Commentary

Whence the Intestinal Intraepithelial Lymphocyte?

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One of the most widely accepted principles of immunobiology is that lymphocytes with phenotypic and functional properties of T cells develop from hematopoietic precursors during a requisite period of intrathymic maturation. This developmental stage appears to prepare T cells for their ability to recognize tissue-borne foreign antigens, and to render the peripheral T cell repertoire tolerant to normal host tissue antigens (1, 2). This is not to say that lymphocytes with properties of T cells cannot develop extrathymically, but rather that when immunologists have sought evidence for extrathymic pathways of T cell development, little has been found. Now, however, considerable empirical evidence supports the likelihood that T cells in the small intestine of mice, the intestinal intraepithelial lymphocytes (IELs), have genuine extrathymic origins (3–9). Yet, many aspects of that process remain highly contentious, and the debate involves issues that are central to how the gastrointestinal tract—one of the largest barriers of foreign antigen invasion—maintains its immunological integrity.

Curiously, the intestinal IELs were one of the first discrete populations of lymphoid cells to be identified in mammalian species (10). However, studies of that lymphocyte population were quickly superseded by detailed exploration into lymphocyte biology using more accessible pools of cells in the spleen and lymph nodes of mice, and in the blood of humans. In fact, the intestinal epithelium with its collection of eclectic T cells containing no less than ten distinct phenotypic subsets (11), many with novel functional properties (12, 13), is still occasionally regarded as little more than a graveyard of dying and effete cells. The rejuvenated interest in the IELs can be attributed to several factors, not the least of which is the potential extrathymic nature of those cells, and the identification of IELs as a major pool of peripheral $\gamma\delta$ T cells (14). Evidence for the former comes from traditional experimental approaches used by immunologists to delineate pathways of T cell development, including studies in congenitally-athymic nude mice, in mice rendered athymic as neonates (neonatally thymectomized [NTX] mice), and in adult athymic radiation chimeras. The basic findings from those studies have been consistent, yet perplexing. Simply summarized, they are: (*i*) IELs in adult athymic chimeras are most similar, phenotypically, to IELs found in normal unmanipulated mice; these include both TCR$\alpha\beta$ and TCR$\gamma\delta$ IELs as well as CD8$\alpha\alpha$ and CD8$\alpha\beta$ subsets (4, 8, 9); (*ii*) IELs in nude mice strongly favor the TCR$\gamma\delta$ and CD8$\alpha\alpha$ lineage(s), although some age-dependent increases occur in the numbers $\alpha\beta$

T cells (15, 16); (*iii*) IELs in NTX mice have low numbers and proportions of TCR$\alpha\beta$ cells, and, to a lesser extent, a reduction in TCR$\gamma\delta$ IELs (16–20). The conundrum posed by this disparity in findings rests at the heart of the issue of IEL ontogeny.

So which model most closely reflects the biology of the intestinal immune system and, therefore, which IELs are truly extrathymic? Of course each model is inherently correct in that each provides clues to different aspects of IEL development. These differences have prompted a new series of experiments and subsequent novel hypotheses. On the one hand the paucity of TCR$\alpha\beta$ and CD8$\alpha\beta$ IELs in nude and NTX mice could be taken to mean that those IELs are T cells derived directly from the thymus, either as mature or immature T cells. That possibility has been addressed in part through studies using fetal, neonatal, or adult thymus tissues grafted into nude mice. Findings from such studies, though equivocal considering the level of variations in results between groups, are nonetheless interesting, i.e., thymus-derived T cells (depending upon the developmental stage of the donor thymus) find their way into the intestine epithelium (17–19). This has prompted some to posit that IELs may be derived from immature thymocytes which migrate to the intestine (16, 21). However, missing from the out of the thymus hypotheses is a credible rationale as to why IEL precursors must venture through the thymus and emerge without TCR gene rearrangement in order to become upstanding citizens of the intestine. Moreover, a major unaddressed concern with thymus grafting experiments is the degree to which the distribution of lymphocytes in peripheral immune compartments is artifactually altered by the introduction of a source of T cells into mice with virtually no T cells, i.e., filling the void in animals with disrupted immune homeostasis. Although at first glance the potential for the latter may appear miniscule, studies in my laboratory indicate that such factors can significantly influence the outcome of migration patterns in ways greater than previously assumed.

A second important consideration in understanding the differences in findings in nude and NTX mice versus adult athymic chimeras is the extent to which epi-immunologic, age-dependent factors directly or indirectly influence intestinal T cells development. Consistent with this is the finding that neuroendocrine hormones of the hypothalamus-pituitary axis, in particular thyrotropin, participate in the formation of an intact intestinal T cell repertoire (20, 22, 23), and that disruption of that immune-endocrine network either through congenital thymus deprivation or by neonatal

thymectomy concomitantly disrupts T cell development within the intestine. In this scenario, failure to receive a necessary thymus-derived, endocrine-mediated signal during some stage of fetal/neonatal life would not only lead to immunological perturbations within the conventional immune system (24, 25), but also would render intestinal tissues non-permissive for extrathymic development. Predictably, that deficit would be evident to varying degrees in nude and NTX mice, but would be inconsequential in adult athymic chimeras in which endocrine-induced maturation of gut epithelia is already complete.

