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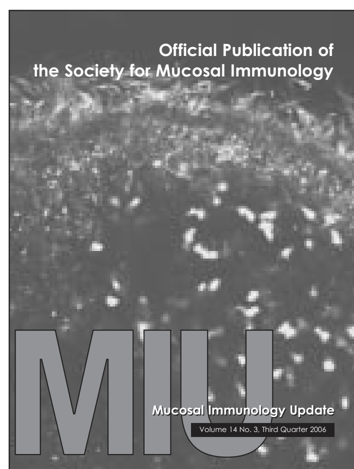

Cover Art: BALB/c mice were adoptively transferred with CFSE-labelled, DO11.10 T cells and fed OVA. Inguinal lymph nodes were taken 20hrs after feeding and imaged by multiphoton excitation microscopy. The location of cells within the intact lymph node was measured in 21 planes per three-dimensional stack of 222 x 178 μm optical sections collected at 2.5 μm intervals between each plane progressing deeper into the lymph node from an initial depth of approximately 100 μm below the surface. The time interval between each stack was 18s. The image shown is a single plane 150 μm within an intact lymph node.

Image provided by: Bernd H. Zinselmeyer[1,2], John Dempster[3], Alison M. Gurney[2,3], David Wokosin[2], James M. Brewer[1] and Paul Garside[1]. [1]Division of Immunology, Infection and Inflammation, University of Glasgow; [2]Centre for Biophotonics, University of Strathclyde,; [3]Department of Physiology and Pharmacology, University of Strathclyde.

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Introduction

We have devoted this issue of the MIU to the legacy of Prof. John Cebra, one of the founding fathers of mucosal immunology. He was critically involved with research outlining the links between various anatomical sites in the gut mucosa and the function of secretory IgA. In particular the Peyer's patches (PP) attracted his interest and many years of innovative and highly talented research led to a number of landmark publications about the properties of this primary site of mucosal IgA responses. In the present issue of MIU you will find articles that highlight the role of the bacterial flora for the development and function of the PP and the gut IgA system. This particular topic was one of the main areas of John's research and much of our current understanding in this field is owed to his pioneering experimental work. We have invited three key investigators of mucosal IgA and its function to give their current view on this topic in the light of John's early observations. These researchers represent close friends and admirers of John's work, people we think are ideally suited to give voice to our great appreciation of John's inspiration and devoted research in the field. We hope you will find their contributions not only affirmative of John's importance to our current understanding of the gut mucosal IgA system, but also interesting up-dates on this, still highly controversial, chapter in mucosal immunology.

Nils Lycke

A Remembrance of John J. Cebra:

Reovirus as a Probe for Understanding Mucosal Immunity

I suppose that if we were to survey the readers of *Mucosal Immunology Update* for John Cebra's most significant contribution to mucosal immunology, the majority of respondents would point to the 1971 paper on Peyer's patches as the source of IgA committed plasma cells [5] or the elucidation of the structure of IgA [4]. Perhaps others with a keen interest in gnotobiology and probiotics would suggest that his work using germfree mice to understand the development of IgA responses and germinal centers, [8,24,25,29] and the contribution of B-1 and B-2 B-cells to IgA defined John's legacy [3,13,27]. For me and many of my colleagues who studied in the Cebra laboratory in the 1980s, the opportunity he provided for us to study mucosal immunity to reovirus infection was equally significant. These studies have helped us to understand the power of viruses as probes to elucidating mucosal immunity, especially during infection of the gastrointestinal tract. In a few paragraphs I will describe work from John's lab using reovirus as a probe to understanding mucosal immunity. Along the way I'll relay a couple of anecdotes that might resonate with those who knew John, or give a sense of his character to those who didn't.

Reovirus (respiratory enteric orphan virus) is a double stranded RNA-containing virus that has been used to understand many aspects of the biology, pathology, and immunology of viral infection. Reovirus was recognized as a naturally occurring isolate of humans prior to 1960 [23]. Despite efforts to link reovirus to diseases in humans, the term 'orphan' still applies when considering its role in human disease. There are, however, a number of disease syndromes in experimental animal infections that have been used to understand viral pathogenesis, including diseases of mucosal tissues. Reovirus serotype 1, strain Lang had been shown to be transported specifically through M-cells from the intestinal lumen into Peyer's patches [30,31] while serotype 3, strain Dearing appears to have a broader cell tropism in the intestine [11].

