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effector T cell desensitization at the single-cell level. Along with single-cell analysis of other parameters, this study sets a path to a richer picture of the immune response that should lead to better design of vaccines and interventions against immunopathology.

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Intestinal Macrophages and DCs Close the Gap on Tolerance

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CD103$^+$ dendritic cells (DCs) must acquire soluble food antigens from the gut lumen to induce oral tolerance. In this issue of *Immunity*, Mazzini et al. (2014), report that CX_3CR1^+ macrophages capture such antigen and transfer it to the DCs by a route involving gap junctions.

To maintain tolerance toward proteins from food, mononuclear phagocytes in the gut mucosa, including the lamina propria, must sample antigens from the intestinal lumen and deliver them to lymph nodes for presentation to T cells.

The lamina propria of the small intestine in mice contains several populations of mononuclear phagocytes (Figure 1). Prominent among those are migratory dendritic cells (DCs)—marked by the expression of the integrins CD11c, CD11b and CD103, and macrophages—marked by the chemokine receptor CX_3CR1 and F4/80 (Varol et al., 2009). Intestinal CD103$^+$ DCs are short-lived cells destined to migrate into the draining mesentery lymph nodes (LNs). Upon arriving there they prime or tolerize T cells, depending on the inflammatory context (Laffont et al., 2010). Thus CD103$^+$ DCs are thought to be critical for maintaining T cell tolerance and for eliciting immune response against gut pathogens (Schulz et al., 2009). The more abundant CX_3CR1^+ macrophages are relatively long-lived phagocytes that do not normally migrate to LNs and are not as efficient at presenting antigen (Ag) to T cells. Their immunological role is debated.

When sampling particulate antigen, DCs can manage on their own. In response to *Salmonella*, for instance, DCs enter the epithelium, send dedicated dendrites to phagocytose the bacteria, and then process their antigens and carry them to the draining lymph nodes for presentation (Farache et al., 2013). In contrast, the DCs are inefficient samplers of soluble antigens, although their presentation is essential for maintaining tolerance toward food antigens. Surprisingly, several studies have shown that CX_3CR1^+ macrophages gather this sort of antigen much more efficiently than DCs (Schulz et al., 2009; Farache et al., 2013). A major mechanism the macrophages use is sending dendrites, dependent on CX_3CR1 signaling, to the gut lumen. Antigen may also directly flow into the lamina propria through goblet cells to be collected there by the macrophages (McDole et al., 2012).

The complementing specialties of the two cell populations, one in uptake of antigen and the other in its presentation, raises an interesting possibility—could CX_3CR1^+ macrophages and CD103$^+$ DCs be collaborating? Could macrophages be in charge of capturing the Ag and delivering it to DCs that would in turn carry it to the lymph node for presentation?

In an elaborate study, the group led by Maria Rescigno now shows that this is indeed the case (Mazzini et al., 2014). Their research follows the extent, mechanism, and immunological consequences of antigen transfer between macrophages and DCs in the intestinal lamina propria of the mouse small intestine.

The study started by verifying, by using histology and flow cytometry, that soluble ovalbumin was taken up efficiently by CX_3CR1^+ macrophages, but not by CD103$^+$ DCs. Interestingly, uptake by macrophages was largely limited to the

