Conference Scene

Recent advancements in immunotherapy

Mucosal dendritic cell–epithelial cell cross-talk Penridge Suite, London, UK, 14–17 October 2011

Dendritic cells (DCs) mainly function to process/present antigens and thus initiate and shape the subsequent immune response. DCs are chiefly present within tissues in contact with the external environment and have a crucial role for maintaining immune homeostasis. Epithelial cells have been attracting considerable attention recently for they express a range of factors that influence the behavior of DCs. Organized by Euroscicon, this meeting focussed on integrating current knowledge and new paradigms surrounding the role of various DC subtypes and epithelial cells; their interaction with one another as well as with lymphocytes, microbial pathogens and commensal organisms. A wealth of experts currently researching this diverse field provided insight into these important interactions, their relevance to the medical setting and highlighted areas for future research.

The meeting was opened by chairpersons Kamal Ivory (Mucosal Immunology Group, Institute of Food Research, Norwich, UK) and Claudio Nicoletti (Norwich Research Park, UK). Their research interests include the study of immunological adaptations favoring healthy aging and, more recently, immune regulation in allergic disorders and the factors contributing to dendritic cell (DC) migration.

Intestinal DCs imprint a 'gut-homing' phenotype

Intestinal DCs constantly survey the luminal microenvironment, acting as 'sentries on the lookout,' acquiring antigens before presenting them to and activating naive T cells in secondary lymphoid tissues. DCs control whether or not an immune response occurs to a particular antigen and modulation of DCs by bacterial products may influence the subsequent outcome.

Andrew Stagg (Bart's and London School of Medicine and Dentistry, UK) presented key data regarding the ability of DCs to imprint a 'gut-tropic' phenotype on lymphocytes and explains how this property is somewhat dependent on the presence of retinoic acid (RA). The ability to activate naive CD4⁺ T cells seems to be restricted to CD103⁺ DCs; however, there appears to be fewer CD103⁺ DCs in inflammatory bowel disease (IBD), a feature that may be reversed with anti-TNF- α agents. In humans, the ability to generate RA is not restricted to the CD103⁺ DC population, although these cells seem to

possess the key enzymes required for the process, notably retinaldehyde dehydrogenase (ALDH1A). Additional factors within an inflamed colonic environment may enhance non-CD103⁺ DC-mediated imprinting, independent of ALDH1A activity. RA can also be produced by macrophage-like cells within the lamina propria and mesenteric lymph nodes. The role of intestinal epithelial cells (IECs) in this process is less clear. Work from Stagg's unit has also shown that colonic DCs have the ability to produce both IL-12 and IL-10 when cocultured with bacteria depending upon the nature of the antigenic stimulus. Given that IBD is probably caused by a dysregulated, cell-mediated immune response to intestinal flora, this supports a central role for DC–T-cell interactions and the development of IBD.

Oral mucosa & novel sights of vaccination

Despite having a microbe-rich environment, the oral mucosa is often falsely regarded as being quiescent with very little inflammatory activity. This concept would contradict its ability to respond to various antigenic stimuli associated with common oral diseases or the capacity to tolerate the vast quantity of material that passes through the oral cavity each day.

Lesley-Ann Bergmeier (Queen Mary's University of London, UK) gave an excellent overview of the distribution of lymphoid tissues within the various compartments of the mouth and provided an example of how *Porphyromonas gingivalis*

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Disclosure

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(an oral pathogen implicated in periodontal disease) may subvert normal DC function. This organism has recently been shown to induce migration of semimature and immunosuppressive DCs carrying *P. gingivalis* bound to DC-SIGN. As a result, periodontal lesions have an intense infiltrate of DC-SIGN⁺ cells and immature DCs express proinflammatory chemokine receptors. Ligation of DC-SIGN has also been shown to induce reverse migration of DCs from tissue to blood and this molecule may provide a link between periodontal and cardiovascular disease.

The second part of this talk revolved around discussions of how we may be able to manipulate the oral mucosa and create a novel sublingual route of vaccine delivery. In a Phase I/II clinical trial in patients with Beçhet's disease, Bergmeier's group were able to induce oral tolerization using a recombinant cholera-bound toxin linked to heat shock protein 70. Patients demonstrated a downregulated Th1 immune response associated with sustained clinical remission on withdrawal of immunosuppressive medication. This may be a future avenue of therapeutic intervention in autoimmune disease.