One of John's longest and most successful collaborations was with Don Rubin (now at Vanderbilt University), who introduced John and his group to reovirus in the mid-1980s. Don was an enthusiastic and devoted captain of the reovirus projects that developed in John's laboratory, and he provided a wealth of expertise and insight. Don had earlier reported that reovirus appeared to preferentially replicate in ileal crypt cells following uptake in Peyer's patches [22], induced significant IgA responses following oral infection [1], and induced oral tolerance if the virus was inactivated [21]. The work was extended by Steve London, then a graduate student with a degree in dentistry, in John's laboratory. With Don and John's mentoring, Steve London produced an outstanding Ph.D. dissertation that reported several new significant findings in a series of papers, including: 1) a demonstration that reovirus-primed IgA-producing cells in the Peyer's patches, 2) enteric virus infection induced virus-specific precursor cytotoxic T-lymphocytes (pCTLs) in the intestine [15], and 3) reovirus stimulated germinal center B-cells and a subset of T-cells to express a novel activation antigen designated, GC-T [14]. Steve also made the second report of virus-induced activation of intraepithelial lymphocytes, which was published in our departed journal of mucosal immunology, *Regional Immunology* [16]. Paul Offit , a regular participant in John's lab meetings first reported rotavirus-specific CTLs in the epithelium [17] shortly before Steve's reovirus paper, and John and his laboratory offered intellectual support to Paul's work.

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I joined John's laboratory in 1988 as a postdoctoral fellow and took up the project where Steve left off. At the same time a number of graduate students and post-docs were using reovirus to probe several aspects of mucosal immunity. I was charged with working on the reovirus project and as per John's modus operandi, he left open just about any avenue I wanted to explore. Initial reports of IELs that expressed the gamma delta form of the T-cell receptor were appearing [10, 2], so we looked at whether the gamma delta TCR-expressing IELs were the reovirus-specific CTLs that Steve initially found (They weren't! [6]). I also worked with a novel isolate of reovirus called 'clone 9' that caused meningoencephalitis in neonates after peroral infection, and assessed the role of mucosal effectors on protection [7]. At roughly the same time, Anna George, and then Jim Taterka, worked on features of the SCID mouse model of reovirus infection [9,26]. These studies showed the contributions of innate and adaptive immune responses to reovirus infection.

An approach to studying mucosal immunity that was used in John's lab (and I have continued using) was to compare aspects of immune responses following oral or systemic infection, and when qualitative differences are identified, hypotheses that explain these differences were hashed out and tested. Peter Weinstein, a graduate student in the lab, used germfree mice infected either orally or systemically and examined the development of IgA and IgG to ask whether preferential IgA production in the gut was due to unique features of the intestinal microenvironment, or whether IgA responses were due to chronic stimulation of the gut [28], which seemed to be John's preferred explanation. This was a test of John's 'site versus sink' hypothesis and the results demonstrated that the microenvironment controlled IgA production. Although John left open both ideas, my impression was that he leaned towards 'sink' rather than 'site'. Even the greatest minds guess wrong! A bit later David Kramer joined the laboratory and did some fantastic work in which he examined immune responses to reovirus infection in immunocompetent neonates that were foster-nursed by SCID dams [12]. Although the main thrust of the report was a demonstration of the immunocompetence of the neonatal Peyer's patch, I was always impressed with the substantial bystander effect of reovirus infection in the neonates.

I left John's laboratory in 1992 and was followed by Sangeeta Periwal who continued work on responses in neonatal infection [20], and then shifted her studies to respiratory infection with reovirus [19]. Her work inspired Adrian Zeurcher, who followed Sangeeta as a post-doctoral fellow. Adrian initially spent time in my laboratory at West Virginia University to learn the ropes with reovirus, and his tour in John's lab resulted in a very nice report describing features of cross talk between mucosal lymphoid tissue in the gut and lung [34]. Adrian also used reovirus to stimulate nasal associated lymphoid tissue in mice [33] and rats [32]. The latter study again used the approach of analyzing qualitative differences in the immune response to reovirus following various routes of exposure in order to better understand mucosal immunity. In the last couple of years of John's life he and I were collaborating on an in vivo correlate to a study reported last year from my laboratory in collaboration with Charlotte Kaetzel in which we characterized reovirus-mediated upregulation of polymeric immunoglobulin receptor expression in HT-29 cells [18]. This gave me the opportunity to be able to spend a little more time in the company of John and Ethel.

