New immune systems: Disease-specific immune responses in invertebrates, and their potential applications in aquaculture

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Immunology that studies how animals defend themselves from infection is in the middle of a paradigm shift. New evidence suggests that invertebrates, including important aquaculture species such as oysters, mussels, abalone and shrimp, have sophisticated immune systems that allow them to accurately discriminate between different types of infection. In the future, it may be possible to tailor these immune systems to combat infection and prevent disease outbreaks that cause huge losses in aquaculture worldwide. This article describes the discovery of entirely new families of proteins that may underpin novel forms of disease-specific immunity. These new proteins are characterized by extremely high levels of variability, meaning that every individual can have hundreds, if not thousands, of subtly different forms of the protein. So far these types of "hypervariable" systems have been found in sea urchins, molluscs, insects and ascidians. Here, we focus on very recent research, which shows that at least some invertebrates can differentiate between infectious disease agents with great accuracy, possibly using molecular hypervariablity. If the results of this research can be used to produce economical, efficacious disease management systems for cultured animals, possibly including immunization against specific diseases, the benefits to global aquaculture will be enormous.

Adaptive Immunity and Hypervariability

Since the 1950s and 60s, the overriding dogma of immunology has been that invertebrates do not have sophisticated "adaptive" immune systems of the type found in humans (Raftos 1993). One reason that this view has persisted for so long is that invertebrates lack antibodies, the key molecules involved in the human immune system. Antibodies are "recognition" proteins. Their job is to identify invaders and label them for destruction. The ability of antibodies to detect the enormous range of infectious agents that inhabit the environment is based on molecular hypervariability. Every individual can produce millions of subtly different antibodies, each of which is able to identify a unique molecular signature associated with a particular infectious microbe. The best way to picture the way in which antibodies work is the "lock-and-key" model. Each different antibody has a unique three-dimensional shape into which a single molecular structure associated with a single type of infectious microbe can fit…like a key fitting exactly into a lock. This type of lock-and-key fit is called specific immunorecognition. Each human can produce millions of different antibodies, so every individual has the potential to identify and destroy millions of different types of disease-causing organisms, or pathogens. In this way, antibodies provide a global coverage of the vast universe of infectious microbes and parasites.

Antibodies give humans the advantage of accuracy and precision in their immune responses, allowing defenses to be tailored to particular types of infection. But antibodies come at a cost in terms of the genetic space they require. The amount of DNA contained within a cell is a limited resource. Each human cell has about 3 billion base pairs, or individual letters of the genetic language. This represents the cell's genome. Three billion base pairs of DNA sounds like a lot. But it still only codes for about 20,000 to 25,000 different genes. That's not nearly enough raw material to be able to support an antibody-based immune system that relies on the ability to produce millions of different antibodies.

The adaptive immune system of humans has gotten around this space issue with typical evolutionary ingenuity. It turns out that there are only about 300 antibody genes in the human genome. Mature antibodies are generated by cutting, shuffling and splicing these different genes into unique combinations. Each mature antibody is made by shuffling seven different types of genes. The genes are randomly shuffled and joined together, primarily during fetal and neonatal development, to form the fully spliced, functional antibodies of adults. If you calculate all of the different combinations of the different antibody genes that are available after cutting and pasting, you come up with a very big number - something on the order of 10 million different combinations. And even more variability is added by other genetic mechanisms that take over after antibody genes have been assembled. The result is a huge number of subtly different antibodies that can match all of the potential pathogens out there in the environment.

Invertebrate Immune Systems

Antibodies have been discovered in all of the groups of vertebrates - fish, amphibians, reptiles, birds and mammals. Hypervariability is evident in all antibodies, even though it is generated

by slightly different genetic mechanisms, and some animals have less variable antibodies than humans. But, in essence, the antibody-based immune system is very similar among all vertebrates.

