Good memories for details improve fish health

Prof. dr *ir.* G.F. Wiegertjes

Inaugural lecture upon taking up the post of Personal Professor of Cell Biology and Immunology at Wageningen University on 15 May 2014

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Rector Magnificus, colleagues, family and friends,

During this inaugural speech, I would like to introduce you to the field of cell biology and immunology and in particular the field of fish health. I will take you on a journey under water and let you experience life as a fish in aquaculture where your health is threatened by dangerous bacteria, viruses and parasites. After this unnerving experience I will introduce to you the different players of your immune system and explain the building blocks that underpin immune-enhancement and vaccination, both excellent interventions available to help you, as fish in aquaculture, fight infectious diseases. I will discuss with you new developments which indicate that memory may also exist for innate immune responses, which provides us with exciting possibilities to protect fish health. I hope that later today you will leave this room with the urgent desire to either become a fish in aquaculture, or become a scientist studying fish health and immunology.

Of immune-enhancement and vaccination

The key to a healthy immune system is its ability to distinguish between own body cells and foreign organisms. Foreign organisms, such as viruses and bacteria, carry nonself "antigens" and should be attacked by the immune system. The immune system can be divided into two categories: innate and acquired. Innate immunity refers to semi-specific defense mechanisms, which come into play very quickly after an antigen appears in the body. Acquired immunity refers to slower but highly specific mechanisms, often based on the production of antibodies. Acquired immunity may develop more slowly, but once developed, it retains a strong memory component for the same antigen based on the rapid clonal expansion of long lived memory T and/or B lymphocytes. Acquired immunity is built up during childhood, when you develop antigen-specific memory for when you grow up to be older and wiser. In contrast, innate immunity is provided from birth onwards and has always been thought to remain unchanged during life. This dogma, however, is challenged by exciting new developments which indicate memory may also exist for innate immune responses.

It is well known that in plants innate immunity bears a form of memory (Muthamilarasan and Prasad, 2013) and also in insects innate immunity can show a form of memory (Pham *et al*., 2007). The most recent observations indicate that also innate immune cells of mice, for example, could preserve memory functions. It is now known that macrophages, cells typical of innate immunity, can adapt and reshape their response upon a second exposure to the same microbe (Netea *et al*., 2011). This phenomenon is described as trained immunity (Fig. 1).

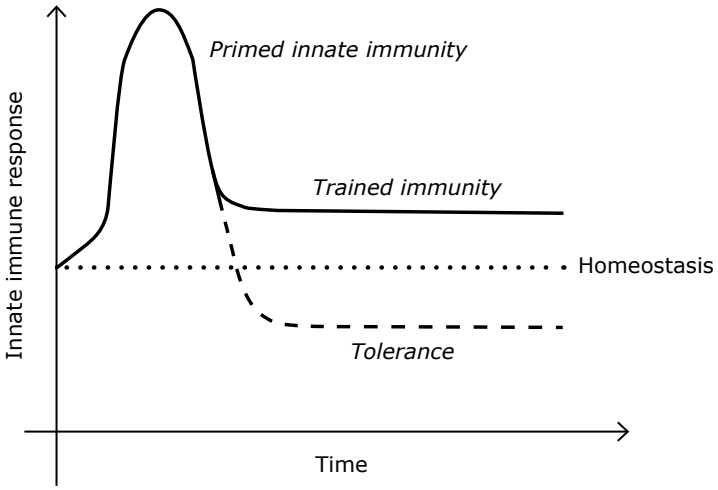

Figure 1. Trained Immunity (from Netea, 2013). During certain infections or shortly after exposure to certain stimuli, innate immune responses can be primed to respond more strongly upon challenge with a second stimulus. Although this priming effect declines rapidly after the infection is resolved, the state of innate immune responses often does not return to basal levels after the infection, remaining enhanced through medium- and long-term reprogramming effects at the level of innate immune cells. Trained immunity can be defined as a type of nonspecific protection against unrelated pathogens. It supports the suggestion that the innate immune system has a memory that can be trained and opens up a series of questions on the evolutionary origin and extent of these nonspecific training effects.

