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Workshop Report

Dialogue on defenses: Report from the workshop on "the impact of the environment on innate immunity: At the defence frontier", 22–27 April 2007

Invertebrate immune systems have been described as primitive and general because they lack the B cells, T cells, antibodies and major histocompatibility complex molecules that are the main components of vertebrate acquired immunity. But does the innate immune system of invertebrates really lack complexity or specificity? Ecologists studying invertebrate pathogens in the field have long suspected the existence of specific host–parasite interactions. But the mechanisms for immune specificity in invertebrates are poorly understood [1]. We know how *Drosophila* distinguishes between broad classes of pathogens, such as fungi and Gram-negative and Gram-positive bacteria, but how do invertebrates distinguish between different strains of the same pathogen? Two European conferences held in 2001 and 2004 brought together ecologists and immunologists to encourage more dialogue between these two fields. These conferences are discussed in two separate workshop reports [2,3]. Both these meetings highlighted the gap that exists between the broad specificity seen by immunologists and the more finely tuned specificity found in whole-organism studies. Three years later, in April 2007, the workshop in Obergurgl, Austria, organised by Paul Schmid-Hempel (Zurich, Switzerland), Joachim Kurtz (Münster, Germany) and Sophie Armitage (Copenhagen, Denmark) was a chance for both fields to report back.

The Workshop—funded by the European Science Foundation and attended by 90 immunologists and evolutionary ecologists—focused on the molecular mechanisms of innate immune defence and specificity of responses, the evolution of innate immunity, and ecological immunology. Many of the talks discussed the tremendous diversity in invertebrate immunity, not only in the immune systems of model organisms, such as *Drosophila* and *Anopheles*, but also in other invertebrate taxa (bed bugs, moths, crustaceans, worms, sponges and bees, among others). Research presented at the workshop suggests that we have underestimated the complexity and diversity of invertebrate immunity, and this diversity may rival that seen in the vertebrate world.

1. Recognising diversity

A high diversity of recognition molecules has always been regarded as a characteristic of vertebrates. Somatic diversification is one way by which vertebrates generate a diverse repertoire of defence molecules. But, it now seems that somatic diversification can occur in invertebrates. Sam Loker (Albuquerque, USA) discussed fibrinogen-related proteins (FREPs) that are produced in the haemolymph by circulating defence cells in response to infections in snails [4]. He showed that an immunoglobulin domain of FREP3 has huge diversity between individual snails. These variant FREP sequences are derived from a small set of source sequences by point mutation and recombinatorial processes.

Dietmar Schmucker (Cambridge, MA, USA) described a different mechanism for generating extreme diversity in an invertebrate immune molecule. He described the molecule Dscam, a member of the immunoglobulin superfamily. In immune cells Dscam is capable of producing 18,000 isoforms by alternate splicing, although only a small subset (~10–50) of these isoforms are ever expressed in a single haemocyte. New structural data suggest that Dscam isoforms have variable recognition sites, which may bind to a specific pathogen surface. If so, this would offer a vast repertoire of recognition molecules in the *Drosophila* immune system. Work from Schmucker on flies and George Dimopoulos (Balitmore, MD, USA) on mosquitoes raises the possibility that the different Dscam isoforms may be important in recognising different pathogens [5,6]. For example, when insect immune cells are challenged with pathogens, the pattern of alternative splicing is different for different bacteria. The

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researchers talk about exciting areas of new research into the role of Dscam in pathogen recognition.

Another way of generating a diverse repertoire of defence molecules is by evolutionary diversification. Dan Hultmark (Umeå, Sweden) discussed the role of the transmembrane protein—Nimrod C1—that is expressed on the surface of a class of blood cells called plasmatocytes. Nimrod C1 is one of a cluster of nimrod genes and is involved in phagocytosis of bacteria in *Drosophila*. There has been both rapid sequence evolution and frequent duplications and deletions of Nimrod C1 between closely related species of *Drosophila,* suggesting that this gene may be important in adapting to different parasites encountered by different fly species. Invertebrates have large population sizes and short generation times, and so they have the potential to generate huge diversity in recognition molecules by change through evolutionary time.