John's cancer became more public in 2001 when he missed the satellite mucosal immunology meeting in Iceland because he had to have emergency surgery. He fought back from a difficult surgery and in the time he had remaining he pushed forward, always putting in front of himself another reason to carry on - the next meeting, the next international trip to a colleague's laboratory, or the next wild set of experiments. Although his health waxed and waned, I sometimes was able to convince myself that he had found a way to become a statistical outlier and fight off his disease through a combination of medicine, luck, and willpower. But many of you saw him at the Boston meeting last summer and perhaps realized that he was making his last stand. John imparted to me a sincere respect for the Society and its members, and I think that he appreciated the recognition awarded to him at the meeting. I last saw John with friends and students at the Marine Biological Laboratory at Woods Hole later in the summer when his former students honored him with an endowed lectureship. The distinguished and heartfelt inaugural lecture was given by Per Brandtzaeg.

Let me finish with a couple of personal remembrances. By his own account, John's major accomplishment was the training of 32 Ph.D. students and many post-doctoral fellows, for which he was presented with the 2005 American Association of Immunologists Excellence in Mentoring Award. Part of John's educational philosophy was to expose his students to the enriching experience of international travel and scientific exchange. One of my fondest memories from Penn included a 6-week exchange visit to Academia Sinica in Taiwan, where John was helping a newly established Institute of Molecular Biology get off the ground. It was incredibly generous of John to enable my wife and me to join him and Ethel there, where we met new friends and future collaborators. A favorite story from that time was when he charged me to take some SCID mice with me on my flight to Taiwan. I really didn't want to fail him at this

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task but was stymied by an animal rights activist who worked the counter of Korean Air at JFK airport, and wouldn't let me ship the mice on the plane. (The mice made it intact a day later thanks to China Air's cargo division.)

Towards the end of my fellowship I also had the opportunity to travel to Groningen, The Netherlands, where John was doing a sabbatical and working with Frans Kroese, and my good friend and another of John's major collaborators, Nico Bos, on the role of B-1 B-cells in mucosal immunity. On that trip John and Ethel introduced me to a meal that John was fond of preparing and serving; a dish that I think they called 'smoked eel tureen'. It was fantastic, and my favorite Cebra concoction.

And finally, John was the center of gravity for the Mid-Atlantic Immunobiology Meeting, which was billed as the longest continuously run regional immunobiology meeting, and ran for some 32 years. I was able to attend the last 17 of them. For me it was part meeting, but mostly family reunion, which provided an opportunity for me and in later years my students, to present our work in oral presentations in front of some of the greatest minds in immunology who would join us as honored guest speakers.

I loved John and do sorely miss him. To me and many of my friends and colleagues, John had an incomparable intellect and was the consummate mentor. Losing mentors is very difficult indeed, but I suppose in the end it is the price we pay for the opportunity to enjoy one of life's most nourishing relationships.

Contributed by Chris Cuff

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I am very grateful to the editors of MIU, especially Nils Lycke, for inviting me to contribute to this issue. Since this is a time of looking back, I also offer my deepest thanks to Don Rubin for all his help and support. My warmest thoughts and condolences remain extended to Ethel and the rest of the Cebra family.

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John Cebra's contributions to Function and origin of mucosal IgA

John Cebra has been an inspiration to many mucosal immunologists in the past 50 years. With so many contributions to the field of mucosal immunology by John in these fifty years, it is impossible to be complete. I will therefore restrict myself to John's contribution to unraveling the function and origin of mucosal IgA and I apologize upfront to all of those that I don't mention.

In John's most early work in the late fifties he was unravelling the structure of immunoglobulins. In 1965, one year after IgA officially got its name he published about the distribution of different isotypes in human lymphoid tissues. Already at that time until his death on 7th october 2005 he was intrigued by the question why there were so many B cells that produce IgA in the mucosal tissues and what the function of these IgA antibodies was. I was fortunate to share that passion with him during the last 14 years of his life. The main questions that were on the top of both our minds were: 1. Where and how are B cells being selected to become IgA producing cells? 2. Is there a difference in reaction towards different commensal or pathogenic bacteria? 3. What is the function of these secreted IgA antibodies?