The same is not true of invertebrates, including key aquaculture species, such as, shrimp, oysters and mussels. Antibodies have never been identified in these animals (Flajnik and Du Pasquier 2004, Litman *et al.* 2005). Most importantly, antibody-like genes cannot be found in the complete genome sequences that are now available for a variety of invertebrates. All of the DNA in sea urchins, sea squirts, worms, flies and bees have now been decoded and thorough gene databases have been constructed. Other comprehensive, albeit incomplete, gene databases are available for a many other invertebrates, including oysters and shrimp. Antibodies have not been found in any of these genetic resources. So, it seems clear that invertebrates do not have antibodies, or even genes that are similar enough to antibodies to operate in the same way (Flajnik and Du Pasquier 2004).

But this doesn't mean that invertebrates are defenseless. In every animal, no matter how simple, there must be mechanisms to fight infection or they would not survive. Infection is inevitable because all animals provide a nutrient rich, stable environment that can promote the growth of infectious microorganisms and parasites. Therefore, it is essential to have immune systems that fight infection. The universality of disease is borne out in many invertebrate aquaculture industries where disease outbreaks represent the greatest challenges to sustained production.

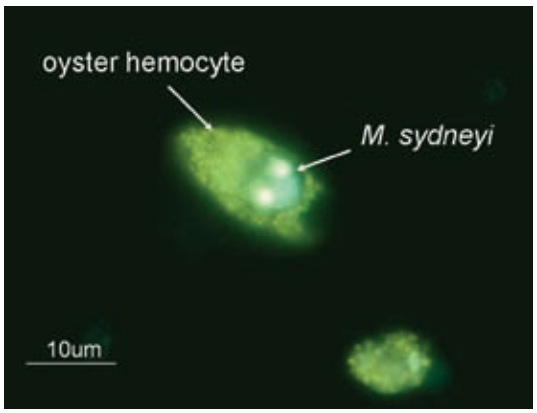

Fig. 1. High magnification image of a Sydney rock oyster blood cell that has engulfed a protozoan parasite.

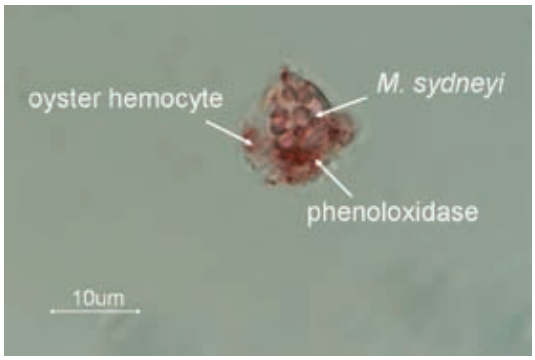

Fig. 2. The defensive enzyme, phenoloxidase, inside an oyster blood cell.

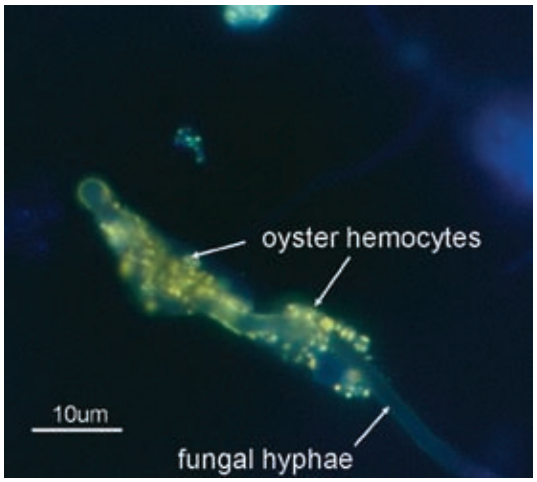

Fig. 3. Oyster blood cells forming a capsule around a fungal hypha.

To combat the threat of infection, invertebrates have many of the same mechanisms that are used by vertebrates to kill infectious agents. Each individual has a variety of these so-called effector systems to cope with highly diverse types of pathogens that need to be killed in different ways. Despite the diversity of effector systems that have evolved in most animals, there are a surprising number of common processes. Many of these processes are undertaken by specialized blood cells (more strictly called hemocytes or coelomocyte in invertebrates). Perhaps the most common cellular defense system is phagocytosis - the ability of defensive blood cells to engulf microorganisms, such as bacteria and fungi. A high magnification, ultraviolet image of a Sydney rock oyster blood cell (hemocyte) that has engulfed by phagocytosis one of the protozoan parasites that causes fatal QX disease (*Marteilia sydneyi*) is shown in Figure 1.