Finally, Brian Lazzaro (Cornell, MA, USA) showed that there is genetic variation between individual flies in their susceptibility to different bacteria isolated from the haemolymph of wild-caught flies. Because resistance is an important fitness trait, we might expect natural selection to result in all individuals being resistant. So why do we see variation in susceptibility to pathogens? He suggests that variation is maintained by a number of factors, including genotype by environment interactions and trade-offs between resistance and other traits that are important for fitness.

2. Going on the defensive

A number of talks discussed defences against dangerous microbes that extend beyond systemic immune systems. Bruno Lemaitre (Gif-sur-Yvette, France) showed that a major contribution to the fly defence against *Pseudomonas entomophila*, which is ingested by the fly, is provided by a local epidermal response in the gut, rather than the systemic immune response. Similarly, George Dimopoulos (Balitmore, MD, USA) identified a handful of Anopheline antimalarial genes that may be responding to the growth in gut bacteria following a blood meal.

A number of talks considered morphological and behavioural defences against pathogens. For example, variation in moth resistance to the granulosis virus (PiGV) may be due to the thickness of the mid-gut wall (Mike Boots, Sheffield, UK), while Hinrich Schulenburg (Tübingen, Germany) discussed the active avoidance behaviour *Caenorhabditis elegans* shows towards Gram-positive *Bacillus thuringiensis*.

To understand the relative importance of morphological, behavioural and inducible defences to immunity, it is important to study natural pathogen infections that are established by natural transmission. Several researchers presented results from coevolved pathogens including *Daphnia*-microparasite systems, mosquitos and *Plasmodium,* and bumblebee parasites. This should clearly be a major focus for future work.

3. Managing immunity

Immunopathology and the energetic costs of launching an immune response matter. Immune responses have the potential to harm as much as they help and so they must be managed. The importance of dampening down immune responses is well documented in vertebrates, but the pathways involved in the negative feedback loops in invertebrates have rarely been examined. Bruno Lemaitre (Gif-sur-Yvette, France) provided an example from a Drososphila peptidoglycan-recognising protein (PGRP). Peptidoglycan is a component of the bacterial cell wall that is recognised by the insect immune system and stimulates a rapid immune response. Lemaitre has shown how PGRP-LB, which is upregulated following infection, digests peptidoglycan and therefore dampens down the immune response [7].

Aside from mechanisms to slow down responses, it is increasingly apparent that many invertebrates maintain their immune systems in an enhanced state following an initial pathogen attack to counter future infections. This can even cross generations with the maternal transfer of immunity to offspring, which was illustrated in the meeting by examples from bumble bees and mosquitoes. In a further example of immune management, Mike Siva-Jothy (Sheffield, UK) showed the anticipatory nature of bedbug immune systems. During mating, male bedbugs pierce the female's abdomen with their genitalia. This process, known as traumatic insemination, introduces bacteria, and so female bedbugs possess an organ, the spermalege, that is thought to offer some protection against the potential pathogens introduced during sex. Siva-Jothy has found that lysozyme-like activity within the spermalege increases prior to mating, in anticipation of an increased risk of infection.

4. No more sitting on de-fence

Workers who have been willing to straddle the fields of ecology, evolution and immunology have made considerable advances to our understanding of innate immunity. And yet, the full diversity of invertebrate immune systems is just beginning to be revealed. New molecules are being uncovered (e.g. DSCAM, FREPs and Nimrod proteins) that might have the potential to recognise and attack specific pathogens, while the roles of better-studied molecules continue to expand (e.g. PGRPs). This challenges the idea that invertebrates are adequately served by broad-spectrum pathogen recognition proteins [8]. Future work must continue to investigate the diversity of environmental and pathogenic challenges faced by invertebrates and discover the potentially sophisticated molecules involved in defences. Finally, credit must be given to the organisers of the workshop for a stimulating and enjoyable 5 days. We hope that the continuing dialogue between immunologists and evolutionary ecologists will further enhance our understanding of the innate immune system and lead to future workshops like this one.

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