If some or all intestinal IELs are extrathymic T cells, there are several possible ways by which that might occur. On the one hand, the IELs may be a repository of extrathymic T cells which have developed elsewhere, possibly within the liver (26), and have homed to the intestine. Alternatively, extrathymic development may occur locally within some portion of the intestine itself. This distinction is far from trivial since it will undoubtedly provide clues as to where those IELs are selected positively and/or negatively, and will help to explain such things as antigen reactivity by IELs. At present several lines of evidence, in particular unique patterns of IEL selection (9, 27–29), distinct mechanisms of antigen presentation (30, 31), and the use of histocompatibility antigens that are common to the intestine (32, 33), indirectly favor a local developmental route. Certainly, far more definitive proof of this would come from identification of the IEL progenitor cells within the intestine, if present. These elusive cells now may have been identified as described in this issue of *The Journal of Experimental Medicine* (34). Detailed histochemical studies by Kanamori, Ishikawa, and colleagues describe a novel population of cells with characteristics of early T cell precursors located in intestinal crypt lamina propria of mice. These clusters of cells termed cryptopatches (CPs), though relatively rare overall are more abundant in the small intestine (~1,650/small intestine) than in the large intestine (~150/large intestine), each cluster containing about 1,000 lymphoid cells. Phenotypically, most CP cells express the c-kit stem cell factor receptor, the IL-7 receptor, Thy-1, and LFA-1, but do not express TCR$\alpha\beta$ or $\gamma\delta$, immunoglobulin, or B220.

Other distinguishing surface markers of some CP cells include heat-stable antigen, a marker previously proposed to be an indicator of extrathymic IELs (3, 35), and Pgp-1. Interdispersed among CP cells is a group of cells expressing the CD11c/CD18 marker associated with dendritic stromal cells (36). Overall, CP cells do not appear to be of B cell lineage, although the extent to which some CP cells are mast cell progenitors remains open, as acknowledged by the authors. In that context, recent studies of mast cell precursors point to a similar though different Thy^{lo}, c-kit^{hi} cell located in fetal blood and, interestingly, a population of uncharacterized c-kit^{lo}, Thy-1$^+$ cells which more closely resemble CP cells (37), raising the possibility that progenitors of CP cells may reside in fetal blood. Regardless, the fact that mature mast cells are absent in W/W^v mice (37) whereas CPs are present in those animals, provides at least

circumstantial evidence that most CP cells are not mast cell precursors. Given that as many as 1.5×10^6 CP cells are present per small intestine, discriminating between CP cells and mast cells is feasible using multi-color cell sorting followed by adoptive transfer into congenic mice.

Interestingly, CPs appear in the intestine between days 14–17 post-birth, or at about the time of the first appearance of IEL within the intestine, and concomitant with the full developmental maturation of the intestine epithelium. Does this mean that CP cells do not arrive in the intestine until that time, or that CP cells are seeded earlier but remain dormant until conditions are suitable for development? To what extent are CP cells a self-replenishing pool? These questions remain to be addressed. However, it is reported that CPs are present in nude mice, severe-combined immunodeficient mice, in TCR-β $\times$ δ- and RAG-2-deficient mice, in *aly/aly* mice lacking organized lymph nodes and Peyer's patches, and in c-kit and stem cell factor-deficient mice. In contrast, CP cells were not found in IL-7R-deficient animals. The apparent dependence of CPs on IL-7, a cytokine used by T cells during the earliest stages of development prior to TCR gene rearrangement (38), implies that CPs may have developmental properties similar to those used by early-stage T cell precursors. Moreover, because IL-7-deficient mice lack $\gamma\delta$ IELs whereas $\alpha\beta$ IELs are present in those animals, albeit in reduced numbers (39), it is likely that CP cells are principally precursors of mature $\gamma\delta$ IELs, although not necessarily exclusively so.

One of the more curious yet strangely logical findings from these studies is the location of CP cells in the lamina propria just below the epithelium basement membrane. In that regard it is interesting that clusters of cells with histologic characteristics similar to CP cells also can be found within ectopic fetal intestine tissue grafts two to three weeks post-implantation into the kidney capsule of nude mice (40). Thus, compartmentalization within the lamina propria may provide the necessary microenvironment needed for antigen-independent selection of IELs in the presence of host histocompatibility antigens and professional antigen-presenting cells. This mechanism would necessitate migration of developing or mature CP T cells across the basement membrane near the crypt epithelia, i.e., at a site of active epithelial cell generation. Passage into the epithelium may be facilitated by acquired expression of cell surface adhesion molecules, possibly the $\alpha_E\beta_7$ integrin which is present on most IELs but few lamina propria lymphocytes (41; Hamad, M., and J.R. Klein, unpublished observations), and which recently has been shown to be required for full development of the gut-associated lymphoid tissues (42). In situ studies into the expression of cell adhesion molecules, and additional studies in genetically-manipulated mice, should be of considerable value in that regard.

Little by little many aspects of intestinal lymphocyte biology are beginning to come together, much more remains to be explained. Perhaps one of the most unpredictable findings to come from recent studies of IELs is the striking interdependence and cellular crosstalk between hematopoietic and non-hematopoietic components of the intestinal

epithelium. Thus, mice which lack γδ IELs have severely impaired development of intestine epithelia (43, 44), whereas epithelial-derived cytokines (45) and local use of hormones (20, 22, 23) have the potential to rapidly yet selectively influence the development, distribution, and function of the IELs. Similarly, the expression of c-kit has been linked to IEL homeostasis and gastrointestinal immunity (46, 47). In many ways the intestine is one of the best models for understanding a dynamic interactive immune system in the context of its natural environment. The identification of CPs should help to further focus studies toward that end.

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