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SECRETORY IMMUNITY

Authorised transcript of a lecture by

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Good morning!

I am very pleased to be back here in Herborn. I was here at the extraordinary 400 years' celebration 4 years ago and it was a great pleasure to be invited.

I shall talk about secretory immunity, which probably to a large extent is separate from systemic immunity. There is a good line of evidence that these two immune systems are at least partly independent. The first glimpse we had of an independent secretory immune system was apparently published in 1922 by a British military doctor called Davies who was stationed in Jerusalem. He found time to collect stool samples from the soldiers and investigated these for antibodies against dysentery bacteria. He found that such antibodies could appear in stools from infected patients several days before they appeared in the blood. He was very pleased because Dr. Besredka, who came from Russia to the Pasteur Institute a few years earlier, had a rabbit model where he had found similar "copro-antibodies" in stools before appearing in serum. He actually published his concept of local immunity in 1919. So Dr. Davies was very pleased that he could confirm this concept in man.

Then there came a long period in which very little happened in terms of local immunity, until the structure of antibodies became known in the late 50s and in the beginning of the 60s. I will particularly mention the findings of Dr. Tomasi in Buffalo, who characterised the secretory IgA antibody molecule and showed that it was quite different from monomeric IgA which is predominant in human serum. He found that secretory IgA was a dimer and that it was associated with an epithelial glycoprotein of about 80 kD, which is now called the "secretory component". It was originally called the "secretory piece", and it is actually co-valently linked to one of the subunits in the IgA dimer. So secretory IgA is an interesting hybrid molecule consisting of a plasma cell product and an epithelial cell product. It contains an additional polypeptide called the "joining" or J chain. This was discovered in 1970 by Dr. Mestecky in Birmingham, Alabama, and independently by Dr. Mariann Koshland in the USA. Dr. Mestecky showed that same peptide was found in IgM. As you will know, both these polymeric immunoglobulins can actually be transferred selectively into exocrine secretion. So both these molecules can appear as secretory immunoglobulins. We have quite good evidence that the J chain is the part of these polymeric molecules that determines their capability to be translocated into the secretions. The secretory component is indeed the

receptor for this external transport, and the secretory component can also become linked to IgM.

This model for the active transport of dimeric IgA, and the same refers to pentameric IgM, is now well accepted; I think this is an important part of the secretory immune system which is internationally agreed upon and which is a real biological mechanism.

The dimeric IgA in man is mainly produced by local plasma cells lying close to exocrine glands. The secretory epithelial cell, not the goblet cells and other mucus cells, but the serous-type of epithelial cell, produces the secretory component as a transmembrane protein in the rough endoplasmic reticulum. It goes through the Golgi complex for terminal glycosylation. It then moves to the basolateral membrane and sticks out as a receptor protein which shows high affinity for dimeric IgA and pentameric IgM; it is in the range of an antibody-antigen reaction and is, of course, primarily a non-covalent interaction. But as I mentioned, in secretory IgA there will be a covalent stabilisation through disulphide bonds taking place during the transport. This is an endocytic type of transport in vesicles. Also excess of receptor protein will be taken through the same vesicles to the lumen. The receptor is about 20 kD larger than the actual secretory component because there is an enzymatic cleavage of the receptor just before it is taken into the secretion. The cleavage site is unknown. We don't know anything about the fate of the cytoplasmic tail or the transmembrane part of the receptor protein. It is apparently degraded. The larger part of the receptor, however, is sacrificed as secretory component to stabilise secretory IgA. This molecule is actually very stable, indeed the most stable immunoglobulin of the body. Some of it will appear in faeces as active antibody, so it can pass through the gastrointestinal tract at least in small children and provide them with antibodies from breast milk to bacteria and viruses. Infants produce first secretory IgM but it is not so stable as there is no covalent stabilisation with the secretory component. So, IgM antibodies in the secretions are much more easily degraded by proteases.

This is a picture of paired immunofluorescence staining to show you what the secretory immune system looks like in the humane large bowel. These are the colonic crypts and we have stained the plasma cells here with a red fluorescence indicating IgA. Note that this immunoglobulin class is being produced in numerous plasma cells. The secretory component has been stained green. We see here the Golgi complexes, which are purely green. Yellow means that IgA has become complexed with the secretory component and is on its way out to the lumen; yellow is thus IgA plus secretory component or, in other words, secretory IgA migrating to the apical part of the cytoplasm outside the Golgi complex on its way to the lumen.