Once pathogens have been engulfed by phagocytosis they can be killed by specialized processes within the cell. The smaller bright dots inside the oyster blood cell shown in Figure 1 are membrane bound granules packed full of defensive molecules, including the enzyme phenoloxidase. The contents of these granules are used to kill parasites once they have been engulfed. In oysters phenoloxidase is one of the most important of these sub-cellular killing processes. Phenoloxidase produces the pigment, melanin - the same pigment responsible for the coloration of human skin. In many animals, particularly crustaceans and molluscs, pigmentation seems to be just one function of phenoloxidase. Its other, perhaps more important job, is to kill microorganisms and parasites. Many by-products of melanin production are toxic to bacteria, fungi and protozoan parasites, and melanin itself is directly involved in microbial killing.

Our research group is particularly interested in phenoloxidase because of its role in preventing the infection of Sydney rock oysters by *M. sydneyi*, the causative agent of QX disease (Bezemer *et al.* 2006, Butt *et al.* 2006). QX is a crippling parasitic disease. Outbreaks can sweep through farms killing up to 95 percent of the oysters within a few months. The disease is so severe that production has been abandoned in a number of prime oyster farming areas on Australia's east coast. Our work suggests that phagocytosis, followed by phenoloxidase-mediated killing, is an effective defense that normally keeps *M. sydneyi* infections under control. Figure 2 shows another high magnification image of a rock oyster hemocyte that has engulfed an *M. sydneyi* parasite. In that

photo, the cell has been stained to highlight phenoloxidase. Sites of phenoloxidase activity are shown as red to pink. Once the parasite has been engulfed, phenoloxidase bombards toxic metabolites onto the parasite, killing it. QX outbreaks seem to occur when this phenoloxidase-based defense of oysters is compromised. A range of common environmental factors, such as low salinity, starvation or pollution can stress oysters, causing a decrease in phenoloxidase activity (Butt *et al.* 2006). This allows the parasite to break free of phenoloxidase's normally effective control and cause QX disease.

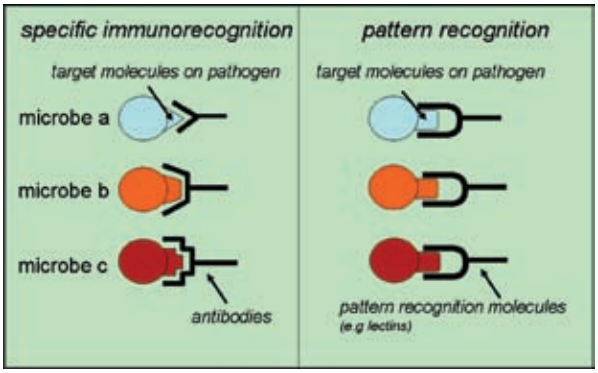

Fig. 4. Differences between specific immunorecognition and pattern recognition.

In addition to the killing systems that operate inside blood cells, invertebrates have a range of effector mechanisms that can be deployed outside cells. This is important because invaders can escape phagocytosis, often because they are simply too big to be ingested by a single blood cell. This is particularly true of large parasites, such as worms. When parasites are too large to ingest, they are walled off from the rest of the body by the process of encapsulation. Large numbers of blood cells surround the pathogen forming a tight seal, or capsule. A photo of this process is shown in Figure 3. In this image, a number of oyster blood cells hemocytes, revealed under UV light by their bright internal granules, are surrounding a fungal hypha to form a capsule. This process of encapsulation isolates parasites within infected animals, preventing them from proliferating and spreading infection. Defensive enzymes and other toxic molecules within the bright granules of the hemocytes are secreted into the capsule to kill the enclosed parasite. Phenoloxidase is one of the mechanisms used during encapsulation to kill parasites. Melanin produced by phenoloxidase is used to make the wall of the capsule rigid and prevent the parasite from escaping. It also helps to kill the parasites inside the capsule.