Vaccinations are widely viewed as the most successful medical advancement in the history of public health: before vaccines were introduced smallpox killed millions of people and polio paralyzed thousands. To date, we routinely vaccinate our children at young age to protect them from childhood diseases such as measles, mumps and rubella and we vaccinate ourselves above middle-age against the annual wave of flu. In fact, we not only vaccinate ourselves but also vaccinate our pet animals: our dogs against rabies, our cats against cat flu and we even vaccinate our pet birds against canary pox. So why should it surprise you when I tell you we also vaccinate our fish?

Yet, when asked, it does surprise most of you and I would dare to take a bet that when confronting most people in this audience with the question, "what to do with a sick fish", most often your answer would be: flush this fish through the toilet.

Yet, we can and we do routinely vaccinate our fish (Brudeseth *et al*., 2013). However, regretfully, fish vaccines often are developed only against those pathogens and for those fish species that are of high economic importance, for example salmon. In other words, against those pathogens that potentially kill many of the most expensive fish. This may sound harsh, but you may appreciate that economy is an important driving factor in our present day society. But for those fish species considered of lower economic value there is hope too: immune-enhancers require innate immunity only, semi-specifically boosting fish health (Rombout *et al*., 2011) and thus can be effective against many different pathogens at the same time. Vaccines, in contrast, need acquired immunity and by definition are highly antigen-specific. Imagine as immune-enhancers not only a healthy lifestyle, pre- or pro-biotica, but also harmless yeast-derived products such as beta-glucans (Dalmo and Bøgwald, 2008). The scientific mechanisms explaining the effects of these immune-enhancers should be sought in the presence of pattern recognition receptors (Akira *et al*., 2006; Pietretti and Wiegertjes, 2014) on white blood cells. I will come back to these receptors later during my lecture. For now, please remember: both vaccination and immune-enhancement are excellent interventions to keep fish healthy.

Aquaculture and fisheries

At this moment I would like to fine-tune the common denominator "fish": when I mention fish, you probably first think of the goldfish in your fish bowl at home. Most of you probably would not imagine there are thousands of fish grown in aquaculture systems. Especially the fish grown in aquaculture are what I will be referring to this afternoon. At this point it is good to realize that approximately half of the world's fish for human consumption is grown in captivity rather than caught in the wild.

The next graph (Fig. 2) from the Food and Agriculture Organization of the United Nations, makes clear that aquaculture is responsible for an ever increasing proportion of the world's consumption of fish products. In fact, there are some eminent professors who are convinced that the future world demand primarily will be on chicken and fish: we recently had prof David Hughes from the Imperial College at London as invited speaker at our annual Graduate School Science Day. He convincingly spoke about the future needs for aquaculture products, to meet the increasing demands for protein.

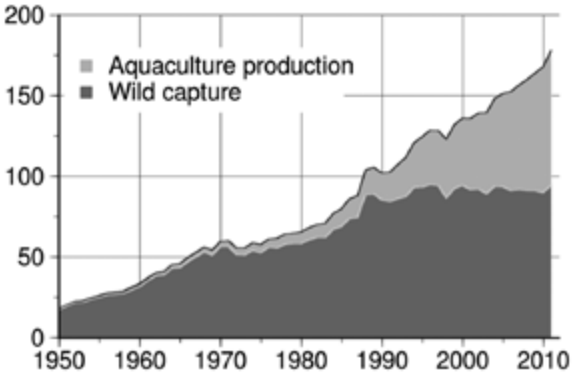

Figure 2. Relative increase of aquaculture production with respect to wild fish capture over the last 60 years (Food and Agriculture Organization of the United Nations, FAO).

To provide you with some more insight in the underwater world of fish grown in aquaculture systems, I will now show you two short videos. The first video shows an extensive farming system of carp in large ponds typical of countries in East-Central Europe: please note the large football field-sized pond from where the carp are harvested prior to becoming a traditional Christmas dish. The second video shows an intensive farming system of Atlantic salmon in sea cages typical of Norway, Scotland, Chile or Canada. Note the contrast of the high-speed intensive nature of salmon farming. Please remember the clear differences between the two aquaculture systems because they influence the best approach on how to keep fish healthy.

