Transcript from interview with Dr. Bienenstock. Completed February 12, 2016 by Dr. Irving "Coy" Allen

Dr. Allen: Dr. Bienenstock, what do you think is the most important research problem for mucosal immunology to tackle in the future?

Dr. Bienenstock: Good question. Because mucosal immunology is a bit of a grab bag at the moment, and always has been, some of the difficulties that we face are the nature and extent to which these mucosal tissues and surfaces are all connected by common systems. That's where we start at any rate. The main issues are to try and assemble as much information about each mucosal tissue, and compare those to each other. Then, see the extent to which the bodily systems, which are interacting with these, are in fact affecting them. It's a systems biology problem. I think mucosal immunology basically has not done well, yet, to start to incorporate the interactive effects of everything from gender, to endocrine, nervous, and other systems, which will equally be affecting the systems that we're all interested in, in immunological terms.

Dr. Allen: Excellent. What would you consider to be your own most significant scientific discovery?

Dr. Bienenstock: We established something, which we then termed "common mucosal immune system", which suggested that the different mucosal tissues were, in fact, part of a system they had in common in terms of traffic of the appropriate lymphocytes between these tissues and, therefore, separating this system from the classical system that people have studied, before getting into mucosal tissues and being parental. Spleen, lymph nodes, and so on. The other thing that, I suppose, received a lot of attention, was the bronchus-associated lymphoid tissue which many people now think is not a primary immunological system. We thought at that time that, and there was good reason to think that, it had very great similarities in the lung to Peyer's patches. That does not seem to be the case. It's nevertheless still retained a great deal of interests, and it's re-surging in interest.

Then, maybe the observation that mucosal mast cells had very different characteristics pharmacologically, phenotypically, and so on, from mast cells seen in tissues, such as skin, and so on. That's received a lot of information. Then, the further evidence that the nervous system was largely connected to, and interacted with, the mast cell systems in general, has received a lot more attention, connecting the nervous system and the brain to the function of mast cells. Whether you regard mast cells as being primary immune cells, or certainly innate immune cells in mucosal tissue, that's up for debate.

Dr. Allen: If you don't mind, could you expand a little bit more about the mast cell nerve interactions? Can you discuss a little bit about the influence of cholinergic and

sensory neurons on mast cells, and the importance of mast cell nerve interactions at specific mucosal tissue sites?

Dr. Bienenstock: Well, the association has been borne out. It's not just in mucosal sites. In mucosal sites the relationship between mast cells. We were the first to bring attention to this association, particularly in the gut, hasn't been so well established, and looked at, in other mucosal tissues. In the gut, certainly mast cell function and activity, activation, secretion of mediators, and so on, can, and is, influenced by, and experimentally, can easily be shown, both ex vivo or in vitro, as well as in vivo; that especially cholinergic and substance P-containing nerves, with the product of substance P can, in fact, influence, and do influence, the mast cell function. Mast cell nerves, as a homeostatic unit in maintaining normal homeostasis in the gut, is very interesting.

Since the nervous connection is established, then clearly, the brain can influence this function. Brain-to-gut biomass cells is a two-way, bidirectional informational pathway. There's much to be learned still about how, therefore, the brain can be involved. It does raise the whole question of brain circle psychological gut-brain activity in stress and in other situations. How many of the functions of the target organ, in this case, let's say it's the gut, can be influenced directly by nervous influence, indirectly then through, say, mast cell function. The area of how the nervous system is affecting primary lymphoid tissues, such as Peyer’s patches and other lymphoid follicles, is again raising this question as to how much influence does the nervous system have, which has been an interest of my mine, for some time. On the function of lymphoid tissues, functions of lymphocytes, function of mast cells, and how this connects together in these very complex, interactive systems that we have to maintain homeostasis.

Dr. Allen: With that in mind, can you speculate a little bit about the role of the microbiota in this process?

Dr. Bienenstock: Absolutely. That's certainly, in the last 10 years, that's what has been our focus of activity. Clearly, the microbiome, as such, in not just single bacterial organisms, but also in clusters and unknown interconnections, affect, and largely control, at least in the initiation of the immune system, because of the difference, for example, between germ-free and mono-colonized or conventionalized animals. The role that the microorganisms have in making things, for example, they can make prostaglandins. They do make neurotransmitters, such as GABA. They make all sorts of things. Their influence, in terms of their secretion, the local epithelial and immune function, by products, such as short-chain fatty acids, propionate, butyrate, as well as these things that they make. Thereby, influence not only the immune system, but the local nervous system, and all the cells contained within it.

Our interest has been to follow this up, and apply this to what is commonly known as the gut-brain axis, the microbiome gut-brain axis. It chases the pathways of interaction up from the lumina up through to the brain. We've

been instrumental in showing that the microbiome in the gut can, at least experimentally, influence through this mechanism, especially vagal connections through the vagus nerve being a primary conductor of information to the brain. If the brain, in turn, can then be changed neuro-chemically, as well as behaviorally, by introducing a single change in the gut microbiome, like for microbacteria or probiotic organism, you can show that, and others have shown that, you can change stress effects in the HPA axis. You can change behavior, and this has become seized upon by the popular press. The thought that you can simply take a bacterium, and influence mood, or mood changes, and even diseases, such as depression. This is a two-way street. The brain can influence downstream, and change the microbiome content. The field is just beginning, but it's very exciting, and has much to offer.

Dr. Allen: In your opinion, is that the future direction for this field? When you think about it, what are some of the potential clinical applications?

