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Editorials

Reduce, Reuse, and Recycle: Shedding Light on Shedding Cells

In the intestine, dead or dying epithelial cells just get sloughed off, drop into the lumen, and die. Or do they? The paper by Iwanaga et al. in this issue of GASTROENTEROLOGY[1] suggests that intraepithelial lymphocytes (IELs) and subepithelial histiocytes are involved in removing all but the most superficial parts of enterocytes. This part of the enterocyte lives on without its nucleus, preserving the integrity of the mucosa until ultimately this remnant of the enterocyte is also shed into the lumen. The basal part of the enterocyte including the nucleus is engulfed by macrophages lying immediately beneath the epithelium. Using pseudopodia that extend to the basal part of the cell, these macrophages absorb the enterocyte with resulting pyknotic nuclear fragments being visible within their cytoplasm. The description of this novel mechanism, currently only documented in the guinea pig small intestine, at a single stroke suggests that our cherished views on cell destruction and turnover in the intestinal epithelium may require modification. In addition, this brings to the forefront possible physiological functions for IELs and the subepithelial macrophages. The function of both of the latter two cells has been enigmatic, with many "probable roles" in a variety of functions each with limited evidence. The study of Iwanaga et al. may be the first to shed light on these issues. At the same time it raises questions such as whether this process occurs in humans and why such a complex process should exist at all. This is certainly a most intricate mechanism, which could conceivably be a mode of conservation or a means of preventing parts of the cell, including the nucleus, from reaching the lumen. If it is a mode of conservation, is this part of a more widespread phenomenon seen in other epithelia? Life without a nucleus is not an entirely new concept; human red cells do it all the time.

The functional integrity of the intestinal mucosa is dependent on the barrier provided by a single layer of columnar epithelium sitting on a basement membrane and held together by tight junctions. These cells serve in the absorption of nutrients, fluid, and electrolytes. The integrity of this barrier is reflected in the selected permeability to molecules. Hence the external world is kept from invading the internal environment. Any disruption in this epithelial layer alters the normal physiological function of the epithelial cells and disrupts its barrier function as seen in inflammatory bowel diseases.

Presumably to maintain a viable epithelial cell layer there is a constant turnover of cells and precursors. Immature cells in the crypts provide new cells that mature into enterocytes and goblet cells that migrate up and over the villus. Cells at the villus tip are sloughed off and shed into the lumen. At least this has been the dogma up until now. The study of Iwanaga et al. suggests that the removal of dying epithelial cells is more complex and occurs as a result of a specific mechanism designed to eliminate enterocytes at a predetermined anatomical site in a very controlled fashion while ensuring that the integrity of the epithelium is never compromised.

The whole process of programmed cell death or apoptosis is complex.[2] The notion that intestinal epithelial cells die and become apoptotic with nuclei that become smaller and fragmented has been known for about 40 years since Leuchtenberger described small inclusion bodies of DNA in large bowel adenomas.[3] However, the process was recognized in embryos as part of normal embryogenesis 3 years earlier.[4] Similar inclusions are also described in excess in other conditions associated with cell death including radiation, chemotherapy, and graft-vs.-host disease and even drug-related disease.[5] This also appears to be part of the normal mechanism of programmed cell death, pathology frequently representing a change in the dynamics of a normal process. The question of how and why this occurs as part of the mechanism of normal enterocyte turnover during the housekeeping of the crypt-villus unit needs to be explored.

Although Iwanaga et al. suggest that because subepithelial histiocytes are present in humans that the same process likely exists in the human gut, this hypothesis requires appropriate confirmation. Nevertheless our knowledge of the origin and function of these macrophages is limited. Macrophages are frequently seen in the human large and small intestine immediately beneath the epithelium and frequently contain nuclear debris, the origin of which has never been clear but which included epithelium, possibly IELs, and other cells dying in the lamina propria. However, the origin

and function of these histiocytes in humans is unclear. There are at least two possible modes of origin, perhaps the most logical being from bone-marrow derived monocytes; however, a second possibility is that they could originate from the myofibroblasts of the pericrypt fibroblast sheath. These cells are readily visible in the basal two thirds of crypts but seem to fizzle out in the upper third of the large bowel mucosa and in villi. In tumor pathology most are aware of the fibrohistiocytic group of tumors (e.g., malignant fibrohistiocytoma) in which it seems possible that histiocyte-like cells are derived from fibroblast or myofibroblasts. Invoking the pericrypt (myo)fibroblast sheath as the origin of lamina propria histiocytes raises another thorny issue, which is whether this population is static (i.e., the conveyer belt of epithelial cells migrate over the sheath towards the lumen) or whether sheath cells are dynamic as well and migrate up along with the epithelium. Evidence from turnover studies and crypt labeling has supported both of these mechanisms, and the question therefore remains unresolved.[6–10] If the sheath migrates, then the question is what happens to these cells when they reach the superficial mucosa. If they die, they can be shed with the epithelial cells (or part of them) into the gut lumen or engulfed by the superficial macrophages. But rather than be ingested by them, could they actually transform into them, engulf part of their neighboring epithelial cell as proposed by Iwanaga et al., and then migrate out? It is easier to postulate migration out than death because this would produce an even greater disposal problem of dead cells and cell products in the superficial lamina propria. There would be even less problem if sheath cells were a relatively static population.