Think like a fish

Now that I have given you a first impression of this underwater life, I would like the people in the audience to imagine you are an individual fish sharing a large pond, or sea cage, with thousands of your direct relatives. Imagine you are fed at several regular time points a day a healthy, nutritious pellet that makes you grow at steady speed. Imagine you are well under way to become a healthy fillet on someone's plate. This is your fate and you are born to be ok with this. Yet, since you are with so many individuals and live so close together, there is a serious risk that if only a few of you catch a cold, soon all of you will catch the same cold. Indeed, aquaculture-based losses due to infectious diseases can be enormous and cost the global aquaculture industry thousands of millions of euros each year (FAO). And have an impact on fish welfare that cannot be expressed in euros. Please remember, this is the moment to refute the idea of being flushed through the toilet only because you feel sick: would you not much rather feel safe? Safe, because you know that you have been given a healthy vaccine?

But now we face a problem. Even if you as an individual have an immune system highly comparable to that of humans (Gratacap and Wheeler, 2014), and even though you realize you can be vaccinated, how will the vaccinators ever find you among the thousands of relatives you share your underwater column with? You do know for a fact that sometimes vaccinators take each and all of you out of the system, to give to you the desired protective injection. But simply the thought of this vaccination route already stresses you out. Another route much preferred by you is when the vaccinators simply pour the vaccine in the water when you are young, when you put your gills wide open to let the vaccine enter your body. Or even better, you simply love the route where the vaccinators add the vaccine or immune-enhancers to your daily meal (Fig. 3), because these protect so well your beautiful skin and keep in shape the mucus covering your body surface. Regretfully, remember that we scientists still find it hard to protect you via this last oral route so much preferred by you.

Figure 3. Cartoons depicting bath vaccination and oral vaccination as routes to routinely vaccinate fish in aquaculture (from Lamers, 1985).

Now, I would like you to imagine that even if you swim in sea water of only 5-10 degrees Celsius, you are never cold because you adapt your body temperature to the environment. Would that not be great? This is important because, although you never feel cold, there is also a disadvantage: your acquired immune system is hardly effective at these low temperatures. Indeed, fish kept at lower temperatures largely rely on innate immunity (Magnadóttir, 2006). However, as I mentioned at the beginning of this lecture, if memory would also exist for the innate immune system of fish, this would provide fish farmers with new forms of intervention other than vaccination, to protect fish health.

Of specificity, memory, and trained immunity

The phenomenon of 'trained immunity' therefore is one of the most exciting new developments in the discipline of immunology. Recent studies have shown that

innate immune memory can be triggered by certain infections and mediated by macrophages (Kleinnijenhuis *et al*., 2012). For example, protection of mice against certain fungi or bacteria could be transferred to mice that have no lymphocytes. Thereby, trained immunity provides protection against re-infection in a lymphocyte-independent manner (Netea *et al*., 2011). Several experiments identify macrophages as a cell type crucial to trained immunity, with epigenetic reprogramming through histone modifications as a central mechanism (Kleinnijenhuis *et al*., 2012).

With this in mind it is interesting to go back to some old, but intriguing, data (Olivier *et al*., 1985) on fish injected with a strong adjuvant called "complete Freunds", an oily mix of dead mycobacteria. Adjuvants, by definition, are compounds designed to help stimulate the immune response to an antigen and are often included in vaccines. In this particular study from 1985, indeed many decades ago, fish were injected with Freund's complete adjuvant and later challenged with totally unrelated, live pathogenic bacteria (Fig. 4).

Treatment	Challenge organism	LD50 (cells/fish)	LD50 increase
Saline	*Aeromonas salmonicida*	$1.8x10^{3}$	-
Saline + MFCA	*Aeromonas salmonicida*	$8.1x10^{5}$	450x
MDP (50 µg) + MFIA	*Aeromonas salmonicida*	$8.5x10^{4}$	47x
Levamisole (50 µg) + MFIA	*Aeromonas salmonicida*	$3.2x10^{4}$	18x
Saline	*Aeromonas salmonicida*	$1.1x10^{3}$	-
Saline + MFCA	*Aeromonas salmonicida*	$3.1x10^{5}$	282x
Saline	*Aeromonas salmonicida*	$5.8x10^{2}$	-
Saline + MFCA	*Aeromonas salmonicida*	$1.7x10^{5}$	293x
Saline	*Aeromonas hydrophila*	$5.1x10^{6}$	-
Saline + MFCA	*Aeromonas hydrophila*	$2.7x10^{7}$	5.3x
Saline	*Vibrio ordalii*	$2.5x10^{2}$	-
Saline + MFCA	*Vibrio ordalii*	$1.4x10^{5}$	560x

Figure 4. Quantitative evaluation of anti-'Aeromonas salmonicida' immunity in juvenile Coho salmon resulting from various intraperitoneally injected adjuvants, and evidence for the non-specific nature of the immunity (from Olivier et al., 1985). Challenges were conducted 30 days post-treatment. Lethal Dose (LD)50 increase was calculated by dividing the LD50 obtained by a given treatment by the LD50 obtained for corresponding fish treated with saline. Abbreviations: MDP = muramyl dipeptide; MFIA = Modified Freund's Incomplete Aduvant; MFCA = Modified Freund's Complete Adjuvant.

