Old Herborn University Seminar Monograph 11:
Immunomodulation of the gastrointestinal mucosa.
Editors: John C. Cebra, Peter J. Heidt, Volker Rusch, Dirk van der Waaij, and Richard I. Walker.
Herborn Litterae, Herborn-Dill, Germany: 123-129 (1998).

OLD HERBORN UNIVERSITY SEMINAR ON IMMUNOMODULATION OF THE GASTROINTESTINAL MUCOSA: MINUTES AND REVIEW OF THE DISCUSSION

JOHN C. CEBRA, DIRK VAN DER WAAIJ, and RICHARD I. WALKER

DISCUSSION PARTICIPANTS (in alphabetical order):

Shahida Baqar, Bengt Björkstén, Philip B. Carter, John J. Cebra, Mogens H. Claesson, Lars Å. Hanson, Peter J. Heidt, Adrian Lee, Alistair Ramsay, Volker D. Rusch, Paul Simon, Helena Tlaskalová, Joseph F. Urban, Dirk van der Waaij, Richard I. Walker, and Agnes Wold

MINUTES AND REVIEW OF THE DISCUSSION

The seminar was opened by Richard Walker. He reviewed known mechanisms of gut/microbial interactions that could contribute to colonisation, "colonisation resistance" *vis a vis* other organisms, translocation, and stimulation of a specific or non-specific *local* inflammatory and/or immune response by the host. He also introduced various strategies for deliberately inducing specific gut mucosal immune responses and use of microbial products as adjuvants. The first speakers: Wold, Carter, and Claesson, addressed the modes by which gut food antigens and microbial flora may interact with the host to initiate:

1) specific or aspecific gut inflammatory/immune responses;
2) oral tolerance; and
3) gut inflammatory responses.

Subsequent discussion considered the enigma of why ingestion of soluble antigens - such as ovalbumin (OVA) - led to oral tolerance while expression of OVA by a recombinant, colonising bacteria led to an immune response (*Wold* et al., 1989; *Dahlgren* et al., 1991). Perhaps uptake at different sites by M-cells over Peyer's patches, enterocytes, dendritic cells in the intra-epithelial leukocyte (IEL) compartment could lead to different modes of antigen dissemination and presentation. Ordinarily, the weanling host does make a humoral mucosal immune response to gut organisms. This response appears to be "self-limiting" and excludes gut microbial antigens from further translocation and immune stimulation (*Shroff* et al., 1995). However, if the neonate is exposed early in life to a colonising microbe for which the nursing dam has no antibodies - such as 06:K13 *E. coli* expressing the OVA gene - it appears that oral tolerance results to both OVA and protein, carbohydrate, and LPS antigens of the bacterium. Probably, the various outcomes of gut microbial/host interactions depend partly on how commensal or pathogenic bacteria interact with the host's gut:

1) do they remain free in the lumen;
2) attach to the brush border of enterocytes or to mucus via "holdfast" organelles or pilli;
3) "swim" in the mucus of crypts, like spirilla (*Helicobacter muridarum*); or
4) produce special "invasins" or "internalins" that facilitate translocation via ligands of host cells?

A particularly detailed analysis of the latter has been made for *E. coli* (EPEC

strains) by *Donnenberg* et al. (1997). Certainly, artificial flushing of mucus, such as with $MgSO_4$, can upset the balance of gut micro-organisms and a particular microbe, *Bacteriodes* sp., can alter the expression of terminal sugars on glycoconjugates made by enterocytes (*Bry* et al., 1996) This may in turn influence the colonisation by other microbes.

Perhaps the importance of that part of the microflora which colonises the gut in the mucus layer and in the crypts has been overlooked. The goblet cells producing the mucus are T-cell dependent in their activity. In SCID mice with $CD4^+$ T cell induced IBD, there is an attenuation of sulphomucin secreting goblet cells in the colonic mucosa in the diseased animals (*Reimann* et al., 1995; *Bregenholt* et al., 1997)