IECs & intraepithelial lymphocytes

The gastrointestinal tract is one of the largest lymphoid compartments found in mammals consisting of lymphoid follicles (Peyer's patches), lamina propria lymphocytes and intraepithelial lymphocytes (IELs). These areas can be further differentiated by anatomical location, cell phenotype and function. Lucie Abeler-Dörner (King's College, London, UK) gave a very insightful presentation on the crosstalk between IECs and IELs within the gut. The function of gut-derived IELs and interactions with surrounding epithelium are likely to be crucial to the fine-tuned balance between tolerance to harmless food antigens, immunity to gut-associated pathogens and overall intestinal immune surveillance. Intestinal IELs comprise TCR $\gamma\delta$ cells and TCR $\alpha\beta$ CD8 $\alpha\alpha$ or CD8⁺ CD4⁺ cells, which have been assigned innate-like immune functions and play key roles in the surveillance of stressed tissue. Unlike conventional T cells, these IELs may initiate an immune response rather

than simply being late effector cells. They are therefore important in elucidating the 'immunological information flow' within the gut. In the past, it has been notoriously difficult to study IELs *ex vivo*. Using a novel culture system that allows the expansion of IELs *in vitro*, Abeler-Dörner and colleagues have been able to elucidate a pathway by which IECs secrete proinflammatory cytokines in response to IEL activation. This newly identified IEL–IEC cross-talk offers a novel source of factors regulating mucosal homeostasis, infection and pathology.

DCs in the lung

Historically, the respiratory epithelium was often overlooked as a key player in host immune responses, many believing this area as being merely a barrier with little or no capacity to respond to pathogenic or proinflammatory material. Langerhan's cells (expressing both myeloid and plasmacytic markers) were the first pulmonary DCs to be described and, in conjunction with other groups, Andrew Thorley (Imperial College, London, UK) and colleagues have also demonstrated the presence of CD141⁺ pulmonary myeloid type II DCs, CD103⁺ pulmonary plasmacytoid DCs and, more recently, pulmonary CD83⁺ DCs.

The distribution of pulmonary DCs is predominantly subepithelial (80%) with lower numbers found within the interdigitating spaces of the respiratory epithelium. DCs located within the upper airways have a high turnover, which is unsurprising since the majority of airborne pathogens will be encountered in this area first. Thorley's group have shown that bronchial epithelial cells release CCL20 (MIP-3 α) in response to certain allergens, which behaves as a chemoattractant for DCs. Low levels of cigarette smoke promotes MIP-3 α secretion from type II bronchial epithelial cells (pneumocytes), although this effect is not observed with higher concentrations. However, a direct link between cigarette smoke exposure and DC numbers has been shown with increasing quantities of DCs in peripheral lung tissue by a mechanism other than MIP-3 α . Increasing amounts of cigarettes smoked per day correlates with increased lymphoid follicle formation and a CD8⁺

T-cell predominant response, as observed in chronic obstructive pulmonary disease. The lungs have become an increasing focus for DC-related investigations and vigorous or inappropriate immune responses may lead to devastating results for these fragile gas-exchange structures.

Intestinal DCs in mucosal clearance

Intestinal DCs not only play a role in the orchestration of mucosal immune responses but are also directly involved in sampling luminal antigens by extending cellular processes (dendrites) between epithelial cells and shuttling them across the epithelial barrier. This event is facilitated by the expression of tight junction proteins that enable sampling without altering the integrity of the intestinal barrier.

DC direct sampling may not be the sole event taking place at the mucosal interface with enteric pathogens. Angela Man (Norwich, UK) and colleagues have used various enteral delivery routes of *Salmonella* and observed a significant number of CD11c⁺CX3CR1⁺CD8α⁺CD11b⁺MHC-II⁺ DCs, which traverse the epithelial barrier and migrate 'as a whole' into the lumen prior to or following internalization of labeled pathogen. Taken together, these data demonstrate that *Salmonella* triggers at least two different events and that transepithelial DC protrusions may not always represent antigen sampling but DCs undergoing migration into the intestinal lumen. Neither *Salmonella* variants lacking flagellin nor transgenic mice that lack MyD88 show evidence of DC migration following internalization of antigen. Interestingly, flagellated variants of *Escherichia coli* or soluble flagellin alone do not appear to induce DC migration, suggesting that other antigenic peptides specific to *Salmonella* are important in this process.