To everybody's surprise, the oily adjuvant mix induced a clear protection. Furthermore, the protection lasted for a period of up to 3 months, much longer than expected based on the classical view on innate immunity and longer than commonly

accepted for fish macrophages. These results can now much better be explained in the context of trained immunity, where memory is defined as a heightened response to a secondary infection that can be exerted both towards the same microorganism, but also towards a different one (Netea, 2013).

Trained immunity also brings me to the topic of macrophage polarization, which refers to the development of a specific macrophage phenotype important for either host defense, or tissue healing (Mantovani *et al*., 2013). As you can see in the next slide taken from a recent review we wrote on this subject (Forlenza *et al*., 2011; Fig. 5), macrophages can be stimulated to develop an inflammatory phenotype, important for the immediate immune response to infectious pathogens. This is shown on the top half of the macrophage. Or, macrophages can be stimulated to develop an anti-inflammatory phenotype to assist the wound healing process, as shown at the bottom half of the macrophage.

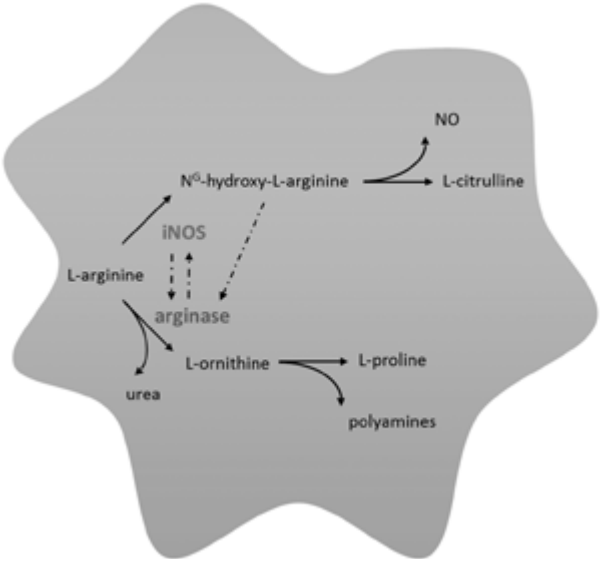

Figure 5. L-arginine metabolism of macrophages: The enzymes inducible nitric oxide synthase (iNOS) and arginase use a common substrate, L-arginine, to initiate different pathways that negatively regulate each other (dashed lines). The pathway initiated by iNOS leads to a phenotype associated with antimicrobial activity and inflammation. The pathway initiated by arginase leads to a phenotype associated with cell proliferation and tissue repair (from Forlenza et al., 2011).

Already some 10 years ago, when I started working on this subject with my former PhD student Maaike Joerink, we noticed that macrophages from parasite-infected carp when re-stimulated with the same type of parasite, could develop polarized

phenotypes (Joerink *et al*., 2006). At that time we spent hours discussing the possibility if individual macrophages could be re-programmed. New evidence now suggests this may indeed be the case (Ifrim *et al*., 2014). No matter what, polarized macrophages exist also in fish.

Balancing diversification and unification

The modern bony fish comprise some 25,000 living species which cannot all be studied. In practice, we comparative immunologists tend to focus on a limited number of fish species, including those most important for aquaculture. For example, in Norway and Scotland we study Atlantic salmon, in Poland and China we study carp. Although this feeds our taste for travel, it makes the discipline of fish health and immunology highly diverse. Where human medicine took a tremendous step forward by studying a common laboratory mouse, progress on fish health has for long been hampered by a diverse approach (Wiegertjes *et al*., 2005). There is, however, a unifying light at the end of this tunnel: the zebrafish. Zebrafish have a number of advantages (Gratacap and Wheeler, 2014): they have a well-described genome and their transparent embryos are small enough to be placed under a microscope. For most types of white blood cells fluorescent labels are available, allowing for real time viewing of immune responses through the microscope.