The apparently paradoxical findings of Carter, that *Listeria monocytogenes* is a particularly effective gut mucosal pathogen, poor at colonising but efficient at causing a systemic disease after translocation - while failing to stimulate an appreciable mucosal immune response, was discussed at some length. Although transit of *Listeria* via M-cells has been detected, SCID mice - which lack demonstrable M-cells - are even more vulnerable than immunocompetent mice. Also, invasive *Listeria* express "internalin", which specifically interacts with a ligand, E-cadherin, on the basolateral surface of enterocytes to initiate uptake by enterocytes. The discussions turned to provocative findings by the group of Pringault (*Kerneis* et al., 1997) that the functional properties of cultured, transformed enterocytes could be modified to resemble some of those displayed by M-cells *in situ*. Nevertheless, it remains possible that the transient translocation of *Listeria*, mainly via enterocytes, allows it to "slip past" the gut mucosal follicles to disseminate and cause systemic diseases (CNS-listeriosis) without being detected by the mucosal immune system. Of some interest is that IFN-γ and TNF-α, probably made by cells in the IEL compartment, are elevated during infection via the gut route and appear to play an immunologically non-specific role in attenuating listeriosis. Finally, an avirulent, virulence-factor "knockout" mutant of *Listeria* (actA-negative) can chronically infect only the gut epithelium of both germ-free (GF) immunocompetent and GF SCID mice. The former mice do display an humoral mucosal immune response (*Manowar* and *Cebra*, unpublished observations). Perhaps either or both humoral and cellular mucosal immunity could, in principle, provide specific protection vs. gut mucosal infection and subsequent systemic disease.

Like *Listeria*, the vast majority of accidentally or deliberately introduced commensal or opportunistically pathogenic bacteria cannot readily colonise and establish themselves in the gut of an immunocompetent host already carrying a fully-established, rather stable gut microflora. However, certain members of the gut microflora such as segmented filamentous bacteria (*Snel* et al., 1998) seem particularly effective at stimulating the development of various elements of the gut mucosal immune system and maintaining their "activated" state. It also seems likely that the ready development of oral tolerance requires the presence of the gut microflora (*Moreau* and *Corthier*, 1988). Thus, the question arises as to whether it would be possible to deliberately administer particular benign bacteria that also were sufficiently competitive to establish themselves in the microenvironment of the gut and be effective there at chronically activating the "normal" or "natural" mucosal immune system? Two promising candidates, *E. coli* 083 (*Lodinova-Zadniková* et al.,1991*)* and *Lactobacillus plantarum* (*Johansson* et al., 1993; *Adlerberth* et al., 1996) were discussed

by Tlaskalová (Institute of Microbiology, Praque) and Wold respectively.

Other micro-organisms in the gastrointestinal tract have immunomodulating effects on the host depending on the nature of the pathology associated with the interaction. For example, *V. cholerae* can attach to epithelial cells and release a toxin which initiates the physiological effects associated with the disease, but also mediates an adjuvant effect which enhances the immune response to antigens being processed in the Peyer's patch. For this reason, cholera toxin and a related toxin of enterotoxigenic *E. coli* have been used as adjuvants for mucosal vaccines. Enteric pathogens eliciting a more damaging or invasive interaction with the epithelium can elicit different mediated responses which may also contribute to an improved immune response. Bacterial invasion of intestinal epithelial cells initiates a pro-inflammatory response by these cells that activates the underlying natural immune system (*Eckmann* et al., 1995). IL-8, for example is secreted by colonic epithelial cells in response to invasive bacteria, but not to non-invasive bacteria. In fact, IL-8 secretion normally induced by *S. dublin* is blocked in the presence of cytochalasin D, a drug that prevents bacterial invasion, but not attachment. This suggests that bacterial entry is required to stimulate IL-8 synthesis, and that neither bacterial attachment nor the presence of bacterial LPS causes IL-8 secretion by the epithelial cells (*Eckmann* et al., 1993).

The discussion moved to consideration of whether gut microbes (i.e., opportunistic pathogens, commensals) that lacked expression of specific molecules facilitating uptake either translocated randomly at a low frequency proportional to their density in the lumen, or translocated in a "gated" fashion that could be regulated by other external stimuli. Influences on the quality of the mucus stratum and the integrity of the epithelial cell layer - perhaps affected by enteric viruses, could indirectly alter frequency of bacterial translocation. Presently, we also do not know whether the rate of pinocytosis by M-cells can be externally regulated.

