

## **Host-Microbial Interactions: CCFA Strategy**

### **“Define the Bacterial-Epithelial and Bacterial-Immune Interactions”**

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#### **Introduction**

The luminal microbial ecosystem is a highly complex community of primarily bacterial microorganisms that communicates extensively both with itself and the host. This microbial community is likely to have major influences on the host including effects upon nutrient absorption, cancer, inflammation, host metabolism, barrier function and gut function (neuromotor, immunologic and vascular), among others. The immunologic effects are perhaps most pertinent. Microbes are continuously interacting with the host through direct interactions with epithelial and subepithelial components of the gut associated lymphoid tissue (GALT) both within the lumen and after translocation and indirectly through the production of a variety of secreted factors which interact with both innate and adaptive components of the GALT. The response of the host to these microbial factors is represented by a normal state of so-called “physiologic (or regulated) inflammation” that is manifest immunologically by various degrees of immunologic ignorance, tolerance and immunity (both humoral and cellular) which is a composite of both microbial regulation of host immunity and presumably host regulation of the microbial ecology. This cross-talk has major developmental effects upon the host that are spatially and temporally related and consist, among others, of effects on the structure, content and function of the GALT including major effects on epithelial cells, antigen presenting cells and lymphocytes (T and B cells). The specific role that bacterial translocation, specific microbial components, breast milk and other factors have on this process is unknown. However, it is clear that microbes have major effects on gut morphology, motility, epithelial differentiation, the immune system (innate and adaptive), metabolism, barrier function and detoxification machinery. How specific microbes (probiotics, prebiotics, microbiotics) differ in this regard, remains unknown.

This relationship between the host and microbes is long-term with its onset early in life such that in large part the community of luminal microbes must be immunologically perceived as self as if it were representative of an organ system unto itself. This is presumably then the rationale for development of potent regulatory systems as manifest by the phenomenon of oral tolerance in general and to microbes in particular. This then creates a major paradox for the host; the need to be both tolerant to the normal luminal ecology but at the same time be able to respond to the pathogens that intervene intermittently. Understanding therefore how microbes and microbial antigens are sampled, the load of antigens regulated for presentation to the

immune system and the antigens deserved of recognition and how they are linked to the development and regulation of physiologic inflammation is highly important. Similarly, the specific details of how microbes normally regulate the homeostasis of adaptive immune components of the T cell compartments (intraepithelial lymphocytes and lamina propria lymphocytes including their recruitment, turnover and apoptosis), immune tone (Th1, Th2, Th3, Tr1), and innate immunity are of high interest. In a corollarily manner, the immune system may talk back to and regulate commensalism.

A key cell type that intervenes itself between the luminal microbial community and the immune system and which is in a way a significant part of the mucosal immune system is the epithelial cell. Intestinal epithelial cells are in a continuous state of response to the normal microbial ecology and through their products (e.g. cryptins from Paneth cells) presumably regulate the composition of this community. In this way, the epithelium is considered as a “microbial sensor”. Defining the range of molecules produced by epithelia in response to bacteria and bacterial products and the mechanism of how do pathogens and commensals differ in their ability to stimulate the epithelium and epithelial barrier function, the role of the epithelium in innate and adaptive functions and how far (distal) from the epithelium can these effects extend remain to be determined.

The mechanism by which bacteria also stimulate IBD remains unclear. In particular, how do microbes (and microbial constituents) drive Th1 and Th2 cytokines, the excess of which cause IBD? What is the role of genetics and genetic modifiers on these mechanisms, the role of bacteria during IBD induction or IBD maintenance, the role of bacterially-driven epitope spreading to self-antigens, if any, the effects of colitis on the composition of bacteria, the regulatory response to bacteria induced relative to aggressor responses, the role of specific populations of microbes and the effects of bacteria on barrier function among others remain to be defined and were the elements of the topic of discussion for this committee.

