Is adaptive-innate lymphocyte cross-talk driving mucosal disease?

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Many nonlymphoid tissues, including epithelial barrier surfaces such as the skin, the lung, and the gut, are seeded by lymphocytes of the innate and adaptive immune system. These cells form strategically positioned local networks for immune surveillance and immediate front-line defense to microbial invasion. The activation of tissue-resident lymphocytes needs to be tightly regulated to prevent collateral damage. Some intraepithelial lymphocytes (IELs), for example, have strong cytotoxic potential and can cause severe tissue damage, particularly upon aberrant expansion and activation as observed in some mucosal inflammatory diseases. Understanding the local cellular interactions that orchestrate and regulate the function of tissue-resident lymphocytes may therefore point toward novel therapeutic strategies. In PNAS, Kooy-Winkelaar et al. (1) investigate potential interactions of CD4+ T cells and IELs of patients suffering from refractory celiac disease (CD), a complex autoimmune-like condition in which an immune response to dietary gluten can cause chronic tissue destruction and, in some patients, lead to aggressive lymphoma. The potential cellular interactions highlighted by this study suggest new additional strategies to treat refractory CD and enteropathy-associated lymphoma, and, more broadly, support the idea that adaptive immunity contributes to the regulation of innate lymphocytes in mucosal tissues.

A Network of Lymphocytes at Mucosal Surfaces

Distinct types of lymphocytes are found in nonlymphoid tissues, especially at mucosal borders. These lymphocytes can be generally divided into innate and adaptive cells. The group of innate lymphocytes is represented by natural killer (NK) T cells, mucosal-associated invariant T cells, and γδ T cells, as well as NK cells, IELs, and recently discovered innate lymphoid cells. In general, innate lymphocytes can seed nonlymphoid organs during ontogeny, have an activated phenotype, and are poised for immediate effector function that can be triggered through germ-line-encoded receptors recognizing ligands and cytokines produced or released during epithelial stress, tissue damage, and infection (2). T cells, in contrast, belong to the adaptive arm of the immune system because they express somatically recombined antigen receptors. To be able to respond to a very broad repertoire of potentially encountered antigens, individual T cells with a given specificity are relatively rare among the pool of all T cells; they recirculate as “naive” cells through secondary lymphoid organs, where they are differentiated into effector cells and massively expanded “on demand” during antigen-specific adaptive immune responses. Activated cells then redistribute and seed inflamed tissues. After the resolution of, for example, infection, most of these cells will eventually undergo apoptosis, whereas a small fraction acquires the ability to persist long term as memory cells that confer "improved protection" if a pathogen is reencountered. It is now being recognized that one key feature of such improved protection is the ability of some T cells to persist in nonlymphoid organs in front-line niches as tissue-resident memory (TRM) cells that can immediately react to their cognate antigen and prevent pathogen invasion (3, 4).

Adaptive T Cells Instructing Mucosal Immune Responses

Because the amount of TRM cells that can populate barrier organs is likely limited, TRM cells of a given specificity may be relatively rare. One question arising is therefore how a limited number of antigen-specific T cells can provide local protection to pathogen invasion. It has been recently proposed that, in addition to their immediate effector function, T cells may act as local antigen-specific sensors that instruct other cells to orchestrate local immune responses, and thereby amplify their antigen-dependent effects (5, 6). This "sensing and alarming" function includes the recruitment of additional immune cells, the activation of tissue-resident lymphocytes, and the activation of epithelial cells (5, 7, 8). Although such amplification is likely beneficial in the rapid response to invading pathogens, T cells reacting to environmental (e.g., food, commensal microbiota) or self-antigens may exacerbate or maintain inflammatory diseases also by coactivating innate lymphocytes (6).