Claesson's presentation focused discussion on whether particular gut microorganisms or food antigens could play a role in the provocation or exacerbation of bowel lesions, especially when the host has a clearly dysregulated immune system. Certain subsets of $CD4^+$ T cells, when transferred to conventionally reared SCID mice, result in a version of IBD. The process is accompanied by a striking polyclonal expansion, cell turnover and accumulation of T cells in the GALT compartment of the recipient. At this stage the cells exhibit a typical mucosa-seeking, activated, memory/effector CD45RBlow $CD4^+$ phenotype. They induce severe inflammatory lesions of the colonic mucosa, leading to death of the animals. Apparently, under GF conditions, little expansion of T cells and no development of IBD results (*Kushnir* and *Cebra*, unpublished observations). In this model, the development of histopathology depends on a particular subset of T cells, influenced by the presence of gut microbes. Helena Tlaskalová and her co-workers presented another model of IBD, especially of celiac disease, in GF neonatal rats or conventionally-reared athymic mice (*nu/nu*) fed gliaden. A correlate of their development of bowel lesions is the appearance of anti-gliadin antibodies, cross-reactive with cytoplasmic elements in enterocytes. Apparently, some sort of molecular mimicry results in an autoimmune phenomenon (*Tuckova* et al., 1995).

Presentations by Urban and Lee focused on pathogen/host interactions, particularly those which indicated some direction - or "misdirection" - of the balance between the host's Th1 vs. Th2 responses and their characteristic cy-

tokines. The outcome of nematode infections caused by *H. polygirus* and *N. braziliensis* appears to be dependent on IL-4 production and can be manipulated via genetic "knock-out" or use of MAbs against IL-4 or IL-4R. There is some indication that IL-4R/IL-13 interactions, even in SCID mice can benefit the host and lead to control of worm burden. Vaccines based on recombinant DNA technology and combining expression of both particular cytokines and protective antigens may permit external distortion of the host's response to favour the most protective/therapeutic modes against a particular pathogen (see *Ramsay*, below). In contrast to infection by these worms, *Helicobacter* appears to distort a host's response in favour of Th1 predominance. Combination antibiotic therapy can be therapeutic, but in some animal models, a vaccine combining *Helicobacter* antigens and cholera toxin can also be therapeutic against gastritis and severe stomach ulcers. Generally, it has been difficult to totally eradicate *H. pylori* from the human host. Discussion initiated by Paul Simon (Neose Technologies, Inc.) concerned the relative roles of attached vs. free *Helicobacter* in the gastric mucosa. Use of polymeric sialoglycoconjugates, administered orally, to interfere with *Helicobacter* attachment, was suggested to complement antibiotic and vaccine therapy.

The presentations by Baqar and Ramsay continued the theme of combining microbial products and cytokines with protective antigens to enhance protective immune responses, particularly those benefiting from a mucosal component. Scarcely 20 years ago, *Campylobacter jejuni* was first recognised as an enteric pathogen of humans, yet today it is known as one of the major causes of diarrhoeal disease and is a frequent cause of Guillain-Barré syndrome. Present promising treatments are suggested by findings that IL-2, IL-4, IL-5 and IL-6 given orally, enhance clearance of *C. jejuni* from infected mice. Perhaps of more practical value were findings that IL-2 given orally with an inactivated whole cell *Campylobacter* vaccine, and CT or LT (*E. coli* heat labile exotoxin) given with the vaccine markedly enhance the gut humoral response and protected mice against oral challenge. Presently, a trypsin-resistant "variant" of LT, with decreased toxicity, seems to be the best adjuvant candidate. Ramsay discussed strategies for enhancing the mucosal immune responses in respiratory tissue and at other mucosal sites including the gut. Recombinant vaccinia virus (rVV), recombinant fowl pox virus (rFPV) and plasmids, each prepared to encode protective antigens and a CK IL-4, IL-5, or IL-6, seemed effective at enhancing the antibody response. Incorporation of the coding sequences for IL-12 showed promise in depressing asthmatic responses in the lungs. He also showed that a consecutive immunisation strategy involving intranasal priming by DNA vaccination and boosting with poxvirus vectors encoding the same vaccine antigens elicited enhanced mucosal responses both locally and at distant sites, particularly in the gut, whilst neither vector alone gave significant levels of specific intestinal antibodies.

The last two presentations, by Hanson and Björksten, concerned various aspects of food allergies, the modes by which we react to ingested antigens, the role of nutrition in influencing our full immune potential and our general well being and possible mechanisms by which previously acquired, suckled maternal antibodies may influence immune responsiveness of neonates.

Discussion centred around the enigmas raised by Björkstén:

1) that food allergies have rapidly increased in incidence in modern times;
2) that tobacco smoking can markedly increase respiratory allergies; and

3) that little correlation can be found between incidence of atopy and general air pollution in middle and eastern Europe.

