that vaccinia virus encodes a protein termed A46R, which interferes with TLR signaling by sequestering MyD88 (refs. 4,5). A46R was shown to contain a domain homologous to the Toll–interleukin-1 receptor (TIR) domain—a domain found in MyD88 and the cytosolic face of each TLR[6]. The interaction of the TLR and MyD88 TIR domains initiates signal transduction, and the TIR domain of A46R seems to interfere with this by interacting with MyD88. There has also been a report of a protein termed TIR-like protein A in *Salmonella enteritica* serovar Enteritidis that also impairs TLR- and MyD88-mediated signaling and, in doing so, promotes intracellular bacterial accumulation[7].

Given these observations, Cirl *et al.*[3] screened bacterial genomes for proteins similar to the *Salmonella* protein. They found such a protein, dubbed TcpB in *Brucella melitensis*, that causes brucellosis (characterized by fever and muscle pain) and another, TcpC, in the uropathogenic *E. coli* strain CFT073. Structural modeling confirmed the similarity of the proteins' TIR domain to the mammalian TIR domain.

To test the function of TcpC, they deleted it from *E. coli* CFT073 and then used this strain to infect a macrophage cell line and a uroepithelial cell line. Infection with the mutant resulted in a stronger induction of the pro-inflammatory cytokines tumor necrosis factor (TNF) and interleukin-6. TcpC was also shown to facilitate the intracellular survival of *E. coli* CFT073.

The authors also transfected cells with genes encoding TcpC or TcpB[3]. These genes blocked activation of the key inflammatory transcription factor NF-κB by the Gram-negative bacterial product lipopolysaccharide (LPS), which acts via TLR4. Neither Tcp protein affected the NF-κB signal when activated by receptors that do not signal via MyD88, pointing to the proteins' specificity. Importantly, TcpC was shown to bind MyD88 directly. All of these results clearly indicate that certain strains of *E. coli* and *Brucella* have TIR domain–containing proteins that target MyD88 to limit innate immunity.

These molecular observations seem to be relevant for virulence. To examine this question, the authors used a mouse model of acute kidney infection. In mice infected with wild-type *E. coli* CFT073, a much higher bacterial burden and a greater likelihood of kidney abscesses were observed relative to mice infected with the mutant strain lacking TcpC. Most interestingly, *E. coli* strains were isolated from the urine of people with kidney infections (acute pyelonephritis), bladder infections (acute cystitis) or asymptomatic bacteriuria, which represent decreasingly severe forms of human urinary tract infections (UTIs). Forty percent of acute pyelonephritis isolates contained bacteria with TcpC, with these bacteria being less common in cystitis (21%) and asymptomatic bacteriuria (16%). These results suggested that TcpC is associated with enhanced virulence, given its association with the clinical severity of UTIs in humans.

How might these extracellular bacteria get all of this to work inside the host cell? The authors addressed the issue of TcpC secretion[3]. Low pH led to the release of TcpC from *E. coli* CFT073. It was also taken up by macrophages and shown to impair TNF induction by various TLR ligands. Finally, a drug that can inhibit the efflux pump in *E. coli*, called phenylalanine-arginine-β-naphtylamide (PAβN), blocked the ability of *E. coli* CFT073 to inhibit TNF production from macrophages and prevented the secretion of TcpC.

The findings suggest that TcpC increases the severity of UTIs in humans, which is consistent with the important role of TLR4 in host defense in the urinary tract[8]. With TcpC, the bacteria can get a foothold in the host, replicate, and cause tissue injury and disease—providing the first clear evidence that human bacterial pathogens target TLR signaling in order to survive and spread (**Fig. 1**).

