

Intestinal Intraepithelial Lymphocytes: The Plot Thickens

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Over the last few years, the characterization of T lymphocytes within the intestinal epithelium (iIEL) has revealed phenotypic and functional heterogeneity that rivals only that observed within the thymus. Of note in this context is that thymic and intestinal epithelia share the same embryologic origin in that they are both derived from endoderm. Several lines of evidence have led to the conclusion that T lymphopoiesis occurs within the intestinal epithelium. This observation raises several fundamental questions some of which have been recently addressed in this and other journals. Specifically, are all iIEL of gut origin, and do the phenotypic and functional peculiarities of the various iIEL subsets reveal novel pathways of T cell development?

Origin of iIEL. The phenotypic and functional heterogeneity of iIEL has been recently reviewed (1, 2). Briefly, as in the thymus, all iIEL are T cells expressing either TCR- γ/δ or TCR- α/β , although the proportions of these two lineages vary among species. In the mouse, a high proportion of iIEL express TCR- γ/δ , the majority of which coexpress the α chain of CD8. Those iIEL expressing TCR- α/β can be further distinguished based on the differential expression of the accessory molecules CD4, CD8 α , and CD8 β . Two of the TCR- α/β^+ iIEL subsets are phenotypically identical to mature thymus-derived T cells: they express CD4, or the heterodimeric form of CD8; they are depleted of cells expressing autoreactive TCR; and they have functional antigen receptor complexes in that they can be stimulated by nominal antigen-, superantigen-, and TCR-specific mAbs (3, 4). Two additional TCR- α/β^+ iIEL subsets are peculiar to the intestinal epithelium: one expresses only the CD8 α chain, while the other coexpresses this chain and CD4. Although all TCR- α/β^+ iIEL subsets express a level of TCR/CD3 similar to that of mature thymus-derived T cells (2), the CD4 $^+$ 8 α^+ β^- and CD8 α^+ β^- iIEL subsets are unresponsive to the above set of stimuli (3, 4), and moreover contain high frequencies of auto-reactive cells (5). In this context the functional status of these four iIEL subsets correlates with membrane expression of CD28 (6). However, all iIEL subsets respond to concanavalin A (7).

There is consensus that all TCR- γ/δ^+ iIEL develop *in situ*. They are found in both congenitally athymic nude mice (8), as well as in athymic radiation chimeras (7). The controversy surrounds the origin of the various subsets of iIEL expressing TCR- α/β . Specifically, Guy-Grand et al. have interpreted the differential repertoire expressed by CD8 α^+ β^-

and CD8 α^+ β^+ iIEL as reflecting the thymic origin of the latter, and the gut origin of the former. Furthermore, in order to explain the high frequency of CD8 α^+ β^- iIEL expressing autoreactive TCRs, these investigators invoke distinct selection pressures applied to gut-derived T cells (5, 8). This brings us to the first new piece of information published recently by the same group (9).

In this study, adult animals lacking the recombination activating gene RAG-2 were either thymectomized or not, sublethally irradiated (400 rad), and then reconstituted with bone marrow from H-2 matched (C57Bl/6) nude donors. The observation is that euthymic recipients contain a normal number of iIEL phenotypically similar to those observed in 6–8-wk-old conventional animals. In contrast, the total number of iIEL in athymic recipients was reduced roughly fourfold, and the authors highlight that these recipients are devoid of TCR- α/β^+ iIEL expressing either CD4, or CD8 β . In support of their original premise, the TCR- α/β^+ iIEL found in these recipients expressed CD8 α exclusively. They conclude that these results unequivocally demonstrate the thymic origin of TCR- α/β^+ iIEL expressing CD4 or CD8 $\alpha\beta$, and the gut origin of TCR- α/β^+ iIEL expressing only the α chain of CD8. Further support for this tenet is derived from the observation that CD4 $^+$ and CD8 $\alpha\beta^+$ iIEL subsets are found in the gut of euthymic nonirradiated RAG-deficient animals injected with lymph node T cells from conventional animals (9).

The concern with the authors' conclusion rests with the extent of reconstitution of the iIEL compartment of athymic RAG 2-deficient recipients. Whereas the reduction in total iIEL was only fourfold, the proportion of TCR- γ/δ^+ iIEL was 20% of that observed in euthymic recipients. More strikingly, only 1–2% of iIEL in athymic recipients expressed TCR- α/β . Thus, one could argue that the development of a large proportion of TCR- γ/δ^+ iIEL and of virtually all TCR- α/β^+ iIEL, is thymus dependent. Consistent with this latter hypothesis is the observation that nude mice are devoid of TCR- α/β^+ iIEL, and contain a reduced number of TCR- γ/δ^+ iIEL (8). Inconsistent with this hypothesis is the observation that all TCR- α/β^+ and TCR- γ/δ^+ iIEL subsets are generated in normal numbers in athymic radiation chimeras, and are derived from donor stem cells (7, 10). In addition, ectopic fetal intestine grafts reconstitute the peripheral T cell pool of athymic radiation chimeras as efficiently as do ectopic fetal thymic grafts (11).