Again, Th1 vs. Th2 balances and imbalances were suggested as determining the likelihood of food allergies: a predominant Th1 pattern of responsiveness appears to favour suppression of gut atopy. It was suggested that correlates of propensity towards food allergies should be sought in distribution of gut commensal bacterial species during neonatal life. One of the obvious deficiencies in our ability to better understand the mechanisms of development of food allergies is the lack of meaningful laboratory animal models for this set of maladies. Hanson offered the first new animal model for many years. If adult rats are colonised with *E. coli* 06:K13, carrying a plasmid that expresses the gene for OVA, the hosts develop an apparent mucosal and systemic immune response against OVA. Subsequent feeding of these sensitised rats with OVA leads to diarrhoea and to the appearance of gut mast cells coated with IgE.

Hanson next stressed the effects of under-nutrition on attenuating normal development and depressing responsiveness and normal functioning of many elements of the immune system. He emphasised in discussions that:

1) comprehensive treatment of children in impoverished regions required not only food-supplementation, but also countering and resolving the many infections to which undernourished children are susceptible; and
2) sub-clinical diet deficiencies, such as for vitamin A or essential fatty acids, could have severe consequences for susceptibility to infections if left undetected during early development.

Finally, the importance of breast feeding in providing passive immunisation and antibody specificities appropriate for the various opportunistic and frank pathogens in the neonate's environment was stressed. Hanson supported a more indirect benefit, namely that maternal antibodies against protective antigens may provide "idiotypes" to prime the neonate and facilitate active immunisation in advance of encounters with the relevant pathogens. Van der Waaij discussed this concept at some length: It is generally accepted that the first steps in the ontogeny of the immune system involve learning of self-nonself discrimination. This may be controlled from the "inside" through the *idiotype network* which produces so-called "natural" antibodies. These "natural" antibodies have been characterised from hybridomas derived from unstimulated lymphocytes of new-born mice. It turned out that many of these antibodies were directed against self-antigens and microbial determinants and were, in a large number of cases, multispecific. In addition, these antibodies appear highly connected through idiotype-anti-idiotype (Id-anti-Id) interactions. This type of recognition might very well explain how the Id-network might be broken in ontogeny:

1) Providing initial recognition signals to the T-cell compartment. This may begin to occur in ontogeny, leading later to the control of the newly emerging B-cell repertoire. Later on, whenever new non-connective monospecific B-cells emerge, all cells expressing one or the other of the original V_H and V_L genes would receive an amplification signal from the complementary Th cells. This would favour the amplification of quite a broad B-cell population. These "secondary type immature B-cells" would not be directly connected to the polyspecific Id-network through classical Id-anti-Id interactions, but indirectly connected to it through "idio-educated" T-cells; and
2) Maternal IgG (for example, directed

against a pathogen) which passes the placenta in a concentration sufficient to destroy the B-cells expressing the corresponding Id's (internal image and its antibodies). Absence of the primary B-cell clone would enable the foetus/new-born to respond after birth to the pathogen directly with Th induced IgG antibody response.

A brief review of the ontogeny of the "primary immune system" followed in a general discussion. Interactions of (high affinity) IgG antibodies produced in the dam to pathogens with homologous idiotypes on IgM of the primary network may help to understand the findings. In the foetus, maternal IgG may have destroyed the corresponding Id and anti-Id in primary B cell clones. This may have caused a T-cell dependent B-cell response to the antigen(s) involved in the foetus/new-born. After birth and gut colonisation (predominantly with maternal bacteria) and translocation, production of IgG to these translocating bacteria may have led to inflammatory responses in the submucosa (instead of a silent non-inflammatory clearance). An IgG response with complement activation/inflammation at the site, could also explain the histological findings. A comparable sequence of events, although less dramatic, may possibly occur in IBD patients. These patients may lack the capacity to respond to a number of "normal intestinal flora components" with clearance upon translocating of these bacteria in a non-inflammatory way. This could come into expression in cases where the innate immune system fails (for example due to antigen overloading of the capacity).

It was clear in this symposium, that the potential for achieving human benefit from learning to better immunomodulate the gastrointestinal tract is great. The task to understand and apply the immunomodulating process presents a challenge. The gastrointestinal tract, it must be remembered, must function in concert with other organs of the body. Neuro-endocrine factors play a role in intestinal immune function and it must be supposed that the liver is also an important part to this integrated system. While this seminar addressed a variety of factors germane to gastrointestinal immunomodulation, it is apparent that much further work and many more seminars will be required to more fully probe this fascinating and critically important subject.

