

Mucosal Immunity

Barriers, Bugs, and Balance

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Inaugural lecture upon taking up the post of Special Professor of Mucosal Immunity
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1. Introduction

Dear Rector Magnificus, colleges, family, friends,

We live in an ever changing world. Despite tremendous advances in modern medicine, developing countries still face high child mortality rates, and Western countries struggle with wealth-related diseases. These differences are linked to an unjust distribution of wealth, healthcare, education and food in the world.

But this picture is changing rapidly, because of global economic, demographic, and geopolitical changes. For example, the population is expected to grow from 6,5 billion today to 9 billion in 2050 (1-3). When you look at the world population increase since 10,000 BC you can see how dramatic this increase is, especially in the last two centuries. Because of this population growth, producing enough food will become a major challenge. Another consequence of population growth is increasing urbanization and travel, which creates the perfect environment for the spread of infectious diseases.

On top of this, the population is ageing rapidly (4). It is estimated that in 2050 16% of the world population will be over 65 – with an estimated population of 9 billion this is almost 1,5 billion people over 65. In comparison, in 1950 this was around 125 million – a more than 10-fold increase ! This will lead to an increasing burden on our healthcare system. So maybe we should start thinking about prevention.

Nutrition and dietary status play important roles in many diseases and conditions, as does the intestinal flora – also referred to as microbiota - that is influenced by our diet. And diet is one of the few environmental parameters that we can change easily. This has led to preventive medicine approaches in which nutrition plays an important role.

Many of the infectious and inflammatory diseases that we can get occur in mucosal tissues. Mucosal tissues are the tissues that are covered by a mucous membrane, and

are in close contact with our environment. The most important mucosal tissues are the respiratory tract (that for example can be affected by asthma, rhinitis, pneumonia and the common cold) and the gastrointestinal tract (that can be affected by diarrhea, inflammatory bowel disease, and celiac disease).

2. Which groups of people are susceptible to mucosal infection and inflammation ?

But who is at risk to get such diseases? Let's start with infections.

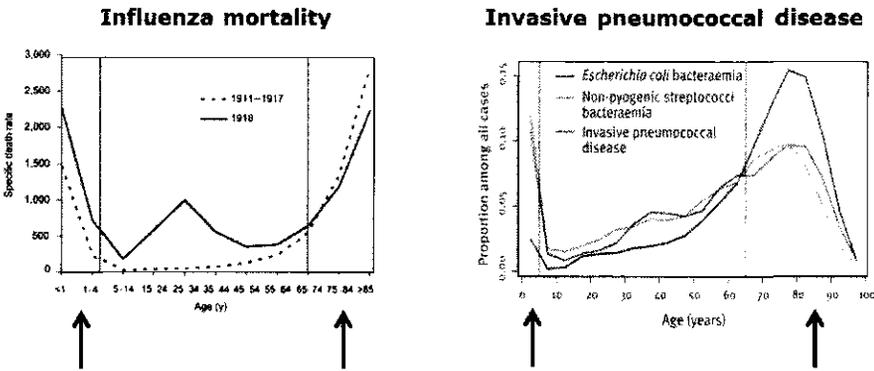


Figure 1. Age-dependent susceptibility to severe viral and bacterial infection (5,6).

As you can see in the left panel of figure 1, influenza-related mortality occurs mainly in children under five and people over 65 years of age (5). The same is true for invasive bacterial pneumonia and other bacterial infections in the panel on the right (6). In 6-65 year old people these infections are less frequent and generally less severe.

If you have been paying close attention, you might have noticed the peak in the 10-50 year old people during the great influenza in 1918. This was because not because of decreased immunity as is the case in the young and the elderly, but rather by an excessive immune responses to this particular virus in healthy people. This is now also seen in recent avian influenza cases – which is why these sporadic cases attract so much media attention.

For diarrhea-related mortality a similar, but less pronounced, age-dependency is seen (7). Here you may note the huge, over 1000-fold difference between diarrheal deaths in low income African and south-east Asian countries and high income American, European and Pacific countries.

