The future of mucosal immunology: studying an integrated system-wide organ

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Over the next 10 years, it will be important to shift the focus of mucosal immunology research to make further advances. Examination of the mucosal immune system as a global organ, rather than as a group of individual components, will identify and characterize relationships between mucosal sites.

The term 'common mucosal immunological system' was coined by John Bienenstock nearly 40 years ago. He suggested the concept when the bronchus-associated lymphoid tissues his group described were found to be similar to those in the gastrointestinal tract. Ironically, appreciation of the importance of this term is only now truly beginning. Since then, the mucosal immune system has received a great deal of attention and is described as an integrated network of tissues, cells and effector molecules that protect the host from infection and environmental insult at mucous membrane surfaces. Mucosal surfaces are immunologically unique, as they act as the primary interface between the host and the physical environment yet also have key barrier functions. It has become increasingly evident that mucosal surfaces are also the main sites of interaction between the host and its associated commensal microbial community. In the past 40 years, the field of mucosal immunology research has exploded and understanding of this key component of the immune system has flourished. Many important findings have come from this research and have aided in the understanding of immune deficiencies and associated diseases and facilitated the design of effective vaccines. The next decade will be important in determining how the knowledge gained is synthesized and which directions future studies take.

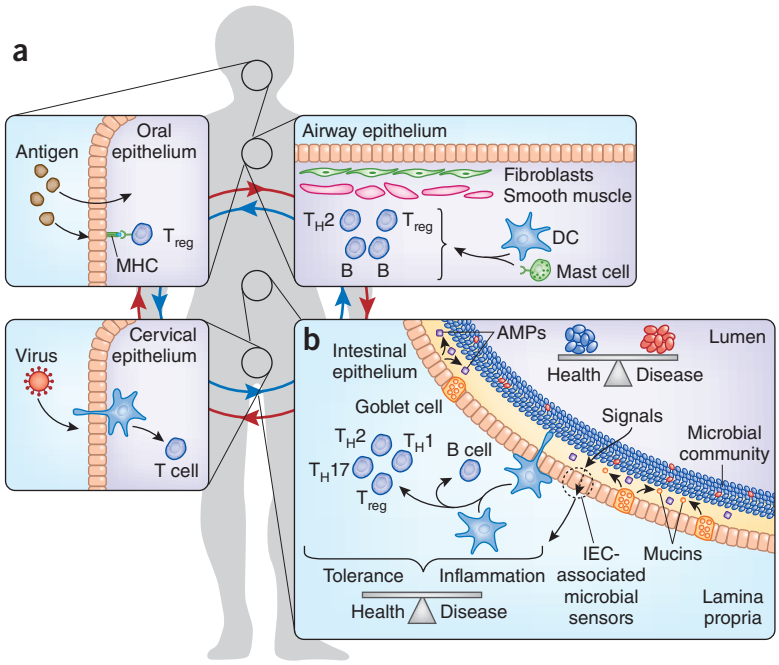

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Figure 1 The mucosal immunological system. (**a**) Recent advances suggest that mucosal sites function together as a system-wide organ. Various mucosal sites throughout the body act as an interface between the physical environment (food, airborne, viral and commensal antigens) and host mucosal defenses. (**b**) The intestinal mucosal interface, a complex system that must integrate interactions among the microbiota, mucus layer, associated protective compounds, epithelial cells and underlying immune cells of the lamina propria. Notably, it has become clear that both the state of the microbial community and underlying immune cells contribute to the health or disease of the host. T, T cell; T_{reg}, regulatory T cell; MHC, major histocompatibility complex; B, B cell; DC, dendritic cell; IEC, intestinal epithelial cell; AMP, antimicrobial peptide.

