Immunity in the Wild

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Over half of all organisms living on Earth today are parasites, and there are hardly any species which are free from them. How can the "wild immunology" approach help us understand them better?

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We, lucky inhabitants of the First World, mainly think of parasites as exotic creatures from biology textbooks, and are only occasionally reminded of their presence closer to home when we deworm our pets. We tend to forget that the human immune system – just like those of other vertebrates – was formed and has evolved under constant pressure from parasites.

According to estimates by the World Health Organization (WHO), around two billion people around the globe are infected by nematodes. In regions where medical care is sparse and famine is common, such infections pose a major threat to health, causing malnutrition, lowered fertility, and even death. This means parasites put major selective pressure on the evolution of their hosts: individuals who cannot fight an infection are less well adapted and produce fewer offspring, and their genes gradually disappear.

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Voles lead the way

In an ecological sense, the term "parasites" doesn't just mean multicellular tapeworms or roundworms; the group includes any organisms which benefit at the expense of their hosts and cause them damage – this also means viruses, bacteria, fungi and protozoa. In vertebrates, the immune system plays a key role in defending the host from parasites; this involves several processes to identify and eliminate any intruders. Many specialized cells circulate in the bloodstream in search of clues, such as fragments of broken down cells of multicellular parasites or cell wall components characteristic of bacteria.

They are detected by specific receptors – proteins on the cellular membrane which bind pathogen-derived motifs through structural fit. A common analogy is that the fragment being detected must fit the receptor like a key fitting in a lock. And here is where an evolutionary "arms race" plays out: parasites try to break down the system by modifying their proteins so that they aren't detected, while hosts try to keep up with those changes. Since isolating and studying protein structures is difficult, the changes are easier to monitor on the level of the sequence of genes which encode them. This allows us to read the protein sequence and recreate the history of the changes which

occurred in the past to reconstruct the evolutionary history of the gene.

In order to gain an understanding of the evolutionary mechanisms shaping this process, it is essential that we study systems which are not affected by human activity – wild animals living in their natural habitats and infected with parasites. The approach has been named "wild immunology" by researchers from the University of Edinburgh. Until recently, the immune systems of mammals had only been studied in laboratory conditions, using highly selected, specially bred strains of mice. In genetic terms, individuals from each strain are almost identical, which makes the differences between experimental and control groups more pronounced; it also means that even minor experimental changes can be observed. The development of state-of-the-art sequencing technologies has made it possible to take immunology experiments beyond the lab. It turns out that the results are nothing like those obtained under laboratory conditions: wild animals are very different from their lab-raised counterparts (and also from one another), and moreover they live in highly diverse environments which pose a range of challenges. In addition to fighting infections, they have to contend with shortages of food, predators, intra-species competition, and so on. The human immune system evolved under similar conditions over hundreds of thousands of years; researchers hope that wild immunology will help them improve their understanding gained by studying clinical cases in humans and highly selected laboratory rodent lines.

I use wild immunology to study bank voles, a rodent species found in forests, as part of the Opus project, financed by Poland's National Science Centre (NCN). Voles are frequently infected by parasites, including nematodes and tapeworms, and also protozoa and bacteria (often transmitted by fleas and ticks). They have short lifespans and bear several litters a year, which means we can study several generation in a short time and observe changes in infection rate and gene frequency.

Fig. 1, 2
Voles are caught in live traps. If any females are found to be pregnant, they are released to ensure the stability of the population.

Fig. 3, 4, 5
Nematodes and tapeworms are extracted, then used as material for further study.

Context defines existence

In my earlier research, I examined the relationship between the presence of certain alleles (variants) of MHC genes and susceptibility to nematodes. MHC genes are an element of acquired immunity, based on recognizing antigens – fragments of proteins originating from parasites. This means that the acquired response is extremely specific, although it acts more slowly and needs time to develop. From the evolutionary perspective, it is a relatively recent development and is only found in vertebrates. We don't know whether the mechanisms describing the evolution of MHC genes are standard or exceptional in the immune system.

In the current project, I am studying the evolution of genes encoding elements of the innate response, in particular in TLR and cytokines. The innate response evolved significantly earlier and it is also found in invertebrates; it is also the first line of defense against pathogens. TLR proteins recognize motifs typical of single-cell organisms (bacteria, protozoa) and viruses. Mammals have between ten and twelve TLR proteins, and each one recognizes a different motif and a different group of parasites. In voles, individual TLR genes differ significantly in terms of polymorphisms; the least variation is seen in the gene encoding TLR7 – the receptor recognizing elements of viruses – while TLR2, involved in response to bacteria, shows the greatest variation. This suggests that in voles bacterial infections are a significantly more powerful selective factor than viral infections. Additionally, certain TLR2 alleles are found more frequently in individuals infected with blood parasites, while others are more common in animals free from infection. This is the result of dynamic co-evolution between parasites and hosts – the alleles which bring "susceptibility" to infection are those which bacteria have adapted to. However, this can change at any point, since "susceptibility" alleles will disappear from the population as a result of a higher mortality of the individuals carrying them.

Researchers hope that the "wild immunology" approach will greatly enrich what we have so far learned by studying clinical cases in people and highly selected laboratory rodent lines.

Helpful nematodes

The system is dynamic across space as well as time. It turns out that the same TLR2 allele can play a different role in different populations of voles: in some it's more common in individuals carrying an infection, while in others it is more common in resistant individuals. This is because the escalating arms race between parasites and hosts isn't global in character; it plays out locally and depends on the original frequency of immunity alleles in the host population and genetic variation in the given area. This can be explained us-


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ing an example from our own species: the immune systems of people living in Africa evolve under strong pressure from malaria, while in Europe resistance to flu is more important for survival. The results of my research show that such differences can be found in animal populations not living continents apart, but separated by as little as 20 km. They also indicate that there is no universal "immunity gene," and the activity of given alleles always depends on context – in this instance on pathogens found in the environment and their diversity.

Under natural conditions, it's also common for individual animals to be infected with several species of parasite at the same time, which also influence one another. In voles, this generally means a few species of gut parasites and blood parasites. Together they form an "internal ecosystem." Some of the inhabitants support one another, others compete. For example, nematodes found in the small intestine of voles – in particular in the duodenum – are negatively correlated with the presence of pinworms found in the cecum and large intestine. Additionally, different types of parasites make use of their hosts in different ways. For nematodes and tapeworms, a long-living host means they can live and reproduce for a long time. This means they are generally not especially malignant, in contrast to blood parasites whose aim is to replicate as fast as possible and move on to the next host, in blood that gets sucked by a flea or tick. Nematodes are even able to modulate the host's immune response such that it eliminates acute inflammation (Th-1 response) and replaces it with a milder Th-2 response associated with chronic infection. This property is being investigated for potential medical applications, for example to treat persistent autoimmune diseases: therapeutic infection with nematodes can relieve symptoms of illnesses such as multiple sclerosis and intestinal inflammation resulting from Crohn's disease.

My project shows that the evolutionary mechanisms shaping the variation of genes involved in immunity, of which only MHC genes (an element of acquired response) have been described in detail so far, seem to act similarly in other components of the immune system, which differ from MHC in structure and act in a slightly different way. Although "wild immunology" does not lead directly to the development of new drugs or therapies, the new understanding may end up being extremely useful, perhaps in the context of bacteria developing resistance to antibiotics and the spread of diseases and immunity to them.

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Further reading:

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