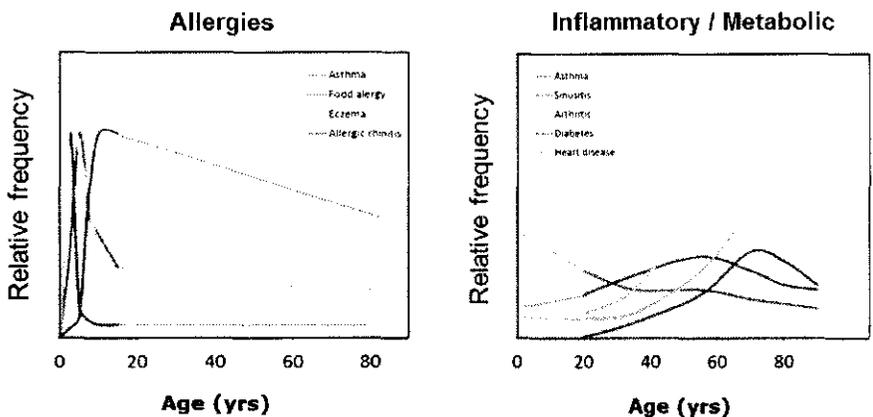


Figure 2. Age-dependent susceptibility to allergies and inflammatory/metabolic diseases (8,9).

For the occurrence of inflammatory diseases the picture is quite different (Figure 2). It is apparent that the development of asthma, eczema, and food allergy is determined early in life (8) – but the development of most of the inflammatory and metabolic diseases is linked to advancing age (9), except for inflammatory bowel disease that generally has an earlier onset and has a clear infectious component.

So young children are susceptible to develop allergy and infections, and elderly people are susceptible to non-allergic inflammatory diseases but also to infection. This suggests that their immune systems function in a different manner.

Indeed, during our lifetime, the function of our immune system changes (10-12). For example, adults have a strong anti-infective immune function, whilst young children and elderly generally have a more anti-inflammatory immune system, which explains that both groups are more susceptible to infections.

Further, in young children that meet allergens for the first time allergic responses can develop, as allergy is the result of type 2 responses that are also linked to early life. The induction of allergy by different environmental and food allergens, as well as allergy prevention, are important study themes in the Allergy Consortium Wageningen (ACW), but I will come back to that later.

The reason infants are immune compromised is because their immune system has not yet matured at birth, but also because it is actively downregulated. There are two hypotheses why this is the case.

Firstly, it may be to prevent that the fetus mounts an immune response against its own mother, – or, alternatively, it may be to remain unresponsive to the colonization of the GI tract with commensal bacteria which occurs shortly after birth. My guess is that it is a combination of the two.

Elderly people are also immune compromised, but that is to a large extent because they are prone to inflammaging – associated with increased levels of pro-inflammatory cytokines – and develop several forms of low grade or overt inflammatory diseases.

Quite the opposite to what one would expect, even though they have increased levels of pro-inflammatory cytokines in their circulation, elderly people have decreased innate immunity to infection, as can be seen by their reduced response to Toll Like Receptor triggering by pathogens (13).

But you may ask yourself if strong immune responses are always what is best?

For infection this may be true – but not for inflammatory diseases.

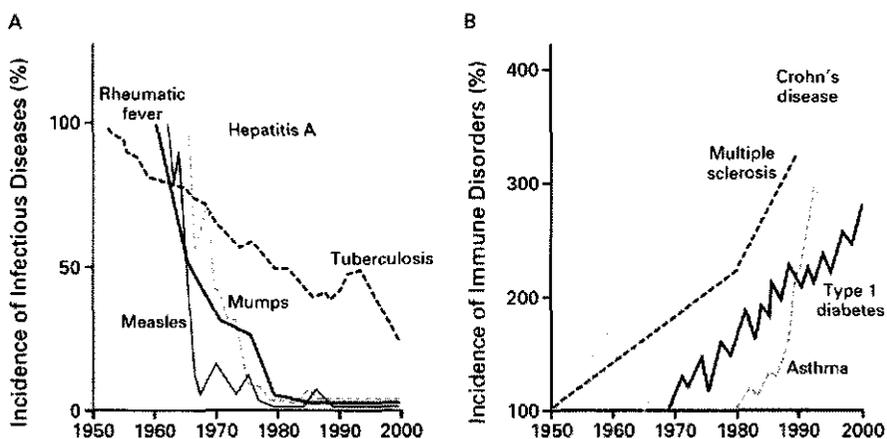


Figure 3. Decreased infectious pressure is associated with an increase in chronic inflammatory and autoimmune diseases (figure from ref 14).

Over the last 50 years the occurrence of severe infectious diseases has decreased dramatically – especially in the western world (14). This is shown in the left panel of Figure 3. At the same time, on the right, a sharp rise in chronic inflammatory and autoimmune diseases is seen. It is now generally thought that this is caused by a

decreased ability of the immune system to regulate immune responses, which is probably the result of the fact that the immune system is not challenged as often by pathogens as it used to be. The result of this is a decreased response by regulatory T cells that produce the tolerance inducing cytokines TGF- β and IL-10, thus tipping the balance towards a pro-inflammatory immune response.