In the landmark paper in 1971 Susan Craig and John Cebra showed that the germinal centers in the Peyer's patches are the places where B cells are stimulated to become IgA producing cells [1] Subsequently, he showed together with Tseng that activated B cells migrate through the circulation to seed the lamina propria as a large number of IgA plasma cells [2].

John and I met in 1991 when John and Ethel, his wife and life-long companion, visited our lab in Groningen. John came because he was fascinated by the finding of my colleague Frans Kroese that a particular B cell population in mice (B-1 cells) could contribute significantly to the IgA production in the gut without being present in Peyers's patches [3]. During John's stay, we visited one the beautiful small islands at the coast of The Netherlands and ended up stranded there because of a storm. That gave me a great opportunity to learn that next to travelling, John and Ethel love nature, especially bird watching and good food. At every place I have been with John and Ethel eversince, there was always some time for birdwatching and good food.

In 1992 John and Ethel spent their sabbatical year at our lab in Groningen in the Netherlands and that was the beginning of our extensive collaboration and friendship. In that year we were able to isolate IgA-producing hybridomas derived from B-1 cells and showed that each of these IgA monoclonal antibodies has its own multireactive pattern in which it binds to commensal bacteria [4]. That led to the idea that maybe these IgA molecules serve another function than the high-affinity IgA antibodies observed in antigen-specific immune reactions against pathogens [5].

John and I both realized that the only way to really understand the complexity of the gut ecosytem was to

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simplify the system by using gnotobiology. In the seventies when John was at John Hopkins he sent out Al Chaney to the NIH to learn how to maintain germfree and gnotobiological animals. Since then he has always maintained his own independently financed gnotobiological facility. Al Chaney went with him from Hopkins to the University of Pennsylvania in 1979 and Al maintained the facility until his retirement and was later joined by Michelle Mikell and Dmytro Kobuley. John has always realized that they were crucial to his work.

I was being trained as a gnotobiologist during my PhD thesis work at the Lobund Laboratory at the Notre Dame University and together John and I were able to conduct a unique set of gnotobiological experiments.

A major step forward was made by Kushroo Shroff. As a postdoc in John's lab he very accurately analyzed the IgA immune response after monoassociation with the commensal organism *Morganella morganii* (MM) [6]. He showed that commensal bacteria can induce germinal center reactions in the Peyer's patches that results in high IgA production. When the IgA production is at a steady level, the MM do not translocate any longer into the tissues, showing a dynamical equilibrium between the production of IgA and containment of the commensal bacteria in the gut. This was extended by Gwen Talham and Han-Qing Jiang who showed that another commensal bacteria, namely Segmented Filamentous Bacterium (SFB), induced an even greater IgA response [7]. Interestingly, only a very small fraction of the IgA induced by SFB could be shown to be specific for SFB. Moreover, the addition of MM to a mouse with SFB led to an independent IgA response against MM, irrespective of the already induced IgA production.

Mano Manohar took these observations one step further to analyze the IgA response against an avirulent form of Listeria [8]. Overall it became clear that all gut microorganisms are able to induce IgA production but that the ratio of specific versus natural IgA was dependent on the characteristics of the inducing organisms [9]. There seems to be a direct correlation between the "pathogenicity" and the amount of specific IgA induced.

In 1999 John was ready for another sabbatical leave and he invited me to run his lab while he was on sabbatical leave to Helena Tlaskalova in Prague. Together with the postdocs Han-Qing Jiang, Natasha Kushnir, Christine Thurnheer and Adrian Zuercher we set up a nice set of experiments to study the functional relevance of IgA production. The role of IgA in controlling the gut flora was studied in an elegant study by Han-Qing in SFB monoassociated mice in which the pups had either immunocompetent or immundeficient SCID mothers [10]. She had adapted this protocol from David Kramer who had shown earlier in such an experiment in conventional mice that maternal IgA forestalls the onset of Ig production by the pups [11]. In Han Qing's paper we could show for the first time the importance of IgA in the behavior of commensal bacteria. When maternal IgA was lacking there was an early rise in ileal SFB colonization. After weaning there was a striking difference between immune-deficient mice and control mice in that control mice clear the SFB from the ileum and immune-deficient mice do not. Although the latter effect could not be directly attributed to IgA, because of the complete immune deficiency, Maaike Stoel during her two year stay as John's last PhD student could confirm this effect completely in gnotobiotic IgA knockout mice (Stoel et al., manuscript in preparation).