### **Inflammatory Bowel Disease**

At the heart of the pathogenesis of Inflammatory Bowel Disease is the possibility that IBD represents an aberrant response to normal gut microbiota due to immunoregulatory defects. Remaining to be defined is whether this aberrant response to the normal gut microbiota is associated with bystander damage of host tissues through the triggering of autoimmunity. Clearly, experimental models of colitis have taught that abnormal balance between aggressor and regulatory T cells would appear to be at the heart of these disorders. Although evidence for these ideas is strongly supported by data in animal models, the data in humans are less clear and are, at best, only inferential. Thus, a major goal would be to identify bacterial antigen-reactive T cells that both promote and regulate the disease and corollarily to define the bacterial antigens to which these T cells are responsive and, downstream, to investigate the manner in which these bacterial antigens and/or macromolecules are associated with immune dysregulation in the IBD-susceptible host. In addition, understanding the components of the commensal anaerobic and aerobic microbiota and the components that are associated with these responses is of high interest. The response to the luminal microbiota is highly complex even in normal circumstances. The response to the normal commensals can be one of immunologic ignorance, tolerance and/or active immunity. Clearly, each microorganism has a specific relationship with the host that is further modified by the overall microbiological community. Given the large number of species of microorganisms that are present in the gut, the overall integration of these responses is no doubt going to be extremely complex. However, a reductionistic *in vivo* approach is probably the clearest and best bet in gaining an understanding of such responses. In

particular, the utilization of germ-free animals that are monocolonized with one or more commensals to evaluate the humoral and cellular responses to these organisms as well as the effects of these microorganisms on the structure and function of the host, in general, is of clear utility. It is thus important to determine which microorganisms have which effects. Whereas some microorganisms may be the provocateurs of the initial response, others may be the final targets. As part of this exercise, it is important to better understand the mechanisms of oral tolerance, in general, and to the microbiota, in particular, and how this response to commensals may alter the response to other ancillary antigens such as dietary antigens, for example. Moreover, as we better understand the immunologic responses to microorganisms, we can begin to categorize these microorganisms into immunologic classes, which presently are limited to the concepts of probiotics, prebiotics and microbiotics. Concepts of probiotics and mechanisms by which probiotics function is not broad enough and needs to be enhanced. As part of these exercises, it is important to begin to build different and better models to understand microbial-host interactions. Other models beyond the mouse may very well include the pig and mini-pig, in particular, which are highly flexible models whose mucosal immune system is highly relevant to human. More gnotobiotic facilities need to be perpetuated around the country to facilitate such types of research. Currently, there has been a dramatic diminution in the number of such facilities. The importance of developing other models is to begin to try to provide more concrete associations with the human given the potential species differences between mouse and human. As we begin to consider the pathogenesis of the disease and the possibility that there is a distinct provocateur, we need to consider the immunologic definition of what a true commensal really is. Clearly, a major question is what triggers the initial event and, subsequently, ongoing lack of regulation. Is it in fact a specific organism? Development of better models to pinpoint initial triggers is thus also needed.

At the core of the problem is gaining a better understanding of the micro-ecology. Given that it is likely that it will take decades to truly gain a handle on this micro ecology, in the meantime, efforts must be made to try to gain a better understanding of some common features of microorganisms that either stimulate and/or perpetuate the disease. Do these organisms have to have invasive characteristics? Do they have to stimulate a particular flavor of immune response, such as a Th1 or a Th2 bias, for example? A true definition of the normal microbiota and those that are natural stimulators of the physiologic inflammation is clearly needed. Moreover, the principles of defining microorganisms in mice may or may not translate to humans. Thus, a better characterization of human micro ecology is clearly needed. As such, it has become very clear that old-fashioned microbiology does not work to this end and better techniques for doing so need to be developed.

As we consider the relationship between the immune system and the micro-ecology, it must still be considered whether IBD is an atypical infectious disease (i.e., a dysregulated mucosal immune response to a component or components of the normal microbiota or an immunologic response to luminal dysbiosis or alteration of the overall gut ecology resulting in microbial imbalance). It also still remains a possibility that IBD is a distinct pathogen and maintaining the interest of the infectious disease community remains important. The fact that antibiotics have some utility in this disease is potentially in support of the latter.

There are a number of ancillary immunologic questions that are clinically relevant and need to be addressed. What is the role of autoimmunity in this disease and how does this potentially explain extraintestinal manifestations of this disease such as sclerosing cholangitis,

for example? What underlies the long symptom-free intervals and the skip lesions for these diseases? Do these have a microbiologic/immunologic basis?

It remains to be determined whether there is a specific provocation or a general aberrant and/or generic response to the microbiota? Is it strictly an immunologic system problem and/or luminal dysbiosis? Is there some specific initiating event such as a change in the microflora? As we begin to consider the latter possibility, that it is related to the microbiota, *per se*, is there a class of microbes that cause this problem, such as their ability to enter intracellular locations and maintain this niche? What is the role of non-bacterial stimuli such as viral infections, in particular, given the Western propensity of these disorders? Is there a group of provocateurs rather than a single provocation, in this regard? Or is IBD simply an overall breakdown in oral tolerance to the microbiota?