Due to the ageing population inflammatory and autoimmune diseases - as well as infections - can be expected to increase even more in the near future. When you add to this the socio-economic changes that developing countries are undergoing - including adaptation of more westernized life styles and diets - it can be expected that this will also happen there.

3. Immune regulation

Should our immune system respond to everything it is exposed to, and should it always respond in the same manner? The short answer is NO – it's all about finding the right balance. Let's take a look at immune homeostasis and immune regulation.

The immune system, and especially the mucosal immune system, is constantly challenged by potentially pathogenic microorganisms, but also by harmless commensal bacteria and by innocuous food antigens and inhaled proteins. In order to prevent a chronic state of immune activation that may lead to inflammation it continuously needs to make choices to either respond to these potential threats, to actively induce tolerance, or to simply ignore them.

When you think about it, the mucosal immune system should react when there is a threat, such as a disruption of the epithelial barrier, or the presence of a pathogen inside the body. As long as a pathogen has not crossed the epithelial barrier there is no need to respond!

So the mucosal immune system is all about Barriers, Bugs and maintaining the right Balance! This is the key difference between the mucosal and the systemic immune system, that should respond to any threat as soon as possible because the threat has already invaded the body.

A tight regulation of immune responses is of crucial importance to maintain immune health. Dysregulated immune responses are associated with infection and cancer when the response is inadequate, and with inflammation, allergy and autoimmunity, when the response is excessive (Figure 4).

This balance is continuously challenged by the presence of pathogens, tissue damage, neoplastic cells and allergens. In addition, genetic and environmental stimuli such as diet and pollution can also have an effect on the immune response. The mucosal immune system should not respond to dietary antigens. This is regulated by the process of oral tolerance induction, a process in which the immune system is educated to tolerate dietary antigens (15).

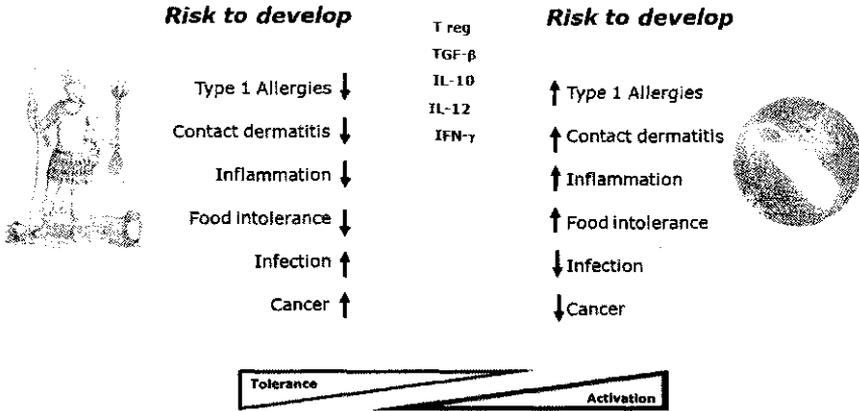


Figure 4: Tolerance and immunity: a double-edged sword.

The induction of oral tolerance is favored by the immunoregulatory environment in the intestine, especially by the presence of TGF-β and IL-10. When tolerance induction fails, food intolerance or food allergy can develop.

A nice anecdotal example of oral tolerance induction is the way Native Americans used to chew on the leaves of poison ivy and poison oak to become tolerant to the irritants in their leaves.

Another one is the story of the scientist – I am not going to mention any names here – that could not induce nickel allergy in mice because they licked the nickel-lined drinking bottles and bars of the cage – and became tolerant for nickel.

It is very important to learn how to balance immune responses and how to induce and/or maintain an adequate level of tolerance induction. At the same time it is important to enhance the resistance of infants and elderly people to infection. But how can we achieve this ?

For protection against specific pathogens there is of course the option of vaccination – although this is not always efficient in elderly people. Further, subcutaneous vaccination

for respiratory and gastrointestinal diseases is not always very efficient. A detailed understanding of processes in the mucosal immune system is needed to improve mucosal vaccination strategies that can specifically target the respiratory or GI tract.

What about food? As I mentioned earlier preventive medicine looks at food as an important factor for preventing the occurrence of immune-related diseases.

Apart from overt malnutrition, even more people have micronutrient deficiencies that compromise immune function. Textbook cases are the nutritional deficiencies that are associated with increased susceptibility to infections - like Zn, Se, Vitamin A, Vitamin D, and others (16,17).