To test the ability of B-1 and B-2 cells to produce antigen-specific IgA responses Natasha orally infected mice with rotavirus. By using chimeric mice with allotypic different B-1 and B-2 cells it became clear that only B-2 cells were able to mount such a T-cell dependent rota-virus specific response, but that both B-1 and B-2 cells contributed to the total production of IgA [12].

Similarly, we also tested the contribution of B-1 and B-2 cells to produce IgA in response to colonization by commensal organisms and showed that these responses can be mounted by both B-1 and B-2 cells [13]. The relative contribution of B-1 and B-2 cells however, depends very much on the model. In neonatal chimeric mice that gave equal chances for B-1 and B-2 cells to grow out Christine Thurnheer showed that the contribution of B-1 cells to IgA production is very limited [13], while in immunodeficient mice reconstituted at adult age, the contribution of B-1 cells is usually higher [14]. Whether B-1 cells need T-cell help to switch to IgA is still a matter of debate [9;15]. It is obvious that mice lacking T cells are able to make IgA antibodies, but whether B-1 cells or B-2 cells or both are responsible for this IgA production is unclear. John and Han-Qing went to extreme lengths to eradicate every T cell in a transferred B-1 cell population and it was obvious that the less T cells were available the less IgA the B-1 cells were able to produce. It is, however, very likely that B-1 cells are not dependent on cognate B-T cell interactions because even non-antigen-specific T cells can provide such help to B-1 cells (Jiang et al, manuscript in preparation).

In the last years, Maaike Stoel from my lab worked for half of her time at John's lab in a collaborative PhD project.

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Maaike set out to analyze the IgA repertoire in conventionally reared mice. In contrast to our expectations it turned out that the IgA repertoire is very limited despite the stimulation by a complex gut flora [16]. More importantly, she found that there might be alternative ways than the classical affinity maturation in germinal centers to trigger IgA production. Next to the antigen-selected B clones that show clear signs of classical affinity maturation, she also observed many B clones with non-shared random mutations.

Our current thinking is that the unique situation of the gut ecosystem in which the commensal antigens never become limiting during the germinal center reactions, will lead to less affinity-selected B clones. This might explain the large amount of natural IgA that is always being induced when the gut is colonized by an abundant number of commensal organisms.

In an ultimately controlled experiment Maaike was able to set up a model in which germfree IgA-knockout and control mice were associated with a defined set of five commensal organisms. Although these experiments are not completed yet, the first results show an unique role for IgA in controlling the immune response against gut commensal organisms.

Nadya Boyko, a collaborator from the Ukrian, using the same gnotobiotic IgA knock-out mice showed that the lack of IgA resulted in increased translocation into the tissues of some commensal bacteria.

Looking back at the three original questions, we can conclude that over the past 50 years John has contributed much to our insights of how IgA functions within the mucosal immune system. His work can be found in 161 peer-reviewed papers and in many book-contributions. John's work will be a great inspiration to further unravel the complex ecosystem of the gastrointestinal tract.

When John was faced some years ago with fighting off colon cancer, he told me that he had thought about what to do with the rest of his life. He decided to continue with the work that he loved so much and he was even teaching his students up to the week before he died. That was exactly what he always wanted and loved to do. Many of his former students and post-docs will remember John as their inspirator and he was awarded the 2005 American Association of Immunologists Excellence in Mentoring Award.

For me, he was even more than that and I will miss him as my scientific father and as my soulmate. I am glad to be part of the large "Cebra family" that came together on the occasion of John's 70th birthday in 2005 and I wish Ethel and the rest of the family strength in dealing with this loss.