Other pathogens escape phagocytosis not because of their large size, but because they have developed ways of evading defense cells, or even worse, hiding inside them without being killed. To counter this, the blood cells of most animals can secrete small antimicrobial proteins that have evolved to kill bacteria, as well as some fungi and protozoans. These proteins often work by "punching" holes in the surface of their targets.

Most extracellular killing molecules are only secreted by blood cells when the presence of an infection is detected. In this way, potentially harmful killing systems are held in check until they are needed. Retaining killing systems within cells until they are required also helps to focus immune responses accurately at the site of infection. Infection usually causes inflammation, a response that increases the numbers of blood cells entering the site of an infection where the cells are stimulated to switch on their killing systems.

Pattern Recognition

As we said before, the effector (or killing) systems that can be brought to bear against pathogens by invertebrates are just as common and just as effective as those of vertebrates. Until recently it had been thought that the major difference between vertebrates and invertebrates was the way in which their immune systems are activated.

In vertebrates, hypervariable antibodies are used to detect infection and switch on effector processes, such as phagocytosis. In contrast, invertebrates had been thought to rely on very different types of detection molecules that use a totally different method to identify infectious agents. This novel type of detection regime has been termed pattern recognition. Instead of using hypervariability to detect exact molecular structures found on the surface of individual microbes, pattern recognition detects structures that are common to large groups of different pathogens. The targets of pattern recognition are molecules, such as lipopolysaccharides (LPS), which are key building blocks that make up the surface of many different types of bacteria. By targeting such broadly distributed molecules, a single pattern recognition molecule, LPS-binding protein, can be used to detect a range of different bacteria. Other

common targets for pattern recognition include the simple sugars found on the surface of many bacteria and fungi. These sugars are detected by another type of pattern recognition molecule, called lectins. Again, a single type of lectin can detect a broad range of different bacteria and fungi because they all have the same type of sugar on their surface.

Pattern recognition is totally different from the process used by antibodies. Antibodies have evolved to detect absolute, fine differences between microorganisms. In pattern recognition, the opposite is true. Pattern recognition looks for molecular signatures that are common to many microbes, not those that differ between them. This is a contrasting, but effective way of achieving the same goal as antibodies; that is, identifying infections and activating defensive responses to suppress them. The fundamental distinction between pattern recognition and specific immunorecognition is shown schematically in Figure 4.

How Important is Pattern Recognition?

Pattern recognition molecules are not inherently less efficient than antibodies in doing their core job, detecting infection. Indeed, they have some advantages over antibodies. They do not rely on the complex genetic machinery needed to generate hypervariability. Just a few genes, maybe as few as 10, are needed by an individual to give it fairly complete coverage of the pathogen universe. And pattern recognition molecules do not need the same complex cellular systems that antibodies require to turn on their production. Unlike antibodies, pattern recognition molecules can be produced continuously, and, therefore, are present at the very beginning of an infection when it is easiest to prevent the onset of severe disease.

The relative advantages of pattern recognition are easiest to see in vertebrates. Even though animals have sophisticated antibody-based immune responses, they also have the same types of pattern recognition systems that are found among invertebrates. In humans and other vertebrates, pattern recognition molecules act as a first line of defense. They are the key molecules used to detect pathogens in the early stages of infection, before antibodies are synthesized. It often takes many days for humans to mount an effective antibody-mediated immune response. Pattern recognition serves as a stopgap during this lag phase. This is shown most elegantly by a common human pattern recognition molecule, called mannose-binding lectin (MBP). The MBP detects a particular type of sugar (mannose) found on the surface of bacteria, fungi and some viruses. When it binds to that sugar, it labels the pathogen for destruction by killing systems, such as phagocytosis. The MBP is produced from birth onward, whereas the full antibody repertoire of humans is not established until later in neonatal life (about three months after birth). It is a critical stopgap in this neonatal period. Its role in detecting infection is so important during this period that individuals with genetic defects in MBP often die from infections prematurely.