This preponderance the IgA-producing cells in all secretory tissues throughout the body from the lacrimal glands, nasal and bronchial mucosae, salivary glands, lactating mammary glands and throughout the gastrointestinal tract, is quite striking. We find that 80% to 90% of the plasma cells producing immunoglobulins are actually of the IgA class. This is in contrast to what we see in spleen and lymph nodes. So there must be some well-tuned regulation of the mucosal immune system to drive the local responses to predominantly IgA. But we see, of course, that there are also other plasma cell classes; IgM and IgG are represented to a small and variable extent. There is one peculiar feature and that is the IgD-producing cells, which are found mainly in the

upper part of a secretory immune system like the lacrimal glands and the nasal mucosa. We very rarely see IgD production in the gastrointestinal tract. This observation has some interesting implications for immune regulation, but I don't think time will allow me to discuss it here today.

When we look at the actual numbers of plasma cells producing immunoglobulins, there are large differences among the various secretory sites. This slide shows the number per mm^2 of tissue section determined by immunohistochemistry. We see, as could be expected, that in the gastrointestinal tract the number is much higher than in the parotid gland. I think that this is a direct reflection of the influence of the indigenous microbiota on the stimulation of the local immune system. We see here, perhaps to your surprise, that the lacrimal glands are also very rich in plasma cells; I think this is a reflection of the fact the conjunctiva is rather heavily exposed to protein antigens from house dust and other stimulating factors from the environment which will give rise to this high number of plasma cells in the lacrimal glands. Conversely, the lactating mammary glands and the salivary glands are rather far away from mucous membranes and are very little exposed to bacteria.

If you consider the total number of immunoglobulin-producing plasma cells in the gut it is really unbelievable. We have calculated that there must be about 10^{10} such immunocytes per metre of small bowel in man, which should be compared with the figure for bone marrow, spleen and lymph nodes altogether, that is 2.5×10^{10}. If you multiply the figure for the gut by let us say 5 or 6 metres, you get a much higher number. Actually at least 70 to 80% of all immunoglobulin-producing cells are located in the gut. We have been a little worried about this high figure for several years, but just before Christmas there was published a paper from the Netherlands showing that more than 80% of the immunoglobulin-producing cells are located in the gut also in the mouse. So our data are very much in agreement despite the fact that they were based on different techniques. The conclusion, therefore, is that in quantity of terms, the major antibody-producing cell system is actually found in the gut. This means that the gut contains our major humoral immune system.

This conclusion fits very nicely with the work of Dr. Dominique Delacroix at the University of Louvain in Belgium. He calculated the actual amounts of antibody secreted into human gut fluid as secretory IgA from local production. That figure was 40 mg per kg of body weight per day, and we know that the total production of IgG per kg is 30 mg. This means that there is more IgA transported to the gut fluid per day as dimeric IgA than the total production of IgG in the body. So IgA is actually our major antibody protein. In the bone marrow there is mainly a monomer production adding up to about 60 mg per day of total IgA. Very little dimeric IgA normally goes from the gut and bone marrow to the circulation - only about 4.5 mg per kg every day. This adds up to about 20 mg IgA to blood and then 40 mg IgA to gut fluid; that means that the IgA system amounts to twice as much immunoglobulin as the IgG system. This is probably a great surprise to some immunologists. In man the liver is mainly involved in catabolism of IgA and apparently very little IgA goes back to the gut through the bile. This is in contrast to the rat in which the liver is actually pumping dimeric IgA back from blood through bile to the gut fluid; there is relatively much more dimeric IgA reaching the blood from the gut in the rat than in man, and the rat liver cells express the secretory component as a

receptor. Human hepatocytes do not have the secretory component as a receptor for dimeric IgA, so there is a striking species difference in this respect.

According to the present concept the secretory immune system has its basis in stimulation of B-cells in Peyer's patches. This slide is from the distal ileum of a 10-year-old girl. There are numerous patches of lymphoid tissue protruding from the mucous membrane like small domes. Collectively, there are large areas of Peyer's patches and solitary lymphoid nodules in the distal ileum, and some of these will remain up to high age.