Therefore a healthy diet with the right levels of essential micronutrients is of great importance to reduce many of these mucosal infections. This was for example shown by the work of Dr. Hans Verhoef in dietary supplementation studies in Africa.

Another way to improve immune function by food is the use of immunomodulatory foods that can support the innate and adaptive immune system. Strengthening the immune system through nutritional intervention is already well accepted in the feed industry to enhance animal health & production - as an alternative for antibiotics.



Figure 5: Variolation of smallpox in ancient China (11th century). The first known form of mucosal vaccination.

4. History of Immunology

OK, maybe this is the right time to introduce a few key discoveries by early immunologists before I start talking more in detail about mucosal immunity.

Humans have been susceptible to infectious diseases since the beginning of time. The first written documentation on immune protection against infection came from the

Greek author Thucydides. In his book “The History of the Peloponnesian war” he describes that when typhoid fever raged through Athens in 430 BC, those who survived the disease could not become ill again. This was, in fact, the first description of the development of immunological memory by the adaptive immune system.

The development of vaccination was a major step forward in our fight against infectious diseases. We all know of the famous experiments by Edward Jenner, who developed a cowpox-based vaccination against smallpox in 1796. He did this after his observation that milkmaids often developed cowpox - a mild smallpox-like disease, but did not get infected by smallpox.

What most of us don't know is that the practice of vaccination against smallpox already existed for several centuries by then. In 11th century China it was already common practice to collect the scabs of smallpox lesions, grind them to a powder and blow this into the nose with the instruments shown in Figure 5. This praxis is called variolation – and is the first form of mucosal vaccination that we know of.

The procedure was quite risky, and many people in fact developed smallpox and died. Edward Jenner made use of the crossreactivity between immune responses to cowpox and smallpox - and thus got effective vaccination responses without the associated risk of lethal infection. But he did not invent vaccination!

Nevertheless, with the exception of just a few vials with smallpox virus that are present in American and Russian laboratories, smallpox has been completely eradicated, showing the immense effects of vaccination on human health.

Ilya Mechnikov (1845-1916)
First described phagocytosis



Paul Ehrlich (1854-1915)
First described antibodies



Figure 6. Mechnikov and Ehrlich, the founding fathers of modern Immunology.

The next major discoveries in the field of microbiology and immunology were done in the 19th century. The field of modern Immunology was pioneered and developed by Ilya Mechnikov and Paul Ehrlich who received the Nobel prize for medicine in 1908 “in recognition of their work on immunity” (18). They are best known for their work

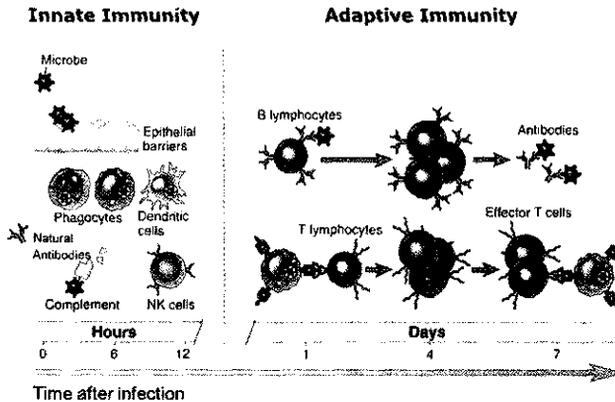


Figure 7. Kinetics of innate and adaptive immune responses. Adapted from Abbas (19).

on phagocytosis of bacteria by macrophages which was described by Mechnikov, and the concept of “magic bullets” or antibodies by Paul Ehrlich (Figure 6).

As you can see in the textbook slide of Figure 7 their work has defined the role of the innate immune system in early responses to pathogens and the role of the adaptive immune system in late responses and the generation of long lived immunological memory (19).

Of course there have been many new developments in immunology since, but still in a way we are filling in the details of what was described in the late 1800s – a humbling thought.

Interestingly enough, in addition to their major contributions to the field of innate and adaptive immunology, Mechnikov and Ehrlich also pioneered nutritional and mucosal immunology.

Mechnikov observed that Bulgarian peasants that had a very high life expectancy consumed fermented milk and yoghurt. The yoghurt was made by adding *Lactobacilli* to milk. This preserved the milk by acidification, but Metchnikoff was convinced that the bacteria conferred a positive health effect. His work is still continued to date and has led to the development of probiotic bacteria. Meanwhile Paul Ehrlich did an experiment in which he vaccinated pregnant mice with toxins, and later exposed their pups to the same toxins - and observed that they did not get sick – but only when they were still being breastfed. He concluded therefore that breast milk can transfer immunity from mother to child (18).