Although worldwide there is a steady increase in zebrafish research units, only few address the use of adult zebrafish. We share a common interest with Professor Johan van Leeuwen from the Experimental Zoology group in a zebrafish core unit at the Carus research facility of the Department. Wageningen University will be unique in its approach to use zebrafish as animal model for studies in aquatic biology and life sciences; an approach that fits very well the recently revived Aquatic Cluster at our Department. Please, remember the zebrafish as the unifying laboratory fish species of the future, not only for the world-wide research area of comparative immunology but also for the Department of Animal Sciences and for our Cell Biology and Immunology group where research on zebrafish unites medical, zoological and zootechnical research on the immune system.

Sometimes, however, we realize that zebrafish simply are too small to work with and at those moments it is good to realize that her larger twin sister carp, not only is worldwide the most cultured fish species for food consumption (FAO), but can easily grow to sizes of 50-100 cm. In an ever intensifying collaboration with Professor Herman Spaink from Leiden University we recently undertook shot gun sequencing of the genome of a double-haploid carp, a clonal fish line inherited from my PhD research (Wiegertjes *et al*., 1994) and confirmed the close genetic relationship between these two fish species (Henkel *et al*., 2012). I am convinced that twinning studies on

both zebrafish and carp will help unravel what is similar and what is different between the immune systems of fish and mammals. But most of all, these twinning studies will help unravel the immune system of fish.

Mucosal immunity and oral vaccination

Only recently, we comparative immunologists, discovered that also fish express a mucosal antibody isotype (Zhang *et al*., 2010). This finding stimulated an explosion of research on oral vaccination. This route of vaccination, so much preferred not only by the fish themselves, but also by the vaccine industry, requires a fundamental knowledge of immune responses at the local level of the gut. For this reason, I am very pleased with the recent appointments at the Cell Biology and Immunology group of Professor Joost van Neerven and of Sylvia Brugman, who are developing new research lines with a focus on immune responses at the local level of the gut, including the use of zebrafish for studies on mucosal immunity.

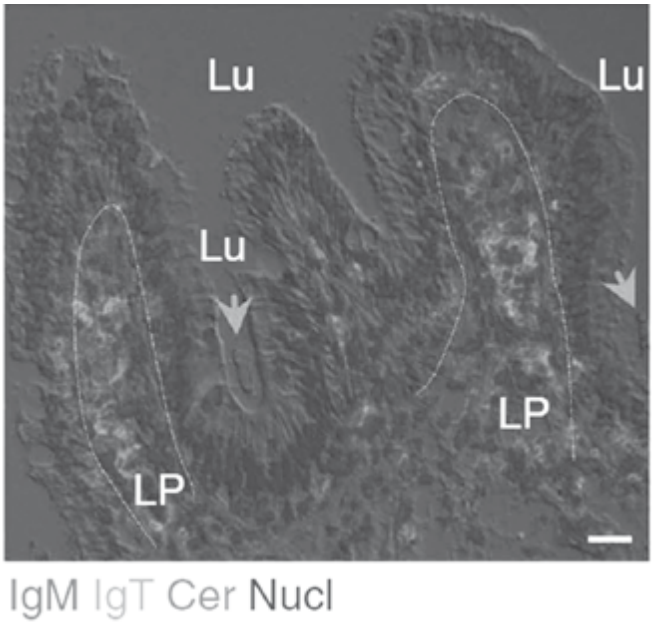

Figure 6. Immunofluorescent image of gut of trout that survived infection with 'Ceratomyxa shasta'. Accumulation of 'C. shasta' parasites (arrows) in the gut lumen (Lu) and of IgT+ 'mucosal' B cells (green) in the lamina propria (LP) (from Zhang et al., 2010).

The picture above (Fig. 6) is taken by a colleague from the United States, from the gut of rainbow trout. For your reference; the upper side is the lumen of the intestine, the folds are typical of the gut and help to increase the surface area of the gut. The nuclei

of the cells are stained blue. Upon exposure to a gut parasite, here shown in pink, the fish develop a mucosal immune response characterized by an antibody isotype, different from the one found in the blood.The antibody molecules are shown in green.

