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Immunology

Toll gates for pathogen selection

Richard J. Ulevitch

All species need an immediate, systemic reply to the microbial pathogens in their environment. This reply — known as the innate immune response — is characterized by the *de novo* production of mediators that either kill the pathogen directly or induce phagocytic cells to ingest and kill it. In the fruit fly *Drosophila melanogaster*, distinct cell-surface receptors belonging to the Toll family mediate separate anti-bacterial and anti-fungal responses in the same type of cell[1]. They do this by inducing genes that encode anti-microbial peptides.

Does this model describe the situation in mammalian systems? On page 811 of this issue, Underhill *et al.*[2] describe the ligand specificity of two members of the mammalian Toll-like receptor family, TLR2 and TLR4. These data show that the situation is indeed similar to that in *Drosophila* — that is, two distinct Toll-like receptors, both expressed in macrophages, discriminate between different microbial pathogens or their products. Whereas TLR4 is the main protein involved in recognizing Gram-negative bacteria and lipopolysaccharide (a glycolipid constituent of the bacterial outer membrane), TLR2 is the key in responses to other types of microbial pathogen, such as yeast and Gram-positive bacteria.

The innate immune response is often driven through recognition, by the host cell, of surface components of the microbial pathogen. Monocytes and macrophages are central to the intensity and specificity of this response. Most — if not all — microbial pathogens are thought to activate the innate immune system using mechanisms with common features. For example, many macrophages contain a surface protein called CD14, which binds ligands such as lipopolysaccharide and triggers an innate immune response[3]. But CD14 is not thought to participate directly in signalling. Rather, one or more of the mammalian Toll-like receptors acts in concert with CD14 to discriminate between microbial pathogens or their products[1] and initiate transmembrane signalling.

Mammalian cells may express as many as ten distinct Toll-like receptors[4]. All span the cell membrane, with repeating leucine-rich repeats in the external domain and a common sequence motif — the Toll-homology domain — in the cytoplasmic tail. Members of the interleukin-1 receptor family, which is another essential element of the innate immune system, also contain a Toll-homology domain in their cytoplasmic tails. Most attention has been paid to the TLR2 and TLR4 proteins, and the importance of TLR4 in responses to Gram-negative bacteria and lipopolysaccharide was first suggested by powerful genetic data. Beutler and colleagues[5] established that TLR4 is encoded by the lipopolysaccharide (*Lps*) gene, and that it controls sensitivity to this molecule. Akira and colleagues[6] also showed that mice in which the *TLR4* gene has been deleted have the same defects as mice with mutations in *Lps*.

But some reports indicate that lipopolysaccharide acts via TLR2. When TLR2 is expressed in cell lines that do not normally produce it, these cells become able to respond to lipopolysaccharide. Moreover, in at least one case, lipopolysaccharide-induced activation of a monocytic cell line is

Earth science

Mercurial vents

R. KELLY

On the land, many hot springs are enriched in trace metals, and have laid down economically important deposits of gold, silver and mercury. Now, Peter Stoffers from Kiel University and co-workers from Canada and New Zealand have discovered the first mercury-producing hot springs under the sea, in water 200 m deep off the coast of New Zealand's North Island (*Geology* 27, 931–934; 1999).

The mercury-depositing vents are part of more than 20 in two groups discovered in the Whakatane graben, a fault-bounded depression on the sea floor. They are inhospitable, if colourful, places, reaching temperatures of 200 °C, laying down thick crusts of arsenic and sulphur, and producing liquid hydrocarbons from the 'cracking' of organic matter — many of the samples came up coated in an oily film that smelt of petroleum. The vents are inhabited by mats of bacteria that can metabolize sulphur.

The vents are also almost saturated with mercury. Much of it is present as cinnabar (HgS; the red crusts in the picture), but Stoffers *et al.* estimate that about 10% exists as free mercury, some of which may have condensed from a mercury vapour formed inside the boiling vents. The water pressure forces some of the mercury into pores in the rocks, silver droplets of which are released when the samples are brought to the surface .

The origin of the mercury is uncertain. The basement rocks beneath the vents are shales and graywackes (a form of sandstone). These rock types have been found to contain large mercury deposits which formed in the past and, as this result shows, formation may still be going on. Shales are rich in hydrocarbons, which would explain their presence in the vents. Mercury could also be entering the vents from nearby volcanoes. Stoffers *et al.* speculate that hydrothermal vents around the world could be adding sizeable amounts of mercury to the sea. That in some places this mercury could end up in fish and eventually humans is an obvious, although unquantified, concern. **John Whitfield**

blocked by an anti-TLR2 monoclonal antibody[7]. So TLR2 has been considered a lipopolysaccharide receptor. But do these findings accurately reflect the physiological pathways of innate immunity?