Contributed by Nico Bos

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In honour of John Cebra: IgA

1. INTRODUCTION
It is obviously with very mixed feelings that I approach writing this article on IgA as part of the series to honour John Cebra. I met John only twice, both last year, and on the first occasion we gave back-to-back talks and fielded questions together as part of the 2005 Old Hebron University Seminar series. It was a great experience on the first evening of the meeting, before the talks, to sit with someone whom I had admired for so long, and who I had only known until then through his papers and reputation. Everyone in mucosal immunology is to some extent influenced by the work that John did and led: for those working on IgA or host-commensal interactions he was and is a guiding light. Many of the experimental rationales and designs in this field have been derived from his work, and in this article I would like to show how heavily our current understanding and our own work is indebted to his ideas and data.

2. IMMUNE GEOGRAPHY OF IGA INDUCTION
It is now self-evident that mucosal immunity is special because of the distinct and integrated nature of the mucosal immune response. Early studies using responses to cholera and diphtheria toxins had shown that the kinetics and levels of serum and secretory antibody titres were independent (1). The discovery of the IgA isotype (2), and the demonstration that it dominated antibodies secreted across mucous membranes and into colostrum (3, 4), confirmed the distinctiveness of the mucosal humoral response.

John's most highly cited publication was the demonstration of the site of induction of the IgA response. He showed with Susan Craig in 1971 that B cells from the Peyer's patches in rabbits switched to IgA from adoptive transfer experiments using Ig allotypic markers (5). This is one of the classics of immunology. The interpretation, that Peyer's patches are the site where B cells are induced to undergo class switch recombination to express IgA and seed mucosal sites with IgA-secreting plasma cells, is now generally accepted. At that time, although IgA induction through oral immunization had been reported, and abundant IgA-containing plasma cells were detectable in the intestinal lamina propria, stains for IgA in the Peyer's patches were paradoxically negative. Two additional points in the discussion section of this paper deserve comment. First, they very cautiously pointed out that 'the concept of there being a local secretory immune system will be further strengthened if it can be demonstrated that Peyer's patches do indeed furnish IgA cells to other secretory lymphoid tissues'. Secondly, they speculated on the homing mechanisms required for the mucosal immune circuitry required for recirculation from the inductive sites (Peyer's patches) to the mucosa. The induction and lymph/blood recirculation system that we now understand is all outlined here. Subsequently the cellular and cytokine interactions required for the specificity of class switch recombination and selective homing have been addressed using cell culture and in vivo systems (reviewed in (6, 7)). The role of the Peyer's patch dendritic cells, shown by John's group to play a key role in the Cα class switch (8), will be further discussed below in relation to the localization of the mucosal IgA response to commensals.

Good experimental evidence of recirculation of IgA+ blasts through the thoracic duct lymph following local induction was obtained in a different experimental setup by Gowans and his colleagues (9). Gowans had shown in a series of papers in the 1950s and 60s that lymphocytes generally home from the bloodstream into the lymph nodes and thence recirculate through the lymph (10). Specifically, they had found that some lymphoblasts isolated from the thoracic duct lymph express IgA and that infused labeled thoracic duct lymphoblasts could also be shown to home to the lamina propria of the intestine. Using cholera toxin as an immunogen, with initial intraperitoneal priming in complete Freund's followed by a intestinal boost without additional adjuvant, they found that lymphoblasts secreting anti-cholera toxin could be isolated from the thoracic lymph 16-19 days after the intestinal boost, followed by increased specific IgA-secreting cells in the lamina propria from day 17. Without the intestinal boost, neither the thoracic duct lymphoblasts, nor the lamina propria IgA plasma cell increases were seen. In a follow-up paper (11), they isolated two intestinal segments in rats by making three transverse cuts spaced along the length of the small intestine, without disturbing the vascular or lymphatic supplies. The small intestine (duodenum) proximal to the first cut was surgically anastomosed to the small intestine (ileum) distal to the third cut, thus restoring the intestinal stream. This left two segments that were separately connected to open onto the skin surface, so they could immunize one segment of intestine without the immunization solution reaching the surface of the other intestinal segment or the main intestinal stream. When they followed their earlier protocol with cholera toxin, most anti-cholera toxin specific antibody-secreting cells were always seen in the immunized loop, but a specific response was also seen in the non-immunized segment and in the main intestinal stream. Both by following the kinetics of the responses in the different loops and by using pulses of tritiated thymidine, they concluded that following recirculation in the lymph and the blood IgA-secreting cells could reach the lamina propria of the intestine remote from

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the site of immunization, but there was also some antigen-specific stimulation of lymphoblast proliferation in the immunized segment.