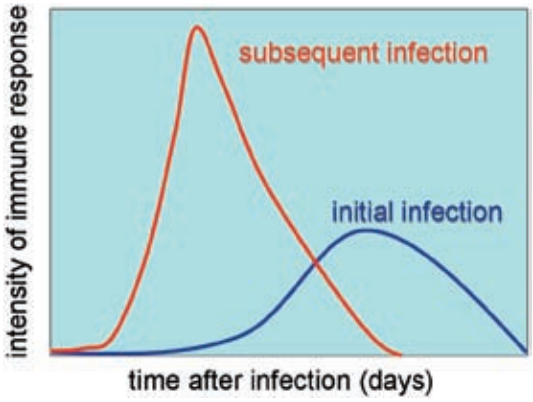

Fig. 5. Vertebrate "memory" responses.

Vaccination and Immunity

Even though the initial lag phase in antibody production may seem to be a fatal flaw, it actually reflects the greatest strength of the antibody-based immune system. Antibodies are produced by specialized blood cells, called B lymphocytes. Each different type of antibody is produced by a very small subset, or clone, of B lymphocytes. A single B-cell clone can only produce one specific type of antibody and every individual needs millions of different antibodies. Normally, each B-cell clone is made up of just a few cells, sometimes as few as 10 in the entire body. This is the reason that it takes so long to mount an antibody-based immune response. When a B-cell clone producing a particular antibody is switched on to counter a particular infection, the clone contains too few cells to have any immediate effect on the infection. When the appropriate B-cell clone bearing the right type of antibody to fight the infection has been selected, it takes time for that clone to proliferate by cell division to build up the large numbers of identical B-cells that are needed to control the infection. In many cases, this process of clonal selection can take more than a week.

Clonal selection tailors the immune system to fight each new infection that the body encounters. The B-cell clone bearing the most appropriate antibody is selected to proliferate and fight the infection. This gives the antibody-based immune system two great advantages over pattern recognition: memory and specificity. Specificity comes from selecting B-cell clones that can produce antibodies that are fine-tuned to fight the particular microorganism being encountered. Memory is generated as a byproduct of B-cell proliferation. When individual B-cell clones are selected and start expanding by the process of cell division, not all of the daughter cells produced go on to make antibodies. Some are 'put to sleep.' They become memory cells that lie dormant until the host meets the same type of infection in the future. When the same infection recurs, memory cells are waiting for it. The effects of this immunological memory are depicted graphically in Figure 5. The graph shows a typical memory response of the type that occurs during immune responses in vertebrates. When an animal first encounters an infectious disease, its immune response is usually quite slow to react, because it takes time to switch on the production of specific antibodies that are fine-tuned to deal with that particular infection. However, when the same infection is encountered again, memory cells produced during the initial infection become activated very quickly, producing a far more powerful and rapid response the second time around. This is the process of

immunization, which allows vertebrate animals to be specifically vaccinated against particular diseases.

The ability of antibody-based immune systems to remember previous infections allows vertebrates to be vaccinated against selected diseases. For instance, humans can be vaccinated against pandemic diseases, such as, polio or smallpox. Attenuated (harmless) versions of the disease causing agents are injected into an individual to trick the immune system into mounting an antibody response that is fine-tuned for that particular disease. This generates disease-specific memory cells targeted specifically against the microbe used in the vaccination. Sometimes the memory response generated by vaccination is so effective that it lasts the remainder of the individual's life. When a real, virulent infection by the same disease agent happens, the immune system is already primed to mount rapid, disease specific responses to that particular pathogen.

Exactly the same process allows fish to be vaccinated against many of the major bacterial diseases that affect aquaculture production, including various species *Vibrio*, *Edswardsiella*, *Flavobacteria* and *Aeromonas*. These vaccines can provide long-term protection from disease, without which many fish aquaculture industries could not remain commercially viable.

Can Invertebrates Mount Disease Specific Immune Reactions?