These uncertainties can now be put aside thanks to the data from Underhill *et al.*[2] and to a report by Takeuchi *et al.*[8] in this month's *Immunity*. These authors found that mice lacking TLR2 respond to lipopolysaccharide in the same way as do wild-type animals. They characterized responsiveness to lipopolysaccharide in both whole animals and macrophages. When the authors looked at macrophages from their TLR2-deficient mice, they found them to be less responsive than those from wild-type mice to cell-wall preparations from several distinct Gram-positive bacteria. These data not only support the idea that TLR4 is the essential element of a lipopolysaccharide–receptor complex that determines subsequent cellular responses to lipopolysaccharide, but they also point to a key role for TLR2 in innate immune responses to other microbial pathogens and their products. And although the initial findings implicating TLR2 as a lipopolysaccharide receptor seem unlikely to be physiologically relevant, they should nonetheless be recognized for having opened a new chapter in innate immunity by showing that Toll-like receptors can activate cells in a ligand-specific way.

Where does this rapidly emerging field go now? Once again, studies in *Drosophila* may lead the way. For instance, Levashina *et al.*[9] have highlighted the importance of proteases in controlling expression of the *spaetzle/Toll/cactus* genes in an anti-fungal defence system. This group showed that the absence of a serine protease inhibitor (serpin) encoded by the *Spn43Ac* gene results in constitutive activation of an innate immune response in the fly. Their findings support two main ideas: first, that the protease system activated by exposure to a microbial pathogen (or a product derived from that pathogen) is an essential control point in innate immunity; and second, that the Toll protein is not itself a pattern-recognition receptor in *Drosophila*[10].

Given the parallels between the innate immune response in flies and in man, it is reasonable to look towards determining the ligand specificity of all the Toll-like receptors. In mammals, are the pathogen-activated protease cascades represented by proteins in the complement and coagulation pathways? Or will careful analyses reveal that cell-surface protease systems work in concert with CD14 and members of the Toll-like-receptor family to generate innate immune responses? Whatever the case, such studies should uncover in more detail the conserved ancestral features of the two systems of innate immunity that are now being worked out in the *Drosophila* and mammalian systems. ■

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Fluid dynamics

Order in chaos

Hassan Aref

Vast quantities of fluid are mixed every day, both naturally and for industrial purposes, and one might think that mixing processes are fully understood scientifically. That is far from the case. New insights are continually emerging, as exemplified by the paper by Rothstein, Henry and Gollub on page 770 of this issue[1]. They have experimentally tackled a type of low-speed fluid mixing known as chaotic advection[2], and describe the surprisingly stable spatial patterns that may arise.

Most mixing occurs in turbulent flows, where the flow field itself is composed of a complex hierarchy of interacting eddies of various sizes. These eddies will each mix fluid on their own scale. But what happens when turbulence does not occur? How does one mix fluids efficiently if the amount of agitation is somehow limited either in spatial extent or amplitude? This may occur, for example, when the fluid is very viscous or tightly confined.

For slow flows we speak of laminar as opposed to turbulent mixing, and the issue here is how to achieve efficient, laminar mixing. Everyday experience provides some ideas. Stirring milk or sugar in a cup of tea involves laminar flow. It is a task we understand intuitively. For an even slower flow consider the process of kneading dough, a mixing process for a very viscous substance. In this case, a different strategy is used in which the various layers of 'fluid' are bent and folded so as to bring initially distant parts close together and separate initially close parts.

This is our clue — laminar flows may be set up so that complex deformations of the fluid arise. In technical terms the deformation may constitute a chaotic mapping, in the sense of the theory of dynamical systems, between the initial configuration and a later configuration (for example the configuration one period later for a periodic agitation process). It turns out that the equations of motion for individual fluid particles may have chaotic solutions even if the flow field itself is a simple, laminar flow. This was

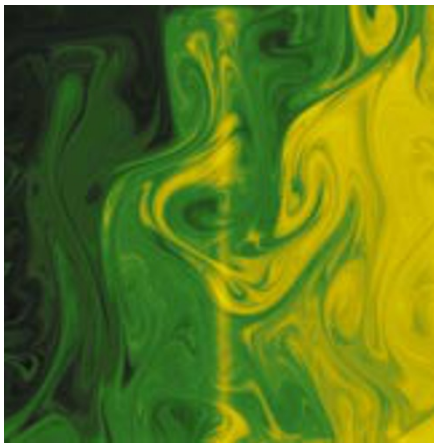

Figure 1 **Recurrent pattern created by Rothstein *et al.*[1] using electromagnetic forces to stir a thin, viscous fluid layer. One side has been labelled by fluorescent dye. The periodic laminar flow exhibits chaotic advection within which a persistent pattern recurs once per cycle of the forcing. The pattern gradually fades out over about 100 cycles, like the grin on the Cheshire cat, while the striations maintain constant thickness.**

shown by Arnold[3] and Hénon[4] in the mid-1960s, albeit for a very special class of flows (in fact, flows that do not contain any viscous effects at all). Their terse papers demonstrating how mixing in a laminar flow can display interspersed regions of regular and chaotic motion were, unfortunately, all but ignored by the science and engineering community.

The terms 'stirring' and 'mixing' enter the discussion, and require explanation. They might seem synonymous, but they have different technical meanings[5]. Stirring is the purely mechanical movement and rearrangement of fluid; mixing, on the other hand, occurs through diffusion — that is, through a homogenizing process at the molecular scale. A fluid system that simply homogenizes due to diffusion, then, is 'mixed but not stirred'. Conversely, if diffusion is essentially absent, agitation gives a fluid that is 'stirred but not mixed'. Chaos allows the creation of such fine striations,