The Odyssey of IgA is completed with its transport across the intestinal epithelium into the gut lumen. Per Brandtzaeg (also present at the 2005 Old Herborn meeting) and his colleagues showed that polymeric IgA or IgM to the polymeric immunoglobulin receptor protein (pIgR) through the joining (J) chain at the basolateral surface of the epithelial cells and transport to the luminal surface where immunoglobulin is released after enzymatic cleavage of the pIgR. They showed that the pIgR is initially synthesised in an uncomplexed form in the Golgi of serous columnar epithelial cells (12, 13), binds immunoglobulin at the basolateral surface and releases it from the complex at the apical surface into the lumen (12, 14). They also showed the essential requirement of J chain in the secreted immunoglobulin, explaining how the transport mechanism is selective for polymeric IgA and IgM that is mostly produced in the mucosa. It is easy to forget that at the time of their original observations the field was confused by at least 5 other (incorrect) models for transepithelial antibody transport (15).

3. IGA AND COMMENSAL INTESTINAL BACTERIA

The significance of large numbers of non-pathogenic commensal bacteria in the lower intestine has intrigued biomedical scientists since Pasteur (16) and Cushing (17). The evidence that mutualism with these bacteria shapes the composition of many body tissues stemmed from the achievements over the last century to derive and maintain axenic animals in a germ-free state (18-20). Comparisons with colonised animals shows that addition to many other ultrastructural (21, 22), immune (23), epithelial (24, 25) and metabolic (26, 27) changes, the content of lamina propria IgA-secreting cells and serum IgA is profoundly reduced in germ-free animals (28, 29). In addition to short-range changes in the content and function of the mucosal immune system including some T cell subsets (30-32), there are also long-range changes in systemic secondary lymphoid structure (21, 23, 33). Systemic immune function alterations have also been shown in experiments by Nico Bos (34-36), John's long-term collaborator and author of another article in this series.

Cebra's group addressed the difficult issues of IgA function in relation to the commensals with some elegant experimental designs. They monocolonized previously germ-free mice with a model intestinal commensal – *Morganella morganii* – and then studied the sequential changes in immunity and parallel bacterial penetration (translocation) from the intestinal lumen into body tissues. As expected, there was progressive induction of IgA from day 14 after colonization, and penetration of live bacteria beneath the epithelial layer were approximately inversely related to the IgA response (37). They showed that secretory IgA was highly effective in coating intestinal bacteria. Despite the persistent accumulation of lamina propria IgA-secreting cells until day 314, the number of germinal centre B cells in the Peyer's patches peaked at day 14 and subsequently decreased. Probably the initial phase of luminal bacterial overgrowth would have settled after day 14, but they showed that secreted IgA would coat the intestinal bacteria and penetration ceased after day 108.

A similar protective mechanism from secretory antibodies probably also works in neonatal mice, although here the endogenous immune system of the newborn animals is too immature to induce and secrete IgA before the succession of bacterial species that colonise the lower intestine start to become established soon after birth. Experiments carried out by Kramer and Cebra (38) showed that scid/+ pups being nursed by a wild-type mother (and therefore receiving antibodies in the milk) did not induce their own mucosal IgA until after weaning at 21 days. Conversely, scid/+ pups being nursed by a scid/scid mother (and therefore receiving antibody-deficient milk) showed early endogenous IgA induction from day 16. These experiments did not formally show that commensals were driving the early endogenous responses, nor excluded a compensatory response from lack of duodenal uptake of IgG in the scid/scid nursed pups (39), however, the most likely explanation is that IgA present in maternal milk protects the pups' mucosal cells from commensal exposure. John wrote a nice review summarizing these adult and neonatal studies on the effect of commensals (40).