From everything presented so far, it is clear that there are two requirements for an animal to be able to mount disease-specific immune responses…

- The ability to produce hypervariable defense molecules that can differentiate between different disease-causing organisms with great accuracy and,
- Ways of linking those molecules to killing mechanisms, so that disease-specific immune response can be generated.

For years after the discovery of antibody-based adaptive immunity in vertebrates, it was thought that invertebrates lacked both of these essential criteria for disease-specific immunity (Raftos and Raison 1992). In hindsight, the failure to detect hypervariable defense molecules and pathogen-specific immunity among invertebrates may end up saying more about our failure to look in the right places than it does about the absence of such systems. Most research in the past has been biased by our own view of what these systems should look like. That view was usually anthropocentric. In many instances, we believed that all disease-specific immune systems should mirror our own.

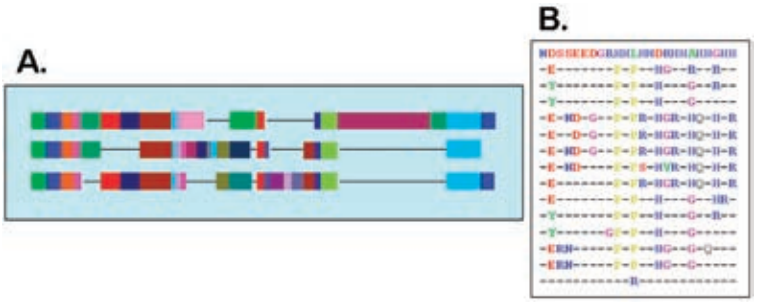

Fig. 6. The generation of molecular "hypervariability" in sea urchins.

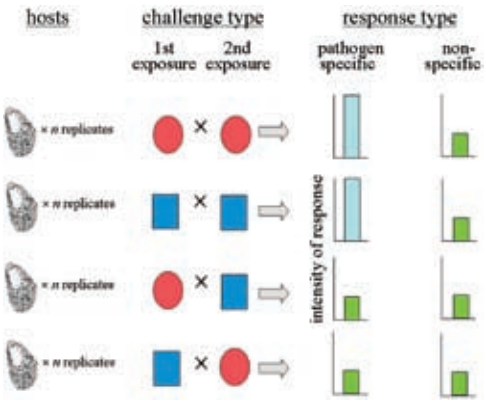

Fig. 7. The design of classical vaccinations experiments.

That view now seems flawed. New technologies are revealing the existence of hypervariable gene systems among a number of different invertebrates that may be involved in anti-pathogen defense. None of those systems produce molecules that look like antibodies. So far, hypervariable genes that are associated with defense have been identified in sea urchins, snails, lancelets, sea squirts and flies. For the remainder of this article, we focus on the hypervariable molecules from sea urchins because one of us, Sham Nair, working with Courtney Smith in Washington DC, was responsible for their discovery and both of us are still closely involved in work on this new system.

The hypervariable genes from sea urchins have been designated *185/333*s, or *185*s for short. This name is based on the original gene lodged in the Genbank gene sequence database during the early 1990s. Even though a single version of these genes had been known for almost 10 years, it wasn't until Sham Nair and Courtney Smith started to characterize the immune response of sea urchins at a genetic level that the true significance of *185* genes became apparent. When sea urchins were vaccinated with bacteria, or bacterial molecules, more than 60 percent of all the sea urchin genes activated turned out to be closely related to the original *185/333* sequence from Genbank. That was unusual. Most often, hundreds if not thousands of different genes are turned on during an immune response. One particular set of genes usually doesn't predominate as *185*s seemed to do in sea urchins. On closer inspection, it became obvious that *185* genes coded for a large family of hypervariable defense proteins. We don't yet know exactly how many subtly different *185* proteins can be made by an individual sea urchin. But we do know that the number is greater than 100, and probably much higher. So far, more than 800 different *185* gene sequences have been identified within populations of sea urchins.