As I mentioned at the beginning of this lecture, vaccinations are widely viewed as the most successful medical advancement in the history of public health. In general, vaccines that make use of live attenuated pathogens are highly effective but not always safe, because the attenuated pathogens might convert back to virulence. Vaccines can also be based on inactivated pathogens, but these vaccines may be less effective. In particular the vaccines based on naked DNA, at least when delivered by intramuscular injection, have proven to be extremely successful in fish, where they are effective at doses a 1000-fold lower than those normally used in mammals. This is particularly true for the G protein of rhabdoviruses which normally infect rainbow trout and carp: in the case of DNA vaccines the antigen, in this case the G protein, is produced by the fish host cells themselves and processed in the correct biological context (Fig 7., Lorenzen *et al*., 2002). Thereby DNA vaccines closely mimic a natural infection in a safe way and trigger complete protection.

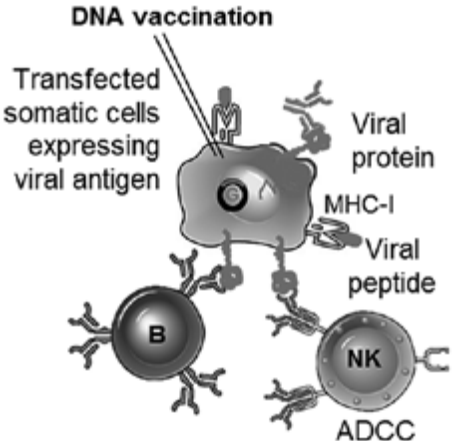

Figure 7. DNA vaccination showing a somatic cell transfected by DNA vaccination to express the viral glycoprotein (G protein) of a rhabdovirus, presenting this antigen by its cell surface major histocompatibility complex type I receptors to a B lymphocyte, or to a natural killer (NK) cell bearing specific antibodies to the G protein.

The idea, however, that integration of this foreign DNA in the host genome cannot be completely ruled out, has hampered the application of DNA vaccination to aquaculture. Despite this practical drawback, DNA vaccines at least provide excellent

means of defining protective antigens. And one day they may find their application also in Europe, maybe even using the oral vaccination route so much preferred by you. This research area is of major interest to my close colleague Maria Forlenza and, no matter what, I am convinced that our collaborative effort on this form of vaccination will bring forward practical applications now or in the future.

Thus, fundamental knowledge on mucosal immunology will be instrumental to the development of oral vaccines, a subject central to the large project named Targetfish funded by the EC 7th Framework programme. For those in the audience who are not familiar with Framework Programmes: these are funding programmes created by the European Commission to support and foster research in Europe and have been abbreviated FP1 through FP7 with the last one, FP8, being named "Horizon 2020". These programmes, from FP4 onwards, have become a major source of funding for research on fish health and immunology, at least within our group. In the Targetfish project we will bring new and improved vaccines closer to industrial application by addressing practical issues such as efficacy, safety and oral delivery route. Having the coordinator's role of this large project places the research on fish health and immunology in Wageningen at the center of the European fish immunology network.

Of pattern recognition receptors and immune-enhancement

Only four years ago the Nobel Prize in Physiology or Medicine went to two immunologists who discovered that a Toll-like receptor seemed conserved all the way from fruit flies to humans: this had triggered an explosion of research on the role of these type of receptors and had caused a paradigm shift by refuting the idea that innate immunity was aspecific. To date, Toll-like receptors are recognized as only one type among many different families of "pattern recognition receptors" all specialized in recognizing antigens common to particular groups of micro-organisms (Akira *et al*., 2006).

The short movie I just showed was made together with help of colleagues at the Biochemistry Department and depicts a three-dimensional view of a heterodimer of Toll-like receptor 1 and Toll-like receptor 2 from carp, in blue and green, binding a microbial antigen, here depicted in red. What is special is that the carp molecules have been modelled on crystallized human Toll-like receptors. In simple words: the carp molecules look very much like the human ones. Which means that indeed, these types of receptors may be well conserved through evolution. Which again means that maybe, the function of these types of receptors may be well conserved also.

The presence of pattern recognition receptors provides the scientific mechanisms that explain the effect of immune-enhancing intervention via animal feed. ß-glucans, for

example, membrane components from fungi or harmless baker's yeast, are frequently applied to this purpose. The research of Professor Jerry Wells of the Host Microbe Interactomics group into the function of different pattern recognition receptors in innate immunity (Wells *et al*., 2011) provides an excellent example where our research interests meet across species borders.