LITERATURE

Adlerberth, I., Ahrne, S., Johansson, M.L., Molin, G., Hanson, L.A., and Wold, A.E.: A mannose-specific adherence mechanism in Lactobacillus plantarum conferring binding to the human colonic cell line HT-29. Appl. Environ. Microbiol. 62: 2244-2251 (1996).

Bregenholt, S., Delbro, D., and Claesson, M.H.: T-cell transfer and cytokine/TCR gene deletion models in the study of inflammatory bowel disease. APMIS 105: 655-662 (1997).

Bry, L., Falk, P.G., Midtvedt, T., and Gordon, J.I.: A model of host-microbial interactions in an open mammalian ecosystem. Science 273: 1380-1383 (1996).

Dahlgren, U.I., Wold, A.E., Hanson, L.A., and Midtvedt, T.: Expression of a dietary protein in E. coli renders it strongly antigenic to gut lymphoid tissue. Immunology 73: 394-397 (1991).

Donnenberg, M.S., Kaper, J.B., and Finlay, B.B.: Interactions between enteropathogenic *Escherichia coli* and host epithelial cells. Trends Microbiol. 5: 109-114 (1997).

Eckmann, L., Kagnoff, M.F., and Fierer, J.: Epithelial cells secrete the chemokine interleukin-8 in response to bacterial entry. Infect. Immun. 61: 4569-4574 (1993).

Eckmann, L., Kagnoff, M.F., and Fierer, J.: Intestinal epithelial cells as watchdogs for

the natural immune system. Trends Microbiol. 3: 118-120 (1995).

Johansson, M.L., Molin, G., Jeppsson, B., Nobaek, S., Ahrne, S., and Bengmark, S.: Administration of different *Lactobacillus* strains in fermented oatmeal soup: *In vivo* colonization of human intestinal mucosa and effect on the indigenous flora. Appl. Environ. Microbiol. 59: 15-20 (1993).

Lodinova-Zadniková, R., Slavikova, M., Tlaskalova-Hogenova, H., Adlerberth, I., Hanson, L.A., Wold, A., Carlsson, B., Svanborg, C., and Mellander, L.: The antibody response in breast-fed and non-breast-fed infants after artificial colonization of the intestine with *Escherichia coli* O83. Pediatr. Res. 29: 396-399 (1991).

Moreau, M.C. and Corthier, G.: Effect of the gastrointestinal microflora on induction and maintenance of oral tolerance to ovalbumin in C3H/HeJ mice. Infect. Immun. 56: 2766-2768 (1988).

Kerneis, S., Bogdanova, A., Kraehenbuhl, J.P., Pringault, E.: Conversion by Peyer's patch lymphocytes of human enterocytes into M cells that transport bacteria. Science 277: 949-952 (1997).

Reimann, J., Rudolphi, A., and Claesson, M.H.: Novel experimental approaches in the study of the immunopathology in inflammatory bowel disease. J. Mol. Med. 73: 133-140 (1995).

Shroff, K.E., Meslin, K., and Cebra, J.J.: Commensal enteric bacteria engender a self-limiting humoral mucosal immune response while permanently colonizing the gut. Infect. Immun. 63: 3904-3913 (1995).

Snel, J., Hermsen, C.C., Smits, H.J., Bos, N.A., Eling, W.M.C., Cebra, J.J., and Heidt, P.J.: Interactions between gut-associated lymphoid tissue and colonization levels of indigenous, segmented filamentous bacteria in the small intestine of mice. Can. J. Microbiol., in press (1998).

Tuckova, L., Tlaskalova-Hogenova, H., Farre, M.A., Karska, K., Rossmann, P., Kolinska, J., and Kocna, P.: Molecular mimicry as a possible cause of autoimmune reactions in celiac disease? Antibodies to gliadin cross-react with epitopes on enterocytes. Clin. Immunol. Immunopathol. 74: 170-176 (1995).

Wold, A.E,, Dahlgren, U.I., Hanson, L.A., Mattsby-Baltzer, I., and Midvetdt, T.: Difference between bacterial and food antigens in mucosal immunogenicity. Infect. Immun. 57: 2666-2673 (1989).