The *185* genes seem to be constructed by a combination of two different processes. When the DNA sequences of different *185* genes are aligned with one another, it becomes obvious that the sequences can be divided up into about 27 different blocks, or elements of DNA. Those elements, and the way

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in which they are arranged, are shown in Figure 6A. Different *185* genes have different combinations of elements. The graphic shows three different 185 genes, each of which is comprised of different combinations of elements, shown as different colored boxes. Some 185 genes have all 27 elements, others have fewer. So far, researchers from the laboratory in Washington, have identified hundreds of different combinations of elements. But that's not the limit of diversity among *185*s. The molecular variability provided by element shuffling is magnified even further by variation within the elements themselves. There are multiple versions of each element based on changes in individual nucleotides, or letters of the genetic code, within each element. That sort of "single nucleotide polymorphism" is depicted in Figure 6B, which shows the changes that occur in the genetic code (represented by letters) in a small region of 15 different *185* genes. In the graphic, a dash means that the same letter of the genetic code is found in this position among all of the genes.

We do not yet have a clear idea of what the proteins encoded by *185* genes do during sea urchin immune responses. But, we do know that they are synthesized by one particular population of sea urchin blood cells. These cells are obviously involved in defense responses similar to the encapsulation reactions that we described earlier and they may also participate in phagocytosis. The *185* proteins are located on the surface of these cells, suggesting that they may be involved in the interaction of these cells either with other sea urchin cells or infectious microbes. Whatever their role, it is clear that cells bearing *185* proteins on their surface are responsive to infection. The numbers of these cells in sea urchin blood increases dramatically when sea urchins are vaccinated with bacterial molecules.

A different family of hypervarable molecules, called fibrinogen-related proteins (FREPs), have been discovered in pond snails by our colleagues Eric S. Loker and Coen Adema from the University of New Mexico in Albequrque (Flajnik and Du Pasquier 2004). Fibrinogen-related proteins were first identified during a long-term study of the relationship between pond snails and the human parasite, *Schistosoma mansoni*. Snails are an intermediate host for *S. mansoni*, which is a major public health problem, particularly in the developing world. During their studies, Loker, Adema and their team found that snails respond to *S. mansoni* infection by producing FREPs. Again, individual snails seem to be able to produce many different versions of FREPs. Similar to *185*s from sea urchins, FREP diversity is based on the combination of different blocks of DNA and on variation within each block. While the exact function of FREPs is still unclear, it is known that they interact with the surface of *S. mansoni* parasites. Critically, the subsets of FREPs synthesized by individual snails changes in response to different microorganisms.

In insects, hypervariability has been found among Down syndrome cell adhesion molecules (DSCAMs), which are involved in phagocytosis (Litman *et al.* 2005). A repertoire of more than 38,000 different DSCAMS is present in the vinegar fly, *Drosophila*. The molecules are constructed by similar forms of DNA shuffling to those seen in snail FREPs and sea urchins *185* proteins. Variable chitin binding proteins (VCBPs) from lancelets and tunicates are produced by similar molecular mechanisms. VCBPs seem to be designed to identify molecules like chitin, which is one of the key components in the shells of crustaceans and insects, and is also found in the cell walls of fungi, molds and yeast. Like FREPs and 185 proteins, the production of VCBPs is greatly increased when individuals are exposed to molecules from infectious microbes.

One of the most important observations to come out of the discovery of these different classes of hypervariable defense molecules is that hypervariability is not particularly rare. There is nothing special about the evolution or life histories of vertebrates that provides evidence that these animals are the only ones to have developed molecular hypervariability. Indeed, the identification of hypervariable gene systems in relatively quick succession, among four different groups of invertebrates, indicates that similar systems will probably be discovered in many other animals in the coming decades.

Another intriguing thing about the hypervariable systems discovered so far is how different they are from antibodies, and from each other. FREPs, DSCAMs and VCBPs have some regions that are distantly related to antibodies, but they are still very different molecules. And sea urchin *185* genes are entirely unique. No similar gene sequences have ever before been reported. This means that it is extremely difficult to predict what the next group of hypervariable genes will look like, or what exactly they will do during an immune response.

Do Hypervariable Defense Molecules of Invertebrates Allow Them to Mount Disease Specific Immune Responses?