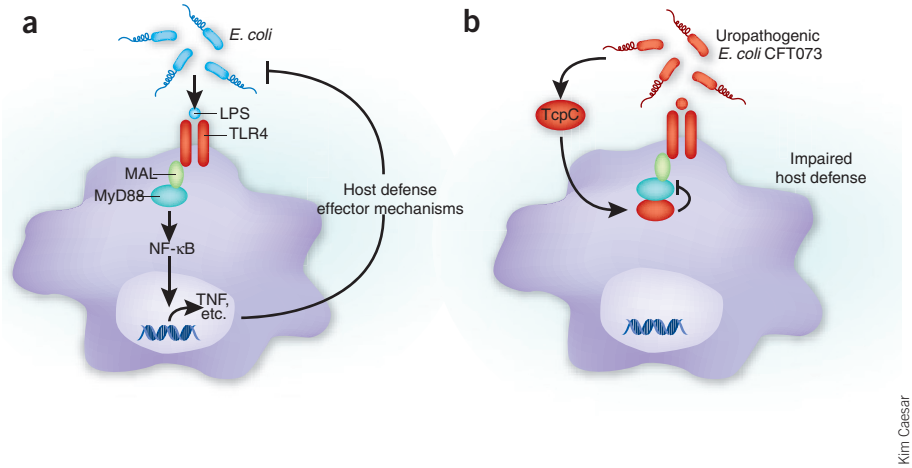

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Figure 1 Bacterial interference with TLR signaling. (**a**) Gram-negative bacteria such as *E. coli* secrete LPS, which is sensed by TLR4 on macrophages. Signaling is then initiated from the plasma membrane by the adaptor proteins Mal and MyD88 (ref. 12), leading to induction of proteins important in host defense (such as TNF) and ultimately to the clearance of the pathogen. (**b**) Cirl *et al.*[3] find that in the uropathogenic strain of *E. coli* CFT073, a protein termed TcpC is secreted and taken up by cells, where it targets MyD88 and prevents signaling. TcpC limits the host defense response, allowing the pathogen to get a foothold, spread and cause pathology. Targeting this process may be useful therapeutically.

A number of issues arise from this study. In viral infections, it's easy to envisage how the decoy protein gets to its target, because the protein will be made in the cytosol of the infected cell. For bacteria such as *E. coli* that are extracellular, at least initially, the decoy must be secreted and then taken up by the target cell. The mechanism of this uptake is still not clear, but may involve the decoy's interaction with lipid rafts.

How widely used is this mechanism in pathogenic bacteria? Cirl *et al.*[3] report that several other human pathogens contain proteins similar to TcpB and TcpC, notably *Brucella suis, Brucella abortus* and the *Staphylococcus aureus* strain MSSA476. It is therefore possible that these bacteria, and perhaps others yet to be described, also avoid innate responses by decreasing the TLR response.

Finally, how useful might these findings be for developing new treatments for bacterial infections—or for inflammatory diseases involving TLRs or interleukin-1 (which also signals via MyD88)[9,10]? For bacterial infections, as explored by the authors, agents such as PAβN that prevent secretion of TcpC could be useful adjuncts to antibiotics. Moreover, bacteria deficient in Tcps may have prospects as new vaccine candidates, as activation of TLRs clearly has potent adjuvant activity[11]. And as for the inflammatory diseases, Tcps might provide leads for the development of selective inhibitors of MyD88, which could have utility as anti-inflammatory agents.

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Robo4 counteracts VEGF signaling

Lisette M Acevedo, Sara M Weis & David A Cheresh

Robo4 expression in emerging blood vessels can neutralize signaling through the angiogenic factor vascular endothelial growth factor (VEGF) and maintain vessel integrity. The findings could lead to new therapeutic targets for angiogenesis and vascular leakage (pages 448–453).

In recent years, it has emerged that guidance cues responsible for neuronal development also have crucial roles in vessel formation and maturity. Many researchers have drawn a comparison between an actively developing axonal sprout and a newly forming blood vessel, as both migrate, elongate or retract in response to cues from surrounding tissues and matrix. Families of axonal guidance molecules influencing vessel development include semaphorins, ephrins and Slit proteins[1]. In particular, the role of Slit proteins in angiogenesis—inducing the sprouting of new vessels from existing ones—has been uddied by conflicting reports implicating them as both pro- and antiangiogenic molecules[2].