Within the epithelium, dispersed between the enterocytes, are isolated mononuclear cells—the IELs. In the epithelium, 2%–3% of the resident lymphocytes actually synthesize DNA in situ; at the villus tip the IELs are a collection of relatively young or recently arrived lymphocytes that transit within 3 days of arrival.[11] This is in contrast to the enterocyte whose average life span is 7 days.[12] This difference suggests that the IELs are not simply shed along with the epithelial cells into the lumen but may reenter the lamina propria, possibly even from the lumen. This issue has not been completely resolved; however, studies in the rat examining the direction of the collagen fibers at the rupture of the basement membrane following passage of a lymphocyte and on the location of the cytoplasmic tail of the moving lymphocytes in relation to the nucleus suggest that IELs reenter the lamina propria.[11,13] Studies in chickens infected with coccidia also provide evidence that IELs are able to migrate back across the basement membrane into the lamina propria.[14,15] Thus IELs are not simply dragged along by the enterocyte escalator; indeed, the enterocytes probably have to migrate over IELs allowing ample opportunity for interactions between these cells. As the resident mononuclear cell, the IEL is an obvious candidate to consider as a cell responsible for the elimination of old, injured, transformed, or dying epithelial cells.

The in vivo function of IELs has, until now, eluded definition.[16] IELs are a heterogeneous population of mononuclear cells that morphologically include granulated cells similar to the large granular lymphocytes (LGL) in peripheral blood.[17] Although the majority of these cells bear the CD8 receptor, which in peripheral blood lymphocytes has been associated with cytotoxic/suppresser T-cell activity, no suppresser activity has been shown in IELs. The majority of IEL express the T-cell receptor (TcR) molecule, and in the mouse this can be the α/β-TcR or the γ/δ-TcR.[18,19] In contrast to the α/β TcR cells, which have quite a wide spectrum of antigen receptors, IELs with γ/δ TcR have much more limited diversity and hence antigen repertoire. This led to the suggestion that these IELs were responsible for the maintaining epithelial integrity.[20] In vivo evidence for such a function, perhaps up until now, has been lacking. If we are to consider the findings of Iwanaga et al. in the context of what is known of IEL function, can we support the hypothesis that IELs are responsible for this role?

In vitro studies have shown that naive IELs have natural killer (NK),[21] cytolytic and cytokines/regulatory functions. Cytolytic ability of IELs expressing either the α/β- or γ/δ-TcR can be shown against the lymphoma cell line Yac-1 that is sensitive to NK cell function[19,21–23] and also against virus-infected cells in a fashion unique to IELs.[24–26] The surface receptors used in these functions are not known. Cytolytic function is also expressed in the ability of α/β-IELs to recognize allogeneic (strain-related) targets in classic cytotoxic T-lymphocyte assays.[27] In addition to this cytotoxic activity, IELs can produce and secrete a host of cytokines that may allow this population to function as a regulatory T cell, influencing B cell function.[28–31] Some of these cytokines, such as tumor necrosis factor (TNF), may also account for the cytolytic activity of IELs.[32]

Given this armamentarium of cytotoxic and regulatory functions, can the IELs recognize and respond to senescent epithelial cells? T cell lines and clones derived from fetal thymus or peripheral lymphoid organs can recognize MHC class I–like molecules such as TL

antigens in mouse and CD1 antigens in humans.[33–36] Since CD1 antigens are expressed on human intestinal epithelial cells it has been suggested that this molecule can function to present a restricted set of self-peptides allowing IELs to respond to self, possibly limited even further to a response to relatively senescent cells. It is also possible that a secretory product of IELs, possibly interferon gamma, can induce expression of MHC class II on epithelial cells.[37] The plot thickens because cell lines derived from neonatal mouse IELs can recognize self-antigen on epithelial cells.[38] IELs also have a high frequency of autoreactive-TcR.[39–42] These IELs are thought to represent cells that have escaped thymic deletion and possibly developed without coming into contact with the thymus at all, therefore representing a thymic-independent lineage. Under normal conditions, these T cells are functionally anergic. Whether such autoreactivity can be stimulated to cause IELs to recognize and eliminate epithelial cells is not clear, but that potential clearly exists.

The study of Iwanaga et al. suggests that epithelial cells undergo a controlled death initiated by apoptotic changes in the nucleus. The ability of IELs to directly kill target cells by inducing apoptosis has not been shown. If IELs were responsible for such a function they would be well equipped to carry out cell killing, since TNF does have cytostatic effects on epithelial cell lines.[43] Furthermore, the granules within IEL, like cytotoxic T lymphocytes (CTL), contain perforins and serine esterases of the granzyme family that can function in target cell killing.[44,45] In addition, CTL contain granule-associated proteases called "fragmentins" that induce DNA fragmentation and apoptosis in target cells.[46] The possibility that such proteins are present in IELs remains to be explored. If present, these proteins would further allow one to argue that IELs are not only well positioned for this function but they are also well armed.

Given the background of IEL heterogeneity in phenotype and function, which includes a cytolytic potential and the ability to recognize molecules on epithelial cells, it is possible that the morphological data presented by Iwanaga et al. represents the first in vivo evidence of a physiological role for IELs, simultaneously suggesting an explanation for a function for subepithelial macrophages and an explanation of the nuclear debris frequently found in them. Further, it may be no coincidence that one of the few diseases in which an excess of IELs and particularly γ/δ-IEL are documented is celiac disease, a disease characterized by a marked reduction in epithelial turnover time.[47] If these findings are related, we should be able to predict that there will be a similar decrease in turnover time in other diseases frequently associated with increased IELs, such as collagenous and microscopic colitis. Even more speculative is the notion that if IELs are responsible for inducing programmed cell death, then in some intestinal neoplasias, initially adenomas, there may be a failure of programmed cell death and therefore may be a primary disorder of IEL epithelial interaction.

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