Mutualism with the commensal intestinal flora at the mucosal surface is multifaceted – presumably co-evolution has ensured fail-safe redundancy in the system. Immune myopia is achieved by limiting expression of microbial pattern-recognition molecules in epithelial cells and the subepithelial macrophages (41). Other antibacterial molecules, including defensins and cathelicidins are also secreted (some of which are also induced with similar kinetics to the IgA response during recolonisation (42)). To look specifically at the role of secreted antibodies we repeated John's recolonisation design, comparing the effects on the $JH^{-/-}$ antibody-deficient strain with the C57BL/6 wild type. To avoid potentially unbalanced overgrowth of a single organism, recolonisation was started by placing a single SPF sentinel (with a modified Schaedler intestinal flora (43)). We found that the antibody-deficient mice had a prolonged

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phase of increased bacterial penetration despite no significant difference in the luminal bacterial densities, although this settled by day 35 of the experiment (44). This is consistent with IgA being one of a series of adaptive mechanisms, and IgA-coated commensal bacteria are overall less able to penetrate the intestine epithelium, or once they have penetrated can be cleared with newly synthesized IgA that is being secreted via pIgR across the epithelial surface. Mice that cannot secrete immunoglobulins owing to targeted deficiency of pIgR also become more susceptible to intestinal Salmonella typhimurium infection because cross-reactive preimmune secretory IgA limits epithelial penetration of this organism (45).

The effects of IgA are not purely those of immune exclusion. In a different in vivo system, the activation-induced cytidine deaminase (AID)-targeted mouse, which is deficient in class switch recombination and Ig affinity maturation, Sidonia Fagarasan has described overgrowth of anaerobic commensals (46). Also, whilst secretory antibodies limit the overall penetration of commensal bacteria at the mucosal surface (comprising the surface epithelium and Peyer's patches), it has been appreciated that M cells have receptors for IgA (47, 48) which can enhance delivery of IgA to dendritic cells in the Peyer's patches (49).

4. CHARACTERISTICS OF LOCAL IGA INDUCTION BY COMMENSALS

In experiments to study the difference between local immune responses to commensals and systemic immune responses, we found that specific pathogen-free mice remained ignorant rather than tolerant of their commensal intestinal flora using Western blots to a model dominant commensal – *Enterobacter cloacae* (50). We found that priming tended to occur spontaneously in mice selectively deficient for IgA, or in alymphoblastic mice which lack most secondary lymphoid structures apart from the spleen and nasal lymphoid tissues: further indirect evidence of an exclusion barrier function. To address the handling of live commensals we carried out intestinal challenge experiments. We found that small numbers of challenge bacteria were sampled by intestinal DC, and Peyer's patch DC containing live bacteria were effective in inducing IgA^+ B cells in culture and in vivo. Heat-killed bacteria do not work in these in vitro or in vivo assays, although it is not yet clear whether the commensals need to be alive when sampled by DC.

In our experiments, Peyer's patches were the main site of sampling (although this may partly reflect ease of access in the challenge setup), but the groups of Maria Rescigno (51) and Hans-Christian Reinecker (52) have also shown sampling across the villous epithelium via DC processes that protrude between the epithelial cells of the terminal ileum. Although intestinal DC loaded with live bacteria can traffic to the mesenteric lymph nodes (MLN), they do not penetrate further to reach systemic lymphoid structures. Experiments in mice without MLN show that these intestinal lymphoid structures form a firewall that limits exposure of the systemic immune system to induction by commensals: in such mice chronic intestinal exposure to commensal results in DC that traffic through the lymph to reach systemic secondary lymphoid structures, resulting in massive splenomegaly and lymphadenopathy. Although MLN are necessary as a firewall, in accordance with John's original data on IgA switch commitment in Peyer's patch B cells, they are not obligatory for IgA induction (44).

IgA is a dichotomous system (53), which is capable of mounting a T-cell dependent response to obtain neutralizing antibodies of high affinity to toxins (54, 55), or a more primitive response that can be independent of T cells (50) or cognate T cell help (56). The groups of John and Nico Bos have shown that much of this primitive response is presumably of low affinity to redundant microbial epitopes, since there is little evidence of sequential acquisition of mutations characteristic of affinity maturation (57).

Nico has written of the direct inspiration that he obtained from working with John. Although I was not part of the Cebra 'family', I have still been very inspired by his work, which is a landmark in our field and a great testament to a special scientist.

Contributed by Andrew MacPherson

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