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An important example of such a condition may be CD, a complex autoimmune-like enteropathy characterized by, among other features, CD4⁺ T cells that react to peptides generated in the duodenum from dietary gluten (further information is provided in ref. 9). One hallmark of CD is the expansion and activation of cytotoxic IELs, which may contribute to the typical epithelial lesions. Some patients develop a severe variant of CD [type II refractory celiac disease (RCDII)] characterized by the expansion of aberrant IELs that can give rise to malignant lymphomas. There is strong evidence that the cytokine IL-15 has a central role in the pathogenesis of CD and RCDII because it can drive the development, activation, survival, and aberrant differentiation of IELs (9, 10). Although IL-15 is often regarded as part of an epithelial “stress response,” it may have additional multifaceted roles in CD (11). A “two-hit” model of CD pathogenesis has been proposed highlighting that disease development requires both adaptive immunity and the aforementioned innate epithelial stress response to drive full activation of cytotoxic IELs and subsequent epithelial damage (12). Emphasizing the pathophysiological role of CD4⁺ T cells, RCDII patients are frequently homozygous for the predisposing HLA allele DQ2.5 and exhibit increased numbers of inflammatory, cytokine-producing, gluten-specific CD4⁺ T cells (13, 14). One important question is therefore whether cells of the adaptive immune system may directly interact with IELs and, independently or synergistically to epithelial stress signals, activate IELs (9, 15). Interestingly, an experimental mouse model suggested that CD4⁺ T cells specific for a dietary antigen secrete cytokines that “help” cytotoxic lymphocytes to promote enteropathy (16).

**Gliadin-Specific T Cells May Activate IELs in Celiac Disease**

In PNAS, Kooy-Winkelaar et al. (1) have now investigated potential interactions of CD4⁺ T cells and IELs from patients suffering from RCDII. Biopsies from the duodenum of RCDII patients were obtained to isolate and establish gliadin-specific T-cell lines. The authors found that supernatants from these cultured cells drive proliferation of previously characterized malignant Lin⁻IEL lines, which prompted them to screen for factors produced and secreted by these cells. Combining gene expression and proteomic analyses with in vitro blocking studies, Kooy-Winkelaar et al. (1) demonstrate that the cytokines TNF-α, IL-2, and IL-21 synergize to drive proliferation of IEL lines. Together, these cytokines potentiated phosphorylation of AKT to a similar level as exposure to IL-15. Culture of IELs in TNF-α and IL-21 was sufficient to induce expression of the antipapoptotic protein bcl-xL as efficiently as IL-15. Interestingly, the strongest induction of bcl-xL was observed when IELs were treated with supernatants of gliadin-specific T cells, raising the possibility that there may be additional T-cell–derived factors that could help the survival of IELs. Supported through a series of in vitro experiments, the authors suggest that synthetic inhibitors that may block signaling cascades of these cytokines, as well as IL-15, may be an additional treatment option for patients with RCDII (1). To extend the insights gained from the use of cell lines to the potential relevance in the patient, Kooy-Winkelaar et al. (1) have isolated IELs from non-CD, CD, and RCDII patients and confirmed the proposed synergistic effects of T-cell–derived cytokines: Lin⁻IELs from about 30–50% of non-CD as well as RCDII patients started to proliferate in response to a mixture of TNF-α/IL-2/IL-21, albeit to a lesser extent and response rate as when exposed to the “gold-standard” IL-15. The effect of T-cell–derived cytokines may, however, be pleiotropic, and in vitro proliferation is only one readout. For example, T-cell–derived factors may regulate the cytotoxic activity and target-cell recognition of innate lymphocytes (17). In addition, it may be interesting for future studies to compare TNF-α/IL-2/IL-21–induced transcriptional changes in IELs with those changes induced by IL-15 to reveal potential additional specific functions of the proposed adaptive-innate lymphocyte cross-talk.

The findings reported by Kooy-Winkelaar et al. (1) are consistent with the idea that signals derived from both innate and adaptive cells regulate the activation of innate lymphocytes in nonlymphoid tissues, and thereby contribute to mucosal inflammation and disease.

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