We need to look forward to taking advantage of phenomenological observations in humans that may provide important clues to disease pathogenesis. For example, in Slovakia, there is a 40-year experience in the Children's Hospital in Prague showing that early colonization with a particular strain of *E. coli* protects the neonatal infant from other pathogens and, as a consequence of early colonization, mitigates against future allergic responses.

Another important group of related questions is how does the normal host handle commensals so well? The role of barrier function is likely to be important in this regard and understanding how luminal contents gain access across the epithelium and how barrier function relates to disease manifestations in terms of both initiation and perpetuation of disease is clearly important. Finding the endogenous pathways that promote barrier function and the predisposing factors that inhibit barrier function are not only of pathophysiologic interest but might provide early markers for disease initiation. In this regard, it is clear that barrier function is constantly disrupted without pathophysiologic consequence. Defining normal and abnormal barrier disruption is thus of high interest. Taking a broader view of barrier function is also very important, including the biophysical characteristics of the epithelium, such as cell surface glycosylation, the importance of mechanisms by which the innate and immunologic factors of the epithelium and subepithelial mucosal lymphoid elements regulate both barrier and luminal microbiota, and the manner in which bacteria regulate the production of these factors.

Given that IBD would appear to be, at least in part, a dysregulated immunologic response that is dysregulated to some component of the microbiota, gaining an understanding not only of the microbial triggers, *per se*, but also the antigenic components associated with these microbial triggers is extremely important. This is limited not only to microbial triggers but also those antigens which also presumably stimulate a regulatory response. As we consider the solutions to these questions, it is important to remain focused on the implications of this information. What will we do with this information? Will this be utilized for development of a vaccine, for example? What is the nature of the target antigen? Although it is possible that it is polypeptide, it may not be, and other possible classes of macromolecules could include nucleic acids, glycolipids and/or others need to be considered. With regard to the latter, what methodological approaches will be necessary to make these definitions? Moreover, assuming that there is a bacterially-derived macromolecule, it will be important to conclusively define non-cross-reactive epitopes that are not present in the human genome and, as a result, not part of a self determinant as we consider diagnosis and therapies. Importantly, it is possible that the microbial antigen or antigens responsible for driving aggression and/or regulation may be stimulatory of innate pattern recognition receptors and not necessarily an adaptive immune response. The end points for definition of immunologic responsiveness must be clearly defined as a T-cell response, *per*

*se*, does not mean anything given that responses occur to normal components of the commensals without evidence of immunopathologic injury. In addition, the immunologic responses that are identified must be associated with the mucosal-associated lymphoid tissues as definitions of systemic immunity do not necessarily translate into mucosal immunity.

Understanding how epithelia interact with specific bacteria, both pathogenic and non-pathogenic, is extremely important. In addition, it is important to determine whether there are general classes of target molecules on the cell surface of the epithelia or molecular determinants from the microbes that are responsible for these interactions. Determining the regional differences in not only the microbial ecology but also the role of the epithelium in this regard needs to be defined. It is generally assumed that the microbiota is stable over time in a given individual and that the endogenous microbiota does not vary very much. Is this also the case for the host immune system? It is important to determine which responses to microbes are associated with pathogenicity and which are not. For example, the host may normally respond to commensals with an immune response that is of no consequence to the luminal microbe(s) such as the observation that IgA coating of microbes in the gut lumen may have no effect upon colonization. It is also important to understand both the manner in which the microbial flora defines the structure and function of the GALT and how the response of the GALT defines, in this regard, the microbial ecology. What are the cell surface molecules in the epithelia, such as lectins for example, that ultimately participate in transmission of these responses?

## **Summary of Concepts**

### Model Systems:

- Animal models will be essential to defining a number of these concepts. In particular, germ-free animal models that are colonized with known microbiota (gnotobiotic) mice, rats or pigs (mini-pigs) to help define the structure and function of the GALT and the immunologic function of these tissues will be extremely important.
- Approaches as used by certain investigators to define these effects that employ animal models together with DNA microarrays are potentially exciting in this regard.
- Such models will also be beneficial for clarifying the development and function of regulatory T cells, understanding of probiotics as well as an ability to examine the response to cocktails of microbiologic strains.
- Working with a limited number of bacterial strains that are corollarily extremely well characterized, including an access to the complete genome of the most interesting microorganisms, may be particularly important.
- On the functional side, utilizing very well characterized *in vitro* assays including working with anaerobic conditions that are more physiologically relevant to the environment within which anaerobic bacteria reside, will be extremely important.
- Functional readouts must focus on mucosa.
- Attempts at extrapolating results into humans and, in particular, the response of humans to bacterial microorganisms will be important.
- Focus on the earliest immunologic events, such as through investigations of aphthoid ulcers, and at the epithelial cell surface in humans and the temporal responses to bacteria in animal model systems is extremely important.