We all know that breastfed infants indeed have fewer gastrointestinal and respiratory tract infections than bottle fed infants, which is probably linked to the many immunological factors that are present in breast milk (20).

In addition, breastfed infants also have a different microbiota composition, a notion that was also described in the 19th century. Theodor Escherich observed that the feces of breastfed infants was different from the feces of meat-eating adults.

We now know that this difference is caused by complex oligosaccharides in breast milk that shape the intestinal microbiota composition of infants. This same mechanism is currently used to modulate the microbiota composition with prebiotics.

These scientific breakthroughs have laid the foundations of most of modern immunology – and as early as the 19th century demonstrated that food can modulate immunity !

5. Mucosal Immunity

By now I have mentioned mucosal immunity several times already, but have not yet explained in detail what I actually mean by the term. Looking at the scientific environment, Mucosal Immunity is a specialty at the crossroads of many disciplines (Figure 8).

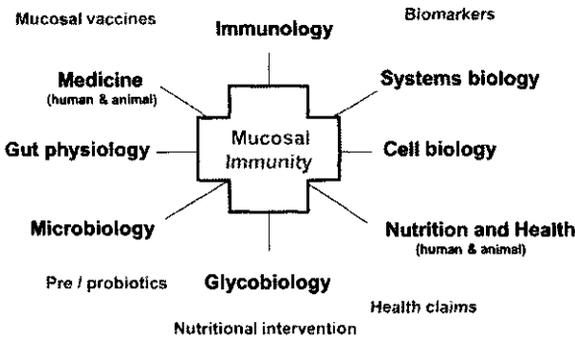


Figure 8: Mucosal immunity is at the crossroads of many different disciplines.

Immunology, medicine, vaccine development, gastrointestinal and respiratory physiology, nutrition & health, glycobiology, food science, and microbiology to name but a few.

As such it is an exciting area, where many disciplines come together and give rise to novel opportunities and collaborations. It is also often at these crossroads where new concepts and applications are developed.

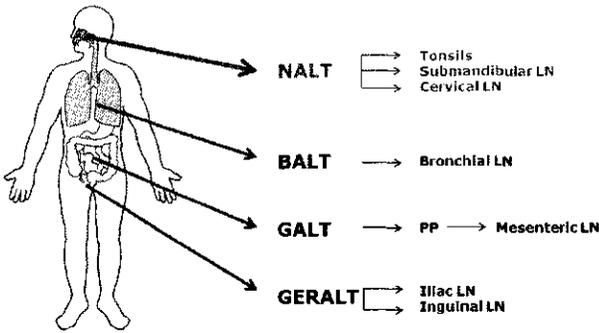


Figure 9. Anatomy of the Mucosal Immune system.

Figure 9 shows the most important mucosal tissues. As I mentioned in the beginning, mucosal tissues are tissues that are covered by a mucous membrane. The largest examples are the gastrointestinal tract (that harbors the gastrointestinal associated lymphoid tissue or GALT) and the upper and lower airways (with the NALT and BALT), but also tissues as the inner ear, the bladder and reproductive organs. These mucous membranes are, in addition to the skin, the sites where our bodies are exposed most frequently to potentially pathogenic micro-organisms – but also to harmless substances, such as food.

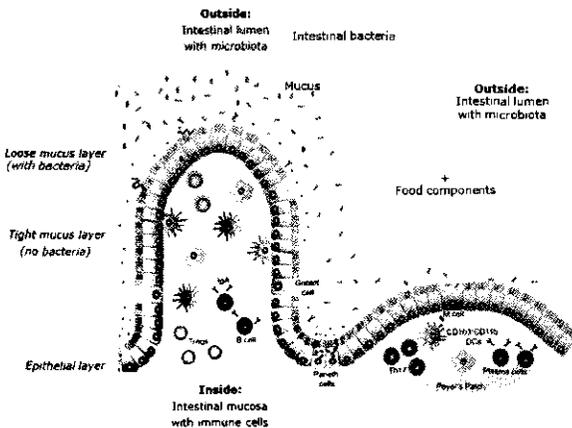


Figure 10. The mucosal immune system in the GI tract (adapted from ref 21).

As you can see in the picture in Figure 10 that represents the gastrointestinal mucosa, the mucus and the epithelial cells underneath have an important role as a first physical barrier between the outside world and the inside of the body – thus keeping

pathogens out. This epithelial surface of our intestines is around the size of one to two tennis courts, which is an immense surface of interaction of our body with the contents of our intestines.