Dr. Bienenstock: We're just into ourselves, as one or two around the world are now, into trying to apply these principles, and ask the question as to whether the results that we found in rodents, for example, actually can be applied to a clinical situation. Clearly, there is beginning information in this area, in the clinic. It's being particularly applied, at the moment, to questions about, for example, autism, and anxiety and depression, psychiatric conditions. The door is only just opening on this. There's very little direct information. We can show, with magnetic resonance spectroscopy, that if we change the microbiome, simply by giving a single organism in a mouse, that we can actually influence neurotransmitter amounts in different parts of the brain.

If we cut the vagus nerve, this actually interrupts these changes, and changes in behavior that we can induce. It's very promising, but it's very early days of attempting to understand this area. The questions are how does the immune system fit into this? Most of the things that we do, like even giving single organisms to a conventional animal, will actually influence the immune system, in a number of interesting ways, promoting regulatory populations of T-cells and other cell types. We're still not sure how the immune system and the nervous system are connected, in this sense, in terms of brain function and behavior. It's very complex, it's very exciting. It promises much, but we have to wait for experiments and results from many people around the world, before can actually start to see the extent to which this applies to diseases in the clinic.

Dr. Allen: Wonderful. We have a few additional questions, just broad questions about the field of mucosal immunology, to ask you. One of the questions, that we were curious about, is what still excites you about your research?

Dr. Bienenstock: I think what's exciting is what was always exciting to investigate or research. That is the unexpected results, whenever you push the system that you're looking at. Trying to explain it, and to discover more, because there's always more. You always think you are right, and you are often wrong, and one thing leads to another. I think the excitement of the unknown, that leads most of us

on. The field, which I was just referring to, the whole microbiome, and effect of the microbiome, is just expanding like crazy. The field is not helped by the fact that, at the moment, while we can recognize different bacteria, for example, by DNA sequencing and so on, and it may have a DNA signature, and you can recognize them, most of these organisms, at the moment, haven't been cultured. Whereas, when I started medical school, there were whatever there were, three or 400 different bacteria. Then, you could then culture them.

By then, you could propagate them, and therefore, you could test out whether that bacterium, by itself, could, in fact, have an effect in a model system. It's not possible yet, in many of these situations, since 70% of the bacteria in your and my gut are, in fact circled, non-culturable. That doesn't mean to say they won't be cultured. Then, we have the additional issue that most people have focused, so far, on bacteria, because that's the way things have gone. Now, it's becoming clear that not just the bacteria is present, but all the bacteria, different forms of bacteria, and changes therein, in different diseases and how they maintain health, but also, that the virome, which was just another huge morass, most of which is made up of phages that affect the bacteria as well. Then, the mycobiome, which is the funguses.

We're really literally only got two or three papers, which are actually describing the virome and the mycobiome yet. We're into the future here, of just not knowing. It's interesting that papers been recently published, for example, showing that a single norovirus can, in fact, completely attenuate, at least, a colitis model, which we normally would never have thought of. That a virus, by itself, could, in fact, replicate the positive results obtained with bacteria, in some of these inflammatory models. The field is just waiting for the next, if you like, discovery, and the push that has to occur in this area. I think that's probably one of the most exciting areas that we'll see develop.

Dr. Allen: We have a couple questions for some of the younger members of the society. The academic job market is getting increasingly competitive. What career advice do you give to your own graduate students and post-docs?

Dr. Bienenstock: I think it's certainly true. It's much tougher, I think, these days, than it was when I was trying to progress through the ranks. The advice is certainly the same. I think you, as a student, have to aim to know more about the particular subject that you're exploring. Or, at the very least, certainly as much as your supervisor. The hope is always that, well, from people like me, is that the student will actually exceed my knowledge, be better read, and so on. That's the depth, if you like, that one needs to go to, as a student, to move, and to obtain confidence. The second thing, basically, I think that's important, is to not just read and learn and know about your particular subject. That's a small portion. To be comfortable with at least being able to know of the breadth of technology that's available now, to support you, potentially, in your future studies, or as the studies progress. Breadth of understanding of the systems, and the technology in support of these systems that can be applied to your question, also helps you, I think, as you go through life. It's certainly tougher these days. That requires

extraordinary amounts of energy expenditure, in order to stay with the course, and hope that you can make it in this very competitive time that we have, at the moment.

Dr. Allen: In your opinion, what should the Society of Mucosal Immunology focus on, as a society, to promote scientific progress in the field of mucosal immunology?

Dr. Bienenstock: It's a good question. I think everybody you ask will have a slightly different view. I think, if you stand back and look at the thing from a great height - your 30,000-foot view - I think that life is becoming very complex. I think one of the problems that we have, in immunology in general, is that we have, with some advantage, but also some disadvantage, tended to focus down - over-focus - on single cells, and what they do. It's hard to ignore that systems biology has to play a major role, because, as I mentioned earlier, you can't just take the cell out of the body, without knowing that's it going to be different at different times of the day. It's going to be different in different local circumstances. It's going to be different, in terms of the gender of the donor. Then, you've got everything from the endocrine system, to the nervous system, and so on, to also take into consideration. I think no human can do that, without really resorting to modeling, and the in-silico approach. One needs to be able to find a way to promote this integrated approach. It's very tough, and not everybody can do it, but I think it's an area that must be emphasized, in order to advance the field.

Dr. Allen: Wonderful. Well, thank you for your time.

Dr. Bienenstock: A pleasure. That was my pleasure. I hope you have a reasonable day where you are. Is it winter where you are?

Dr. Allen: It is, yeah. It's quite chilly here. I think it'll be about eight degrees over the week