If we look at the histology, we can see that a Peyer's patch contains several lymphoid follicles with germinal centres because the local B-cell system is activated. According to the present dogma, these structures are the origin of most B-cells that become disseminated to the secretory immune system all over the body. I am not sure that this concept is completely true, but this is what is implied by the notion "a common mucosal immune system". A specialised follicle-associated epithelium is covering the lymphoid tissue; it contains very few goblet cells in contrast to numerous such cells in the epithelium covering the villi.

The follicle-associated epithelium is supposed to take up antigens actively and bring them in contact with the immune system. This process gives rise to stimulation of B-cells which migrate rapidly from the Peyer's patches through the mesenteric lymph nodes to ductus thoracius lymph and into blood. They are then taken into secretory tissues probably by specific receptor mechanisms recognising determinants on endothelial cells. In this way we have an integrated sort of immune system; you can for example have an antigenic stimulus down in the distal ileum and end up with specific antibody production against the same antigens in the lactating mammary glands. However, there are of course other sources of stimulated B-cells. We should not forget about the tonsils, and we have bronchus-associated lymphoid tissue which probably contributes some of the B-cell blasts that circulate briefly and become activated by second singles locally in various secretory tissues. But the main source of these blasts are, according to our present concept, the Peyer's patches.

The follicle-associated epithelium is specialised as I mentioned and it contains certain cells that are called the "membrane" or M cells. This slide shows an electron microscopic demonstration of these cells made by Dr. Owen in the USA. These bell-shaped cells allow the lymphoid cells to come very close to the gut lumen and they perform inward transport of antigenic material. In this case peroxidase has been taken up and passed through the vesiculo-tubular system of the M cells directly to the underlying lymphocytes.

It is not possible to see the M cells at the light microscopic level; but there is one trick you can do and that is to look for alkaline phosphatase. The brush border with alkaline phosphatase that we have on the villi is also present on the follicle-associated epithelium except where we have the M cells. Breaks or interruptions in the surface staining for alkaline phosphatase will thus indicate the M cells according to what Dr. Owen has shown by electron microscopy. We find on an average about 5 such M cells per done of human Peyer's patches, ranging from 3 to 15.

We wanted to look at the immunological activity adjacent to such breaks in the brush border representing M cells. Firstly, we studied the number of T-cells with CD3 determinants and found clusters of such cells near the

breaks. In contrast, the intra-epithelial T-cells are found scattered along the basement membrane in the villi. T-cells, therefore, apparently accumulate adjacent the M cells in and beneath the follicle-associated epithelium.

We would like to think that the M cells might have an antigen-presenting function. In that case these cells would necessarily have to express MHC class II determinants such as HLA-DR. These self-determinants bind foreign antigens and the complex is seen by the T4 ($CD4^+$) or "helper" cells. This is what we call genetically restricted antigen presentation, which represents a very important genetic aspect to the immune response. Cells like macrophages and dendritic cells, and also certain epithelial cells that express MHC class II determinants, can actually present antigens to the T4 cells. The crucial role of the T4 cell is unquestionable. We know this from AIDS patients in whom these cells are destroyed. The whole immune-system finally breaks down. So this is a key cell in the immune system as a helper cell giving regulatory signals to T8 ($CD8^+$) suppressor/cytotoxic cells and also to the B-cells. We will hear more about these interactions by Dr. MacDonald and Dr. Kiyono later on today. Such interactions are an important initiating stage to get the B-cell system going.

The major MHC class II determinant in man is HLA-DR, which shows considerable polymorphism. We find it expressed on the gut epithelium in the small intestine including the follicle-associated epithelium. In this slide HLA-DR determinants are shown in green immunofluorescence. At the same time alkaline phosphatase is stained red and we see that the breaks indicating the M cells show no green fluoresecence. The M cells, therefore, are apparently negative for class II determinants and hence cannot be antigen-presenting cells. They probably mainly perform antigen transport. But there are, of course, numerous other cells in the Peyer's patch dome area that express class II determinants such as dendritic cells, B-cells in the follicle, and also the rest of the follicle-associated epithelium which is HLA-DR positive.