Underlying the epithelium is the mucosa that contains a large part of the body's immune system. Sampling of pathogens and antigens present in the intestine primarily takes place in the Peyer's patches of the small intestine and results in the excretion of IgA antibodies into the intestinal lumen. These non-inflammatory IgA antibodies can then neutralize bacteria, a process that is known as immune exclusion.

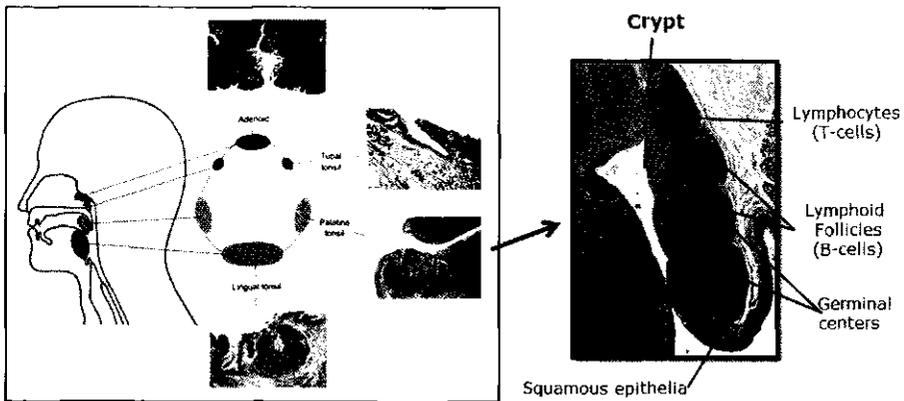


Figure 11. Local immune responses are induced in Waldeyer's ring (adapted from ref 22).

In the upper airways similar processes take place (Figure 11). The back of our oral cavity is surrounded by a number of tonsils, that are collectively called Waldeyer's ring. Immune sampling takes place in the antigen retaining crypts of these tonsils (22). In contrast to the situation in rodents, in humans the contents of the nasal and oral mucosa are sampled simultaneously in Waldeyer's ring, as a result of which upper airway pathogens come into close contact with the immune system - but also with food components.

But how can our diet influence the immune system in the respiratory tract - and what are the mechanisms involved? This is the focus of the chair on Mucosal Immunity. Understanding these mechanisms will help us to define novel nutritional strategies to help prevent infections and inflammatory diseases.

Studying mucosal immunity in relation to the respiratory tract may sound a bit strange to you, as most mucosal immunologists have a tendency to study immune responses in the GI tract.

This is not surprising, because that is the site where our food ends up, and where we have the largest microbial community in our body. However, my primary interest is in relation to mucosal immunity in the (upper) respiratory tract – and how food that is ingested through the GI tract can influence respiratory immunity.

Through work on probiotics, prebiotics, and polyunsaturated fatty acids we know that it is possible to modulate gastrointestinal health and immune function. But more recently, and especially interesting from my perspective, several foods or food ingredients have been shown to confer protection against respiratory infections and allergies – thus linking the GI tract to the airways! For example, breastfeeding and pre- and probiotics have been shown to reduce the severity and prevalence of respiratory infections in children (23-25), and raw milk consumption has been associated with decreased prevalence of asthma and upper respiratory tract infections (26-30).

A general strengthening of the immune system through micronutrient supplementation will contribute to a well regulated immune system – and this will affect immunity in the entire body. For specifically achieving effects on respiratory immunity through the GI tract we should learn more about the mechanisms that regulate tissue-specific homing of immune cells.

Only a few years ago it was thought that all immune cells can recirculate through the blood to all mucosal tissues, the so called Common Mucosal immune system (31). But now we know that it is much more complicated than that!

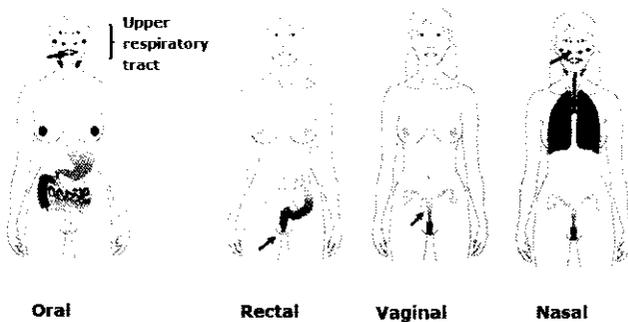


Figure 12. Route of vaccination determines the location of the immune response. CTB-specific IgA detected on mucosal surfaces after administration of CTB on different mucosal surfaces (from ref 32).