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A global organ

Much of the research undertaken in the past several decades on the mucosal immune system has focused on specific individual components of the system. For example, the mechanisms responsible for initiating immune responses to invading pathogens in the gastrointestinal system have been characterized. Similarly, the means by which the respiratory tract deals with bacterial and viral infections have also been established. However, the mucosal immune

system has yet to be examined from a holistic viewpoint as a global organ (**Fig. 1**). Although much knowledge has been derived from study of the individual components of the mucosal immune system, future research should examine how the different components affect each other and how crosstalk is achieved between individual components and, more importantly, between various mucosal sites. As many features are shared by distal mucosal compartments, it is likely that these aspects may have compelling roles in mucosal immunity, and there could be unappreciated levels of communication between mucosal compartments.

There is ample evidence suggesting that the mucosal immune system is a system-wide organ. Studies have demonstrated that stimulation in one compartment of the mucosal immune system can lead to changes in distal areas. For example, intranasal immunization results in vaginal protection against genital infection with herpes simplex virus type 2 (ref. 1). Clinical studies of patients infected with human immunodeficiency virus have shown that high concentrations of human immunodeficiency virus–specific immunoglobulin A are found in various mucosal secretions, including the vaginal secretions, nasal washes, saliva and endocervical secretions[2]. Furthermore, the use of antibiotics in neonates has been associated with a greater risk of developing asthma[3], which suggests that alterations in the microflora of the gut can have an effect on the lungs and highlights the potential for an undetermined link between mucosal immune compartments. Collectively, such studies suggest that the mucosal immune system is actually one large interconnected network and that the individual components are very efficient at sharing information distally.

Understanding the communication between mucosal sites is fundamental to the next phase of disease characterization and vaccine development. Appreciation of the mucosal immune system as a global organ will involve determining what factors link one area of the mucosal immune system to another and the intricacies of this communication. This aspect needs to be addressed sooner rather than later.

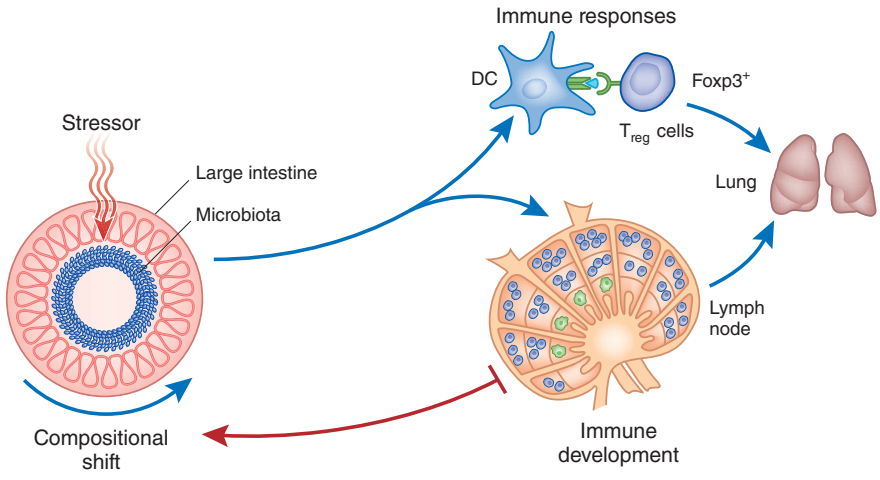

Figure 2 Reexamining the hygiene hypothesis. Investigations that take into consideration the cyclic relationship between the mucosal immune system and the microbial community make it possible to identify previously unknown mechanisms of disease onset. Changes in the microbiota could result in differences to downstream immune responses or, alternatively, immune development. Both of these aspects can affect immune mechanisms in distal mucosal sites, such as the lung, and result in asthma. Alternatively, deficiencies in the immune system can affect the microbiota composition, which can then again have an effect on distal mucosal sites and have a role in the development of atopic conditions.