DC mobilization in infection

Trichuris trichiura is a large intestine-dwelling whipworm transmitted via the feco-oral route and affects over 1 billion people worldwide. *Trichuris muris* is a closely related nematode that affects mice and a validated laboratory model of IBD. Sheila Cruickshank and colleagues (Manchester Immunology Group, UK) have extensively

studied this parasite and observed altered patterns of DC recruitment and different IEL phenotypes in susceptible compared with resistant mouse strains. In resistant mice, which mount a Th2 predominant response, the numbers of infiltrating CD4⁺, CD8⁺, B220⁺ and F4/80 IEL and lamina propria lymphocytes are generally highest around the time of worm expulsion from the gut and associated with rapid DC recruitment. This increase appeared to be sustained 1 week postinfection in resistant mice when the majority of the DCs were mature. In susceptible mice, which mount more of a Th1 response, the number of IELs and lamina propria lymphocytes increased more gradually and were highest after a chronic infection has developed. There are also marked differences in the expression of colonic epithelial chemokines in resistant mice and susceptible mice. Resistant mice have significantly increased levels of epithelium-derived CCL2, CCL3, CCL5 and CCL20 compared with susceptible mice. Furthermore, administering neutralizing CCL5 and CCL20 antibodies to resistant mice prevented DC recruitment. This large body of work provides clear evidence of differences in the kinetics of DC responses in hosts inherently resistant and those susceptible to infection with enteric parasites.

Hepatoenteric immune system

In the progressive cholestatic disorder primary sclerosing cholangitis (PSC) the majority (>80%) of patients (in northern Europe) also have IBD. Any model attempting to explain the development of PSC in the context of IBD needs to take into consideration that the liver disease runs an independent course to that of bowel inflammation.

David Adams' group have contributed a large body of work illustrating how mucosal lymphocytes within the inflamed bowel may enter the liver and contribute to hepatobiliary inflammation in PSC. In adults, although the liver and gut have distinct endothelial phenotypes, there may be overlapping expression of certain adhesion molecules and chemokines. Under normal conditions, VAP-1 expression on hepatic endothelium is far stronger than that observed on mucosal vessels. Gut expression of VAP-1 is greatly increased in IBD

suggesting that liver-derived lymphocytes expressing the VAP-1 receptor may be able to enter the inflamed bowel. Similarly, expression of MAdCAM-1 and CCL25 is normally restricted to the gut, but in PSC, MAdCAM-1 is also found on portal vein endothelium and CCL25 on periportal sinusoidal endothelium. Ligation of CCR9 on lymphocytes by CCL25 triggers conformational changes in $\alpha 4\beta 7$ -integrin promoting firm adhesion of these cells to MAdCAM-1. In PSC, 20% of liver-infiltrating lymphocytes are also CCR9 $^{+}\alpha 4\beta 7^{+}$, most likely being recruited to the liver by aberrant expression of CCL25 and MAdCAM-1. This would allow immune surveillance across both sites in a process where long-living, mucosal-derived memory cells are recruited rapidly to the liver if a trigger leads to upregulation of hepatic MAdCAM-1. If these memory cells were to become reactivated, by cross-reactive antigens in the liver or by gut antigens entering via the portal circulation, this could initiate an inflammatory response and result in chronic hepatic inflammation. Adams' group has recently demonstrated that deamination of methylamine (a compound found in various foodstuffs,

wine and cigarette smoke) by VAP-1 in the presence of TNF- α induces the aberrant expression of functional MAdCAM-1 on hepatic endothelium.

Conclusion

Insight into DC and epithelial cell biology continues to expand at a furious pace. The road from bench to bedside is particularly challenging and although scientists can draw from an ever-expanding 'toolbox' of techniques, the real trial will be to promote these cellular manipulations to clinically applicable and acceptable strategies. Any attempt to manipulate these cells for therapeutic purposes must maintain the delicate balance between excessive immune suppression and sparking off autoimmunity.

There is still a lot to be investigated in terms of cataloguing different DC populations and documenting their respective function, origin, and relationship to other cells. As daunting as these tasks may seem, the reward will more than compensate for the efforts and DCs may hold the key to fighting some of the world's major health issues in the present and foreseeable future.