### Disease Initiation:

- It is important to determine whether initiation of IBD is due to an infectious pathogen, a response to luminal dysbiosis, a specific or class of provocateurs, a loss of (T cell) control versus any or all commensals (an overall loss of immune regulation) and/or is aided by 'insults' (e.g. DSS and barrier disruption in animal models or rotavirus in humans).
- Gaining an understanding of the luminal factors (bacterial and host) that initiate and/or perpetuate disease (including antigens derived from the bacteria, *vide infra*) from the host that perpetuate disease and animal model systems to test these, is extremely important.
- Understanding endogenous host-derived antimicrobial substances and the way in which they maintain balance of the microbial ecology as well as potentially to promote dysregulation in the microbial ecology is extremely important.
- Gaining a handle on the initial provocateur, whether it is bacterial or non-bacterial, including the possibility that it is a pathogen or an overgrown commensal, is extremely important. Moreover, the specific immunologic event that is induced by this microorganism and the characteristics of this factor need to be elucidated.
- What is the molecular epidemiology of the mucosal ecosystem and how does this differ between individuals with IBD and normals? By molecular epidemiology what are the factors that determine this ecosystem and how does it relate to the initiation of IBD?

### Markers:

- Can markers be identified that are indicative of a propensity to develop IBD or predictive of near-term development of IBD?
- Can bacterially specific immunologic markers be developed to monitor and predict disease activity?

### Epithelium:

- What are the range of interactions between microbes and epithelial cells that ultimately define both disease initiation and perpetuation?
- Defining the microbial flora and gaining an appreciation of the factors that are either contained by the microbes and/or secreted by the microbes that regulate the immune response and barrier function is extremely important.
- What are the distinguishing features of pathogens versus nonpathogens (commensals) and their relationship to the epithelium?
- With respect to understanding epithelial behavior and response to microbial organisms, generation of primary epithelial cell lines would be an important advance as they would represent better tools with which to examine these interactions.

### Tolerance/Regulation:

- It is important to consider not only how do microbes stimulate disease but also how do they stimulate physiologic inflammation, tolerance and also wound healing repair and recovery.
- Gaining a better understanding of mucosal immunoregulation (both innate and adaptive) is extremely important. Understanding this will allow for gauging the regulatory mechanisms with more facility. For example, Toll receptors on dendritic cells suppress the function of regulatory T cells thus theoretically promoting autoimmunity.

- Beginning to gain an understanding into the genetic regulation of the host in influencing both the components of the microbial milieu as well as the anti-microbial response needs to be considered.
- If probiotics or antibiotic/benign microbial replacement therapy seems worth trying based upon clinical trials, then we should try to determine the basis for efficacy of any promising probiont.
- We should aim to better understand the basis/specificities of oral tolerance to particular commensal microbes (if it exists) and how gut microbes may mediate/facilitate oral tolerance versus other microbes/dietary antigens -- is there a bystander effect?

#### Antigen Specificity of the Response:

- Discriminating whether there is a provocateur or target, whether there is an anti-self response, whether the microbiota induces disease or becomes a target of disease, or if it is a typical infectious disease are all open-ended questions.
- Defining whether the microbiota is different in disease, that is a dysbiosis that stimulates disease, or is a consequence of disease, is extremely important.
- Cloning bacterially responsive and regulatory T cells from mice and humans to define antigen specificity and defining their cognate antigens should be a major goal.
- Distinguishing between a primary bacterial pathogenesis or an aberrant response either directed at the microbial flora or the microbial flora as a bystander needs to be better defined.
- In animal models -- and the human condition -- we should try to determine whether T cells are mostly specific for a microbial provocateur or reactive with self/altered self, i.e., is IBD an auto-immune disease that is driven or promoted by bacteria? The class II associations in rats and humans do suggest a role for CD4+ T cells.
- We should try to identify/distinguish specificity of initiation ('turn-on') versus specificity at the terminal effector stage versus any specificity of controlling (T cell) elements. Note that it has been shown in one model that OVA-peptide specific Tr1 cells can ameliorate development of IBD in the CD45RB<sup>hi</sup> model.