For example, as shown in Figure 12, administration of a protein in the rectum leads to IgA responses (these are marked in red) in the rectum only, and the same is seen

for vaginal administration (32). But when you apply the exact same protein orally you can also see immune responses in the upper airways and in breast tissue – but not in the lungs.

IgA induction in the lungs is only seen after intranasal administration. So mucosal immune responses may in fact be rather tissue specific – and you can apparently reach the upper airways via the oral route.

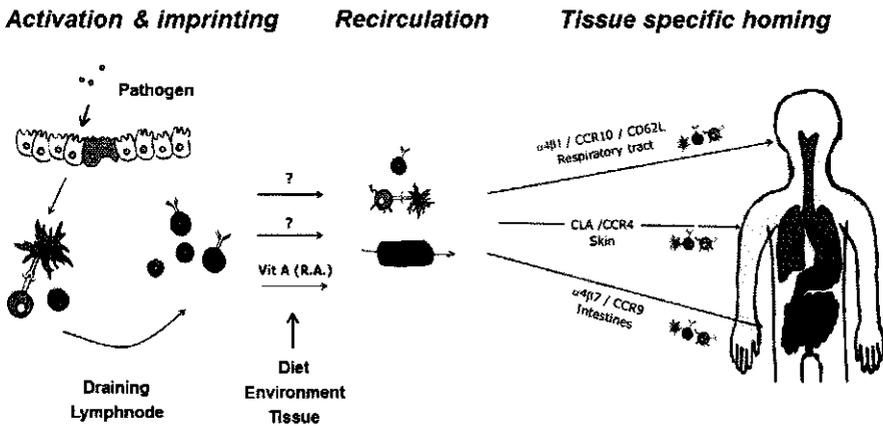


Figure 13: Tissue-specific homing of immune cells.

Recent work from several groups has shown that homing of immune cells to the airways *vs* the gastrointestinal tract *vs* the skin is regulated by the expression of chemokine receptors and adhesion molecules expressed by the immune cells (Figure 13). Some of these markers can be modulated by dietary vitamins.

For example, the vitamin A metabolite retinoic acid can redirect an immune response from the skin to the intestine through the induction of specific homing receptors on immune cells (33).

If that is possible, maybe we can also redirect immune responses to the airways. Or maybe we should find ways to specifically activate only the cells that migrate to the airways, or target Waldeyer's ring, or modulate the microbiota that can indirectly affect immunity in the airways? I don't know the answer to these questions – but I am very happy that I have been given the opportunity to study these questions and mechanisms.

6. Positioning the chair on Mucosal Immunity

Now how does this type of research fit in the strategic mission of Wageningen University? The mission statement of the University is:

'To explore the potential of nature to improve the quality of life.'

I think that the chair on Mucosal Immunity does just that – both in relation to human and animal health. Let me go into a bit more detail, on how the chair fits in the Animal Science Group and links to other chair groups within the university. The chair on Mucosal Immunity aims to study the mechanisms via which mucosal immune health can be improved. The chair is part of Cell Biology and Immunology (CBI) of ASG, and is headed by Prof. Huub Savelkoul.

I have known Huub Savelkoul since my PhD research in the early nineties that I performed in the group of Prof. Martien Kapsenberg in the AMC on the role of the immune system in respiratory allergies - a collaboration with Prof. Henk Jansen of pulmonology and Prof. Rob Aalberse of Sanquin (34). Huub Savelkoul has always had an interest in allergic sensitization and allergies, with a focus on food allergies – and is one of the founders of the Allergy Consortium Wageningen together with Prof. Harry Wichers of Food and Biobased Research.

The research carried out under Huub Savelkoul's supervision by Dr. Gerco den Hartog - and co-supervised by myself – has linked the allergy research in the group to mechanisms in mucosal immunity such as the role of IgA in asthma (35).

Another link to mucosal immunology in the Cell Biology and Immunology group is the work of the Fish Immunology group, led by Prof. Geert Wiegertjes together with Dr. Maria Forlenza and Dr. Lidy van Kemenade. Their work on carps and zebrafish provides novel models to study the role of innate immunity and barrier function. The recent addition of Dr. Sylvia Brugman to the group provides a link between the chair on Mucosal Immunity and the Fish Immunology group.

The veterinary immunology in the group, supervised by Dr. Edwin Tijhaar, studies resistance in farm animals and has a keen interest in identifying markers for immune health in these animals.

I hope that with the work on mucosal immunity, we can identify how to improve resistance to infection via nutritional intervention. As similar mechanisms are expected to be relevant to human and animal health the results can be translated into new approaches to improve animal health as well.