In humans, ß-glucans are recognized by the receptor Dectin-1 (Taylor *et al*., 2007). The Dectin-1 receptor is clearly different from the Toll-like receptors I have just shown, although all are pattern-recognition receptors. Sometimes, as shown in the slide, they work together. In fish, it is well recognized that ß-glucans do stimulate innate immunity but the prototypical Dectin-1 receptor seems absent from fish genomes. Identification of its fish equivalent would help to better define the optimal structure of β glucans for use as immune-enhancers in fish. This exactly is the line of research central to a new Biobased-Economy project with Brazil funded by the Netherlands Organization for Scientific Research and supported by the Research and Development departments of the feed industry. I see great opportunities to expand this line of research, for example in cooperation with the Aquaculture and Fisheries group of Professor Johan Verreth with whom we share a common interest in the use of enriched feed for improvement of fish health. More than ever, molecular approaches allow for an easy switch between fish species, breaking down physiological barriers from the past.

Studying host-pathogen interactions

Protective immune responses are best studied in the context of pathogens including bacteria, viruses and parasites. Carp are the natural host of two trypanosome parasites that can best be considered equivalents to the blood parasite which causes sleeping sickness in humans (Wiegertjes and Forlenza, 2010). Since we can infect carp with known numbers of these parasites and can monitor parasitaemia by blood sample counts, these infections allow us to carefully examine protective immune responses in fish. At present we are collaborating with Professor Mark Carrington from the University of Cambridge to develop fluorescent trypanosomes for infection of zebrafish, making use of the suitability of zebrafish for microscopic imaging of live processes.

Virus infections of carp

Over the last few years we have also started to work on protective immune responses against two economically very important viruses of carp, in collaboration with members of this University's Virology group of Professor Monique van Oers and in collaboration with Professor Alain Vanderplasschen from the University of Liège as well as with Research and Development departments of the vaccine industry. Our

efforts to perform fundamental but strategic research on immune responses to important fish viruses will help provide the scientific rationale necessary for the development of safe and efficacious vaccines against these two deadly viruses.

In Europe, experimental studies on vertebrate animals are under strict guidelines of governmental committees, safeguarding animal welfare. The current view is that experimental studies on animals are allowed only if there really are no alternatives. I fully support this view. It is, however, good to realize that many animal experiments on mice are performed to rule out toxicity of compounds for humans. Presently, the predictive value of these type of experiments is heavily debated. Please, do remember that the type of experiments we perform in Wageningen are essentially different, because we study fish not primarily as a model for humans but as a 'model for fish'.

To provide researchers with the relevant training, we have under development at Wageningen University, a module of laboratory animal science which will help to train specifically those scientists that work with fish. This will help them design experiments using fish in a manner that will respect reduction, replacement and refinement. I have offered my help developing this module at Wageningen University. The availability of highly specialized fish facilities operated in a perfect manner by Menno ter Veld and his team, united in the research facility Carus, allows us to address these issues in fish.

Why good memories?

You may not have realized but for the last approximate half an hour, by pure coincidence, I have been exploring with you the potential of nature to improve the quality of life, taking you through an underwater tour explaining the mission of our university. So what is my take home message? Fish are the first vertebrates in evolution to share with us humans the basic principles of the immune system. First, as in humans, their acquired immunity typically relies on specific memory and forms a sound basis for successful vaccination of fish grown in aquaculture. Second, although fish kept at lower temperatures are forced to rely largely on innate immunity, I have discussed with you recent discoveries which indicate that memory may exist not only for acquired but also for innate immune responses. This concept provides exciting possibilities for renewed forms of immune-enhancing interventions to protect your health. In other words: I have explained to you the importance of developing a good memory for details. This and I hope I managed to convince you, is certainly true for fish.

But it is no less true for human beings. Now, I am not aware of how good your memory is but I dare to state you cannot remember how often I used the word

'remember' during my lecture. That would be exactly seven times. Now, if your personal count is far off, please remember that fish oil improves your memory and thus I urge you to include, in the near future, some oily fish in your diet. During my lecture I have explained to you how I think research on fish health and immunology will improve the quality of life, of fish grown in aquaculture. I hope I managed to convince you that we will try everything we can to provide you with healthy fish and therefore, a healthy life.