One clue about the role of the newly discovered hypervariable genes in immune responses has come from recent work of Courtney Smith and her group in Washington DC. They have found that the types of hypervariable *185* molecules being synthesized by sea urchins changes when the urchins are vaccinated with different pathogen-associated molecules. Similar results are being uncovered by our work on *185* proteins and by Loker and Adema's studies of FREP synthesis in snails. The data suggest that the repertoire of hypervariable proteins being synthesized by sea urchins or snails may change in response to infection. Perhaps the suite of genes responding to an infection is tailored to meet the demands of a particular infectious agent in much the same way that antibody responses are fine-tuned by clonal selection. If this is the case, it is likely that invertebrates are able to mount disease specific immune responses using hypervariable proteins to discriminate between infectious agents.

Remarkably, the possibility that invertebrates can mount disease specific immune responses has never been tested exhaustively, until now. In the last few years, reports have begun to appear of

responses among invertebrates that fit the expectations of disease-specific immunity (Little and Kraaijeveld 2004, Little *et al.* 2005). This recent research differs from previous studies because they exploit well-defined host/parasite interactions that allow strictly controlled experiments to be undertaken with infectious microbes or parasites that are relevant to the host species being studied.

These new studies have used vaccination to test the outcomes of host/parasite interactions. Figure 7 shows the design for a classical vaccination experiment of the type that has been used to distinguish between disease-specific immune systems and other non-specific types of immune response. In this experimental design, the immune system of host animals, oysters in this case, are primed by exposure to one type of infectious microbe. They are left to recover and then exposed to either the same species of microbe, or a different pathogen. If the host has a disease-specific immune system, enhanced responses will only occur when the same microbe is used in the second exposure, while non-specific responses do not discriminate between pathogens. In the past, this type of experiment has failed to provide useful information because the added involvement of non-specific pattern recognition responses clouded the results. The most recent trials have employed closely related microbes that cannot be distinguished by pattern recognition systems to overcome this problem. As a result, they have been able to test far more accurately and precisely for target specificity. In one of the recent studies, it was shown that infections of the copepod crustacean, *Macrocyclops albidus*, with the tapeworm, *Schistocephalus solidus*, were far less severe if the hosts had been primed with siblings of the worms used for the subsequent infections. This acquired protection was not evident if the tapeworms used in the initial and subsequent challenges were genetically distinct. Similar vaccination experiments in the shrimp, *Penaeus monodon*, identified discriminatory responses to virulent white spot virus. Injecting an envelope protein from the virus, VP28, provided substantial protection against white spot infection, whereas a closely related protein, VP19, did not. Protection against white spot syndrome in another species of prawn, *Litopenaeus vannamei*, can be elicited by injecting a special form of RNA, called double stranded RNA (dsRNA). Even though randomly generated dsRNA has some effect on reducing mortality, disease-specific protection is induced only when dsRNA that mimicked the genetic code of white spot virus are used. In a final series of vaccination experiments, it was shown that induced, specific protection against pathogens in the water flea, *Daphnia magna*, can be transmitted from mother to offspring.

What Lies Ahead?

Future developments based on our recent research are still unpredictable, even though the potential benefits seem very large. The production of vaccines for specific disease agents and systems to deliver these vaccines to large numbers and a wide variety of commercially important invertebrates will be difficult, perhaps even impossible. Effective, economical large-scale delivery systems for vaccines simply may never become available. It is also possible that the hypervariable gene systems identified so far have evolved to control very specific host/parasite relationships, such as the interaction of FREPs with *S. mansoni*, and may have little relevance to the types of diseases that threaten aquaculture industries. Finally, it may prove feasible to induce disease-specific responses in some species only to find that the protective period provided by specific vaccinations is too short to make any real difference in the context of aquaculture management practices.

Despite all of these considerations and potential obstacles, the only sensible way forward is to continue and scale up research in the area, simply because the potential benefits of positive outcomes far outweigh the initial investment in research. Think about it this way. An effective vaccine to control White Spot Virus Syndrome in shrimp would save the industry billions of dollars per year in lost production. Next to that, the cost of discovery is cheap.

Notes

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