The immune system and the microbiota

It is known that mammals contain millions of commensal bacteria, called the 'microbiota', but only recently has the importance and complexity of the microbiota been recognized, with a growing appreciation for its importance in mammalian health and disease. The microbiota form an organ system, one that is essential for nutrient acquisition, metabolism of indigestible compounds, defense against colonization by pathogens and development of intestinal architecture and the immune system[4,5]. Despite increasing knowledge in this field, important questions still remain unanswered: Given the diversity of the microbiota and the signals they can produce, what methods are used to interact with the host? Furthermore, how are these interactions monitored and controlled? Studies using knockout mice defective in mucus production, germ-free mice, and probiotics suggest that the mucus layer is a major mediator of interactions between epithelial cells and microbes and that its function is affected largely by the microbiota. The mucus layer in the large intestine consists of two stratified layers, composed mainly of the secreted mucin Muc2, which is heavily *O*-glycosylated with complex oligosaccharides[6], a characteristic that is crucial to its structure and function[7]. The inner layer has a dense composition and is devoid of commensal bacteria, whereas the outer layer is built as a loose matrix housing commensal bacteria[6]. Future studies will be crucial for further characterization of the mucus layer and its function in binding to and sequestering growth factors, differentiation factors and mediators of inflammation[7] (**Fig. 1b**). Stimuli that result in the production of these protective compounds secreted by epithelial cells, including cytokines, trefoil factors and antimicrobial peptides, are poorly understood but probably involve the microbiota (**Fig. 1b**). Furthermore, insults that result in a breach of the mucus layer may facilitate the release of these compounds; this possibly functions to both alert the host and control penetration of the microbiota to underlying tissues. The interaction of the microbiota with the mucus layer and the role of the microbiota in modulating the production of both the mucus layer and the biologically active molecules sequestered therein are intriguing concepts that require future clarification. The mucus layer is truly at the interface between the microbiota and host, which emphasizes its importance.

As there is a new appreciation of the importance of the microbiota, knowledge of the mucosal immune system and immune development must be reexamined. There has already been a great deal of research on the importance of the microbiota in immune development in the germ-free mouse model. These mice lack commensal microbes and have poorly developed mesenteric lymph nodes, Peyer's patches, cryptopatches and isolated lymphoid follicles, the four main structures that make up the gut-associated lymphoid tissue[8]. Studies have shown that colonization with a single bacterial species is able to reverse those defects[8]. Furthermore, various groups have demonstrated a role for the microbiota in intestinal epithelial homeostasis, angiogenesis and the development of the innate and adaptive immune systems of the gut[9–12]. Such findings suggest that the microbiota is influential in the management of immune development at mucosal sites. Future research will need to identify the members of the microbiota that affect the development of the immune system.

From the findings described above, the importance of the microbiota in immune development is evident, which suggests that

the methods by which experiments are executed may require reassessment. The commensal communities present at mucosal sites and how these communities may be affecting the immune response generated must now be taken into consideration. Research suggests that the immune system can also have a role in the generation and maintenance of stable microbial communities[13,14]. Thus, removal of host factors, such as in knockout mice, may result in alterations to the microbiota that can affect the response to other immune stimuli. Moreover, the microbiota of mice with identical genetic backgrounds can differ depending on the commercial supplier, and this may lead to downstream effects on immune responsiveness. The knowledge that much of the research in the past ignored the effect and role of the microbiota is truly daunting and frightening. Researchers must be cognizant of the variability inherent in research animal models, as changes in the microbiota affect the immune response and vice versa. This will at least begin to ensure that conclusions being presented are not skewed by simple differences in the microbiota.

Effect of recent findings

The next decade of mucosal immunology research should not only focus on examining the new concepts outlined above but also revisit established models, taking into consideration recent advances. For example, concepts such as the hygiene hypothesis and even tolerance may benefit from greater scrutiny.

The hygiene hypothesis has historically suggested that the development of allergies is dependent on exposure to antigens, although the exact mechanisms are not completely understood. However, reexamination of this hypothesis while taking into consideration the mucosal immune system as a global environment should determine what role sites such as the gut have in, for example, the development of asthma in the lungs. Furthermore, with the emergence of microbiota studies, the hygiene hypothesis can now be reassessed to understand whether certain components of the microbial community are more likely to cause or protect from asthma and allergies. Finally, assessing the importance of the microbiota in the immune response may afford an explanation of why changes in the gut microbiota can lead to the development of asthma and other allergic responses. It is possible that changes to the microbiota resulting from antibiotic treatments could result in changes to the immune response generated to allergens. However, changes in the microbiota composition may cause changes in the development of immune system components, and these deficiencies could result in the onset of atopic diseases, including allergies and asthma (**Fig. 2**).