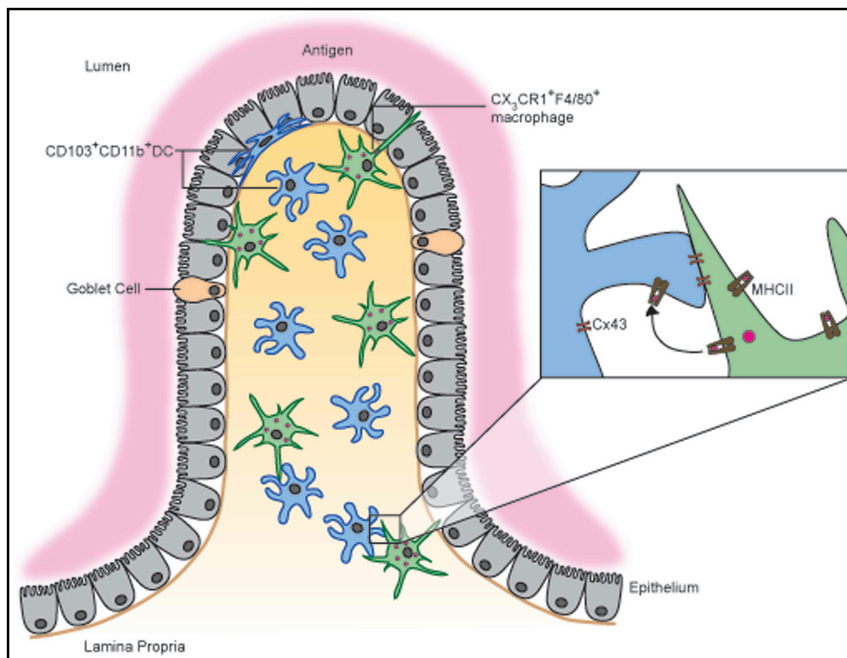

Figure 1. Intestinal Macrophages and DCs Collaborate to Sample and Present Luminal Antigens
To collect soluble antigen from the gut lumen, intestinal $CX_3CR1^+F4/80^+$ macrophages residing in the lamina propria send dendrites through the intestinal epithelium. Alternatively, goblet cells in the epithelium flow the antigen into the lamina propria. Rather than collecting this antigen themselves, $CD103^+CD11b^+$ DCs present in the lamina propria receive it from the macrophages. The transfer of antigen depends on the gap junction molecule connexin 43 (Cx43). Surprisingly, connexin seems to relay the antigenic peptides already complexed to MHC-II molecules in a process involving transfer of cell membrane. DCs would subsequently migrate into draining lymph nodes, present the antigen to T cells, and, under noninflammatory conditions, induce oral tolerance.

duodenum, the uppermost part of the small intestine; in the jejunum and ileum, it was mostly enterocytes that captured the antigen.

The researchers next showed that even though macrophages excelled in taking up the intact antigen, it was their neighboring DCs that could effectively present it to $CD4^+$ T cells. To make this point, the researchers made use of $CX_3CR1^{gfp/gfp}$ mice. The lamina propria macrophages of these genetically targeted mice, lacking the chemokine receptor CX_3CR1, cannot extend dendrites into the gut lumen (Niess et al., 2005), and indeed exhibited reduced uptake of ovalbumin. Although in such mice $CD103^+$ DCs had no intrinsic defects, when purified from mice injected with ovalbumin into the intestine, the DCs could not present the antigen. In line with this finding, $CX_3CR1^{gfp/gfp}$ mice could not develop oral tolerance against ovalbumin. Taken together, these results strongly suggested that the antigen was somehow transferred from macrophages to DCs in the intestine.

Proceeding to check this possibility more directly, they focused their effort on gap junctions—one of the possible routes for antigen transfer among myeloid cells. Gap junctions have been implicated in transferring antigenic peptides between adjacent cells in vitro (Neijssen et al., 2005), but their physiological importance in cross presentation in vivo has not been well documented. The researchers verified that macrophages and DCs indeed express gap junctions, particularly of the connexin-43 (Cx43) variety, and observed $Cx43^+$ patches in contact areas between DCs and macrophages ex vivo. To more directly implicate this molecule, they employed two complementing methods, pharmacologically blocking gap junction communication by using hepatnaol and by using a Cre-flox approach to obtain *Gja1*$^{fl/fl}$ *Itgax-cre* mice whose Cx43 was conditionally deleted in $CD11c^+$ cells, which include $CD103^+$ DCs and CX_3CR1^+ macrophages.

After performing all the necessary control experiments to verify that macrophages and DCs are normal in these mice, the group performed the linchpin experiment in the project—injecting ovalbumin into the intestines of the above conditionally targeted mice and checking whether isolated DCs and macrophages could induce the proliferation of antigen-specific T cells. The striking results showed that deletion of Cx43 abolished antigen presentation by DCs and increased it in macrophages, as if antigen was trapped in the macrophages and could not be handed over to DCs. These results were further solidified by showing that in the absence of Cx43-mediated antigen transfer, regulatory T cells could not be induced by DCs migrating to the draining lymph node and tolerance did not form normally.