Teaching at Wageningen University

Since 1998 I have organized an annual series of international fish immunology workshops for most years together with my colleague Maria Forlenza. These workshops, supported by the Graduate School WIAS and the International Society of Fish and Shellfish Immunology, have probably become the best known educational event for PhD students in the research area. This year (2014), the 500th participant has left Wageningen with the idea that he was guest of one of the most active comparative immunology groups in Europe. It is my wish to continue on this successful path and strive to bring 500 more participants to Wageningen.

It is a great pleasure, to work together with the Teacher of the Year, Professor Huub Savelkoul, who teaches me how to address an audience as an immunoglobulin molecule while still preserving one's dignity. With my children presently being Bachelor students I have become even more aware of the needs for good supervision and it is my wish to contribute to the personal development of students at all levels, as confident of the Graduate School and as mentor in the recently started BSc Honours programme. I see it as a challenge to help further internationalize our University not only at student but also at staff level. To keep science flowing talent must stream freely. It cannot be a coincidence that Oxford, Cambridge, and Wageningen all are situated near the sides of a large river.

Words of gratitude

As a young boy I always said I wanted to be a forester browsing through the woods, far away from office work, a suit and computer screens. Who would have imagined I would spend my days behind the computer finding diversity only in preparing teaching, writing grant proposals, budgeting consumable costs, correcting manuscripts and reading literature? Several young scientists tell me they never want to end up like me and yet they will. I hope that one day, like me, they will be able to see the horizon beyond the small Zodiac office and appreciate the challenge of stimulating a research area as interesting as fish health and immunology. Despite my initial wish to become a forester there is one aspect of my present work that has

always been central to my life, already since I was a young boy of 6 years old: fish. Together with my best friend Paul Meek I spent endless hours staring at large carp swimming out of our reach. Who would have imagined that I would spend the rest of my life building a scientific career around the very same fish species?

I would like to thank the Board of Wageningen University, the Rector Magnificus Martin Kropff and the BAC for their confidence in me and my ability to build a group around fish immunology. I was the first person at the Department of Animal Sciences to step into the tenure track and I appreciate the new and exciting career possibilities it has offered me. The basis for my scientific career was formed as a student at the Aquaculture and Fisheries group. Carel Richter: as acting head of the group you had a profound impact on me and it is a great pleasure to know you are present here today. My internship period as a student I spent close to Oxford where Roger Sweeting and Anne Powell ran a consultancy in fish health. Their attitude and intense combination of private time with working life changed my attitude forever. After my graduation I got offered a part-time position as assistant professor at the Cell Biology and Immunology group, with continuous support of Willem van Muiswinkel who later became my promoter. Wim: I am proud that today I wear your toga and I hope to continue expanding the research area that you shaped here in Wageningen many years ago. I promise to take good care of your legacy.

During the later years at the Cell Biology and Immunology group I developed a strong working relationship with René Stet who later moved to Aberdeen University. It is a real shame he cannot be here today to celebrate with me this happy occasion. Already more than 10 years ago, Huub Savelkoul became the head of our group and we changed focus from being a fish immunology group to become a comparative immunology group and with success. His never ending enthusiasm and support make it a pleasure to work together now and in the future. It may be clear that my scientific career is built on foundations laid by the Aquaculture and Fisheries and the Cell Biology and Immunology groups. I look forward to the coming period where opportunities offered by a joined Aquatic Cluster will form a sound basis for expanding our research on fish health and immunology.

Last but not least, it is my greatest pleasure to share today's occasion with my family and friends, in particular my mother who may have turned 80 but still has the spirit of a 50-year old, tante Geertje, my beautiful and smart children Renske, Kim and Jidde, my partner and best friend Maria, Paul en Bernadette, Imke and Paul and many others, including several who are present online. Last but not least, I would like to thank all of you in the audience and those listening via WUR-TV who

willingly imagined themselves to be individual fish sharing a large pond or sea cage with thousands of direct relatives. And imagined to end up a healthy fillet on someone's plate. That took some courage!

Ladies and gentlemen, thank you for your attention.

Ik heb gezegd.

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Prof. dr *ir.* G.F. Wiegertjes

'Evolutionary, fish are the oldest vertebrates to display both innate and acquired immune responses. Acquired immunity typically relies on specific memory of previously encountered pathogens and forms a sound basis for successful vaccination of fish grown in aquaculture. Fish kept at lower temperatures, however, rely largely on innate immunity. Recent discoveries indicate that memory may also exist for innate immune responses, providing exciting possibilities for additional forms of interventions to protect fish health.'

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