Tolerance is an immune mechanism that could also benefit from reexamination, especially as the incidence of food allergies has increased greatly in the past two decades. 'Tolerance' refers to the process by which the immune system does not react to an antigen. Although the roles of antigen-presenting cells, in particular dendritic cells and regulatory T cells, have been described as being essential to the development of tolerance, questions still remain about the mechanisms involved. The greater prevalence of allergies could be due to changes in lifestyles (diet, sanitation and stress) that could be altering commensal microbe populations and hence immune responses. In fact, studies have shown that germ-free mice, which lack all commensal microbes, have a deficiency in regulatory T cells, which are important in the development of oral tolerance[15].

Conclusion

Together, the examples presented above illustrate the need to take into consideration the different components of the mucosal immune system in the process of understanding how this remarkable system functions. Treating the mucosal immune system as a global organ that affects immune development and has an intimate relationship with the microbiota and the environment may shed light on present models and at the same time broaden understanding of how the mucosal immune system operates. Furthermore, the concept of using the relationship with commensal flora as an advantage by designing therapies that exploit microbial counterparts is one that is slowly coming to the forefront. Many years have been spent ignoring the term 'common mucosal immunological system', and the idea that crosstalk between mucosal compartments is critical for mucosal immune functions is now finally beginning to be appreciated. Mucosal immunology has come a long way in the past 40 years, but there are many exciting avenues left to explore for the next decade and more.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

ACKNOWLEDGMENTS

We thank P. Ernst, L.C.M. Antunes, A. Bhavsar, M.A. Croxen, R.B. Ferriera, S.R. Shames and B. Willing for discussions and revisions. Supported by the Canadian Institutes of Health Research (B.B.F. and operating grants), the Crohn's and Colitis Foundation of Canada (M.W. and operating grants), the Howard Hughes Medical Institute (B.B.F. and operating grants), the Michael Smith Foundation for Health Research (N.G. and M.W.) and the University of British Columbia (B.B.F.).

1. Gallichan, W.S. *et al. J. Immunol.* **166**, 3451–3457 (2001).
2. Artenstein, A.W. *et al. J. Infect. Dis.* **175**, 265–271 (1997).
3. Sobko, T. *et al. Paediatr. Perinat. Epidemiol.* **24**, 88–92 (2010).
4. Round, J.L. & Mazmanian, S.K. *Nat. Rev. Immunol.* **5**, 313–323 (2009).
5. Pedron, T. & Sansonetti, P. *Cell Host Microbe* **3**, 344–347 (2008).
6. Johansson, M.E. *et al. Proc. Natl. Acad. Sci. USA* **105**, 15064–15069 (2008).
7. Hollingsworth, M.A. & Swanson, B.J. *Nat. Rev. Cancer* **4**, 45–60 (2004).
8. Mazmanian, S.K., Liu, C.H., Tzianabos, A.O. & Kasper, D.L. *Cell* **122**, 107–118 (2005).
9. Hooper, L.V. & Gordon, J.I. *Science* **292**, 1115–1118 (2001).
10. Cebra, J.J. *Am. J. Clin. Nutr.* **69**, 1046S–1051S (1999).
11. Ivanov, I.I. *et al. Cell Host Microbe* **4**, 337–349 (2008).
12. Rakoff-Nahoum, S., Paglino, J., Eslami-Varzaneh, F., Edberg, S. & Medzhitov, R. *Cell* **118**, 229–241 (2004).
13. Rawls, J.F., Mahowald, M.A., Ley, R.E. & Gordon, J.I. *Cell* **127**, 423–433 (2006).
14. Dethlefsen, L., Huse, S., Sogin, M.L. & Relman, D.A. *PLoS Biol.* **6**, e280 (2008).
15. Ishikawa, H. *et al. Clin. Exp. Immunol.* **153**, 127–135 (2008).