Classically, gap junctions allow passage of small molecules between cells by coupling their cytosols through pores. In that way, short linear peptides can diffuse between macrophages and DCs to be cross-presented. This scenario is easy to envisage for presentation of antigens to $CD8^+$ T cells, where transferred cytosolic peptides can be directly loaded onto major histocompatibility class I (MHC-I) molecules. In the case of presentation on MHC-II molecules, which normally cannot load antigens from the cytosol, this route seems less plausible. So, in a final twist to the plot, Mazzini et al. suggest that instead, the gap junction protein Cx43 serves as a docking molecule, which allows the DCs to unidirectionally acquire membrane domains containing preformed peptide-MHC complexes (Baker et al., 2008). This intriguing proposition, although demonstrated only in vitro with HeLa cells, suggests that trogocytosis is the basis of antigen transfer.

It would be interesting to test whether these findings hold true for all soluble proteins. Ovalbumin, a popular model antigen, is not necessarily a typical one. Being highly glycosylated, macrophages collect it using dedicated scavenger molecules such as mannose receptors. It remains to be seen whether proteins that exhibit no particular affinity to macrophages behave the same. In this respect, it is important to realize that most food proteins, having gone through chemical and enzymatic digestion in the upper intestinal tract, would reach the small intestine as short peptides and single amino acids. The most abundant intact proteins in the lumen

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are likely the highly-glycosylated mucous proteins, which ovalbumin injected into the intestine more closely mimics.

On a more theoretical note, one might ask what purpose is served by the division of labor between macrophages and DCs—why can't the DCs take up the antigen, or conversely, the macrophages carry it to lymph nodes? Here Mazzini et al. speculate that this relay race might serve to prevent DCs from contacting the gut microbiota and becoming needlessly activated. Because the DCs have also been observed sending extensions into the lumen, and because transfer of antigens between myeloid cells has also been documented in other settings (Allan et al., 2006), this might not be the whole story. Regardless, Mazzini and colleagues have revealed that in the case of developing tolerance to ingested nutrients, food antigens must mind the gap.

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The Battle in the Gut

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Our molecular understanding of how pathogen-microbiota-immune system interactions influence disease outcomes is limited. In this issue of *Immunity*, Behnsen et al. (2014) report that the cytokine interleukin-22, which usually plays a protective role, promotes pathogen colonization by suppressing related commensal bacteria.

We are beginning to understand the complex interplay between mammalian immune systems, indigenous microbial communities and microbial pathogens at a molecular level. The human gut is teeming with trillions of bacteria that are essential for the maintenance of our health. For example, commensal bacteria are key participants in the digestion of food and extract nutrients and other metabolites that we need to stay healthy. Many of the metabolites and nutrients that commensal bacteria provide are implicated in the development, homeostasis and function of our immune system. Thus, our indigenous gut bacteria can provide protection to invading pathogens by influencing immune and nutritional barriers. In addition, commensal bacteria can provide increased resistance to bacterial pathogens by occupying their required niche. However, many bacterial pathogens have the capacity to disrupt or bypass homeostatic, immune, and colonization resistance mechanisms (Sansonetti, 2011). In this issue of *Immunity*, Behnsen et al. (2014) explore the complex interactions between an important mucosal immune factor, the commensal bacteria and the enteric pathogen *Salmonella enterica* serovar Typhimurium (referred as *Salmonella* from here on) in the guts of mice.

Salmonella is an important food-borne pathogen that causes a self-limited gastroenteritis in humans. The mucosal immune response to *Salmonella*, as with other pathogens, is orchestrated by T cells that express the cytokines interleukin-17 (IL-17) and IL-22. IL-17 promotes the recruitment of neutrophils and prevents the dissemination of *Salmonella* to the reticuloendothelial system. IL-22 is produced by immune cells, including T-helper cell subsets and innate lymphocytes, but acts only on nonhematopoietic stromal cells; in particular epithelial cells, keratinocytes, and hepatocytes (Rutz et al., 2013). IL-22 is usually beneficial to the host because it elicits the expression of proinflammatory epithelial defense mechanisms that are essential for host protection. IL-22 promotes epithelial proliferation and helps to maintain and restore the integrity of the epithelial barrier function during the invasion by pathogens. In addition, IL-22 synergizes with other cytokines, such as IL-17 or tumor necrosis factor-α (TNF-α), to induce expression of antimicrobial proteins involved in host defense in the skin, the airways, and the intestine. For example, IL-22 induces the expression of S100A7, S100A8, S100A9, β-defensin-2, and β-defensin-3 in the skin. It also promotes the release of RegIIIβ and RegIIIγ from intestinal cells and stimulates the production of protective mucus (Muc1, Muc3, Muc10, and Muc13) from

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