In this issue of *Nature Medicine*, Jones *et al.*[3] shed new light on the role of Slit proteins in new vessel formation. They show that the Roundabout 4 (Robo4)-Slit2 signaling axis regulates vascular integrity by counteracting the effects of vascular endothelial growth factor (VEGF)[3].

VEGF is a potent angiogenic factor that induces endothelial cell proliferation, survival, migration and invasion. Although VEGF-mediated angiogenesis is necessary for development, it can be detrimental in the adult by, for example, promoting tumor progression. VEGF is also causes vascular permeability, a disruption of the vascular barrier that can lead to edema formation and extensive tissue injury, as seen in ischemic tissue after myocardial infarction or stroke[4]. In cancer, VEGF-induced vessels are leakier, allowing for tumor cell extravasation and enhanced metastatic disease. Antiangiogenic agents targeting VEGF signaling are proving effective in the treatment of various cancers and wet acute macular degeneration; however, some tumors develop resistance to anti-VEGF drugs, and these drugs must be used in combination with more traditional chemotherapeutics[5]. Therefore, exploiting Robo4-Slit2 signaling in vessels may provide new options for therapy.

There are three vertebrate Slit proteins (Slits 1–3), which are secreted ligands for the four members of the Robo family of receptors (Robos 1–4); to date, it seems that any Slit protein can interact with any Robo receptor. Researchers interested in angiogenesis have focused on Slit2, as it is expressed in angiogenic tissue[6]. Of the Robo receptors, Robo4 has received the most attention because it is expressed specifically in the vasculature and upregulated at sites of angiogenesis. Although Robo1 is expressed in angiogenic vessels, it is also expressed in many other cell types[2].

A previous study by Park *et al.*[7] showed that Slit2 inhibits migration of endothelial cells through its interaction with Robo4, suggesting that Robo4 may negatively regulate new vessel formation. However, since then, studies have identified Slit2 as a positive regulator of angiogenesis through interactions with either Robo4 or Robo1 (refs. 6,8). These contradictory findings are further complicated by studies in zebrafish that show that either knockdown or overexpression of Robo4 impairs vessel

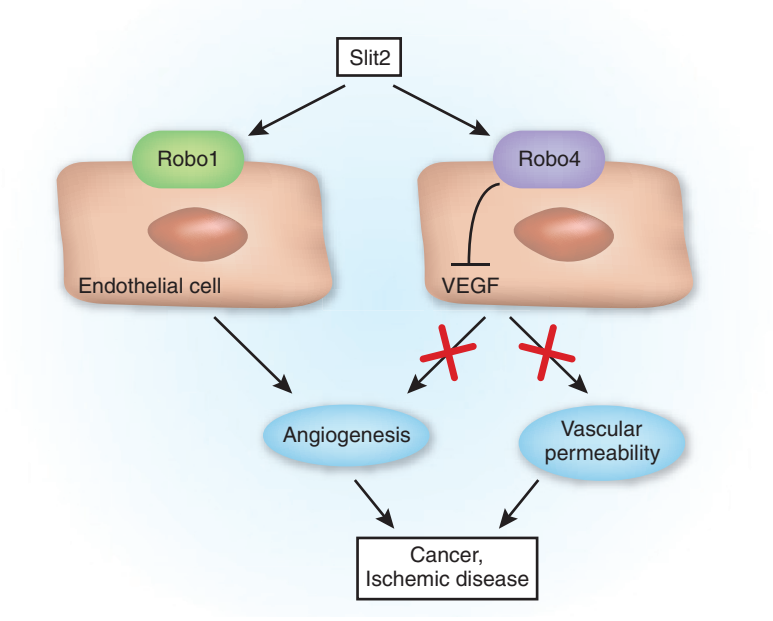

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Figure 1 The work of Jones *et al.*[3] suggests that Slit2 can both positively and negatively regulate angiogenesis by binding to Robo1 and Robo4, respectively. Activation of Robo4 curtails VEGF signaling; this, in turn, blocks VEGF-induced angiogenesis and vascular permeability, processes which severely affect conditions in cancer and ischemic disease.

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