For farm animals a reduction in respiratory tract infections may contribute to a better productivity and a reduction in antibiotic use. For this reason there is great interest in both the fish and veterinary world to add immune enhancers to animal nutrition. At CBI we are currently setting up a collaborative study with Professor Wouter Hendriks and other chair groups within the department of Animal Sciences to study respiratory immune health in farm animals.

Since my appointment in May last year I have been able to attract funding from NWO in the form of an STW project, and additional funding from FrieslandCampina and the Stichting Kernhemfonds. As a result my own research group at Cell Biology and Immunology now consists of Marloes van Splunter and Olaf Perdijk – both PhD students – and Dr. Sylvia Brugman as Post-Doc.

Marloes van Splunter is studying the effect of food ingredients on airway immunity in *in vitro* cell cultures as well as in a new mucosal vaccination model. This is a collaboration with Dr. Els van Hoffen and Dr Harro Timmermans from NIZO, Dr Laurien Ulfman from FrieslandCampina, and Prof Jerry Wells from the Host Microbe Interactomics group.

Another important part of the work in my group will be to study the effects of milk components on mucosal immune function and their potential application for children and elderly people. The main functions of milk are to support growth & development and to provide immune support when the child's immune system is still developing.

Next to breast milk, cow's milk is also linked to immunity in the airways because raw milk consumption is associated with a lower incidence of asthma, hayfever, upper respiratory tract infections and otitis (24-27). As such milk is a very good model to study the effects of nutrition on mucosal immune function.

Olaf Perdijk and Dr Sylvia Brugman work on the STW project I mentioned earlier. They study the effects of milk ingredients on allergy and respiratory infection, with special attention on mechanisms of tissue-specific homing and the regulation of IgA class switching. This project is a collaboration with Prof Willem de Vos and Prof Hauke Smidt of Microbiology, as well as with Dr. Jeanette Leusen and Maaïke Nederend of the Translational Immunology group in the UMC Utrecht. I am very happy that the public and private organizations RIVM and the Longfonds have agreed to join the users committee of the project together with the industrial partners FrieslandCampina and ALK.

7. Words of thanks

I hope that I have been able to convince you that the field of Mucosal Immunity is an intriguing, but complex, research area that has many potential applications that may contribute to the prevention of infectious and inflammatory diseases in humans as well as in animals. Having said that I think it is about time to wrap up my speech, but before doing so I would like to express some words of gratitude.

I have already mentioned quite a number of people, and will mention several more in the next minute or two. In all reality though, I realize that I have probably missed a few names that I should have mentioned – in that case please forgive me.

First of all I would like to thank The Rector Magnificus Prof. Martin Kropff and the Board of Wageningen University, as well as the appointment committee chaired by Prof. Willem de Vos for installing the chair on Mucosal Immunity and the confidence in me as a suitable candidate.

I am especially grateful for the collaboration with and support of Prof Huub Savelkoul. Without his limitless enthusiasm and commitment the chair on mucosal immunity would not have existed at all.

The same is true for Martin Scholten and Menno van Manen of ASG and Tjeerd Jongsma, Rolf Bos, Toon van Hooijdonk, Emmo Meijer and Margrethe Jonkman from FrieslandCampina. Thank you for your support and giving me the opportunity to do more fundamental research at Wageningen University.

Further I would like to thank my current colleges from CBI and FrieslandCampina for a good working atmosphere and a stimulating working environment. Over the years I have also built a large national and international network in the field of immunology and biotechnology, especially in relation to allergy and immune regulation, a network that may help me in further building my group and attracting new grants and research initiatives.

I would specifically like to mention my long standing scientific collaboration with Dr. Edward Knol from Utrecht, Prof. Erika von Mutius from Munich, and my former colleges at ALK in Denmark, collaborations that I really value and appreciate.

I would also like to thank my former colleagues from Bioceros with whom I have really enjoyed an eight year long roller coaster ride in biotechnology ranging from the largest biotech IPO on the NASDAQ to the founding of Bioceros.

And last but not least I would like to thank my family and friends. My parents and mother in law - who are not with us anymore, I know you would have been very proud if you could have been here today !

My brother Jan, who is sitting on the stage over there and his wife Silvania and their children Matthijs and Leticia in the audience, and my father in law Gerard and Mia, and Pieter and his family.

And finally my wife Hetty and my children Thomas and Inge. Without your love and support I would not be standing here today. Thank you for being there for me - and being who you are - I love you !

Mr. Rector, Ladies and Gentlemen in the audience and those that are watching on WUR TV, thank you for your attention and please join me downstairs to moisten our mucosal surfaces !

Ik heb gezegd !

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