If the follicle-associated epithelium and the dendritic cells in the dome area are antigen-presenting cells in a MHC class II-restricted manner, which cells do they actually trigger in the Peyer's patches? Dr. Strober in the USA has proposed that there are particular regulatory T-cells which he has called "switch" cells; they can be directly stimulated by class II determinants as indicated in this slide and they will drive B-cells expressing surface IgM directly to an IgA-expressing stage. Dr. Kiyono will probably discuss with you that there are other subsets of T-cells such as Tα cells that can drive IgA-expressing B-cells to terminal differentiation, thus giving rise to IgA production. So there may indeed be particular immunoregulatory T-cells in the Peyer's patches. There is currently a lot of discussion about this topic. Some scientists think that there is a sort of sequential differentiation along the chromosome in the order of the Ig-heavy chain genes, and that environmental factors may drive the B-cells to terminal IgA differentiation without the help of any particular T-cells. So this is an interesting area but we have actually very little specific information. There are probably various possibilities to end up with IgA as the predominant class of immunoglobulin-producing cells in secretory tissues.

If we look at the distribution of T-cells in the gut epithelium, we see as mentioned before that their number is much higher in the Peyer's patch epithelium than in the villous epithelium. I also mentioned before that there is a concentration of T-cells adjacent to the

M-cells. There is also another interesting difference between villous and Peyer's patch epithelium, namely that the CD4-to-CD8 ratio, or "helper"-to-"suppressor" phenotype ratio, is much higher in the latter than in the former epithelium. In the villous epithelium there is this remarkable dominance of the T8 or suppressor phenotype. We don't quite know the importance of this observation, but it is quite striking and very intriguing. There is really a fantastic selection of T8 cells as you can see from this slide where the villous epithelium is indicated in red staining for keratin. Here you can see the CT4 positive cells of the helper phenotype; they are located in the connective tissue and very few are going into the epithelium. On the right we have the T8 suppressor phenotype; there are very few positive cells in the lamina propria and a tremendous selection into the epithelium. I think it means something in biological terms that we have this selective migration of T8 cells into the villous epithelium.

At the same time we also have expression of MHC class II, in this case HLA-DR, on the villous epithelium. The plasma cells producing IgA are red in this slide; they are found mainly in the crypt region and you can see IgA transport in the crypt epithelium. But the villous epithelium, where we have the T8 cells, is class II positive and IgA negative. There is thus a very interesting spatial relationship between class II expression and T8 lymphocytes in the villous epithelium. The idea which has been discussed in several papers is that the intra-epithelial T8 cells perhaps are stimulated by presentation of luminal protein antigens in relation to epithelial class II molecules, giving rise to suppression of delayed type hypersensitivity and IgG and IgE immune responses. This phenomenon is called "oral tolerance" but we don't know much about it in man. Our information comes from experimental animals. But the theory is that soluble antigens induce suppression of potentially dangerous immune responses, which may give rise to immunopathology in mucous membranes. Conversely, the IgA responses are in some way released from downregulation, perhaps by contra-suppressor cells. In this way we may have a continuing IgA response in the face of a suppression of IgG and IgE responses and delayed type of hypersensitivity. However, there is not much evidence that bacteria can induce oral tolerance, except that in man IgM responses are apparently being suppressed during long-lasting infection with endotoxin-producing bacteria. But we don't have any good evidence for immunological suppression to the endogenous microflora. So perhaps "oral tolerance" is mainly relevant to food antigens.

We can probably discuss for days theories of immune regulation in the gut. But the fact remains that it works in practice. When an infant gets breast-feeding, it will be protected to a large extent against bacteria in the environment. Immunity is induced in the Peyer's patches of the mother and stimulated B-cells will end up in the lactating mammary glands and produce specific IgA antibodies. These are to a large extent directed against the gut flora and afford protection of the infant before its own IgA system has developed. This takes at least 1 month, perhaps 2-3 months, depending on the individual and the environment. But the situation in the developing countries is that breast-feeding is decreasing and bottle feeding increasing, which is a very sad development. Because bottle milk often is prepared under the most primitive conditions with filthy water, the babies contract severe diarrhoeal diseases from bacterial and viral gastro-enteritis. So I will end up with the next slide that gives

us a glimpse of the severe reality existing in the developing countries where 500 infants die from intestinal infections every hour; this means 4-5 millions per year. And in the face of the fact that in some of these countries less than 10 dollars are used for health services per inhabitant per year, this gives us some perspective of the real world. Employment of the intestinal immune system via breast feeding on the basis of better education combined with better health services are needed to reverse this unfortunate situation.