These discrepant results are difficult to reconcile unless one invokes fundamental differences in the T lymphopoietic capacity of the intestinal epithelium in athymic radiation chimeras, nude, and RAG-2-deficient animals. The intestinal epithelium of adult thymectomized radiation chimeras has been exposed to T lymphocytes and/or a thymic influence during pre- and postnatal development. This exposure may predicate the development of a fully competent intestinal epithelium. It is not implausible that in the absence of these putative influences, both the T lymphopoietic capacity of the intestinal epithelium, as well as the pattern of circulation of T cells within the gut, are altered. This could explain the observation that while thymus-derived T cells can access the gut of RAG-2-deficient animals (9), they fail to do so after the parabiosis of conventional euthymic animals (7).

Distinct Structure of the TCR/CD3 Complexes in Various iIEL Subsets. The next series of potential insights with respect to the possible distinct origins of various iIEL subsets are derived from results obtained in ζ chain-deficient mice. It has been clear for some time that ζ expression is required for appropriate intracellular assembly, trafficking, and membrane expression of the TCR/CD3 complex (12). Consistent with these results are recent observations from four independent groups analyzing the effects of dysregulated ζ expression on the development of thymus-derived T cells (13–16). Whereas the peripheral T cell pool in these animals is slightly reduced in number, the proportion of CD4⁺ and CD8⁺ T cells is unaffected. However, the level of expression of the TCR/CD3 complex on these cells ranges from undetectable (13), to 10% of wild-type levels (16). The decreased expression of TCR/CD3 correlates with a comparable reduction in both early (calcium mobilization) and late (DNA synthesis) activation signals induced through the antigen receptor complex on T cells from these animals (13, 16).

In marked contrast, these animals contain normal numbers of iIEL. The expression of TCR/CD3 by TCR- γ/δ ⁺ iIEL is unaffected in the absence of the ζ chain, and their phenotype is indistinguishable from that observed in wild-type animals (13). The level of TCR/CD3 expression on TCR- α/β ⁺ iIEL is also not drastically affected in the absence of the ζ chain in that it is reduced roughly twofold (13, 16). The two groups that further characterized the phenotypic heterogeneity of the TCR- α/β ⁺ iIEL in ζ -deficient mice report discrepant results. One group demonstrates that the four major subsets defined by the differential expression of CD4, CD8 α , and CD8 β are present in normal numbers (13). The second group failed to detect expression of TCR- α/β in iIEL expressing CD8 β or CD4, and use this observation in support of the thymic origin on these two subsets (15).

These experiments led to the biochemical characterization of the TCR/CD3 complexes expressed on the various iIEL subsets in ζ -deficient and wild-type mice. The novel observation indicates that the CD3 complexes within iIEL subsets of normal animals are heterogeneous, and in contrast to peripheral thymus-derived T cells can contain Fc ϵ R1g chains (g) present as either homodimers or heterodimers in combination with the ζ chain (13, 15, 17). While clearly differentiating peripheral T cells from iIEL, and demonstrating that

both TCR- γ/δ ⁺ and TCR- α/β ⁺ iIEL express the γ chain as part of their CD3 complexes (17), these studies did not determine whether CD3 complexes containing γ - γ and γ - ζ dimers were differentially expressed among the various TCR- α/β ⁺ iIEL subsets. This analysis has been performed by Guy-Grand et al. and their results were reported recently (18).

It appears that CD3 complexes using the γ chain are restricted to TCR- γ/δ ⁺ and CD8 α ⁺ β ⁻ TCR- α/β ⁺ iIEL. However, CD3 complexes within these two subsets also use the ζ chain, present as either a homodimer or a ζ - γ heterodimer. In combination with the evidence provided by the reconstitution of the RAG-2-deficient animals discussed above, the authors conclude that the exclusive association of the ζ chain with TCR/CD3 complexes expressed by CD4⁺ and CD8 β ⁺ iIEL correlates with their thymic origin, while the expression of the γ chain indicates that the T cell is of gut origin. Given that the TCR/CD3 complexes expressed by unfractionated iIEL in ζ -deficient animals are nonfunctional (13), the question arises as to whether the TCR/CD3 complexes expressed by TCR- γ/δ ⁺ and CD8 α ⁺ β ⁻ TCR- α/β ⁺ iIEL in conventional animals are heterogeneous at the single cell level. If they are not, results obtained from ζ -deficient animals suggest that a proportion of cells within these two subsets are nonfunctional.

These authors have argued in this and previous studies from their laboratory that the CD8 α ⁺ β ⁻ TCR- α/β ⁺ iIEL are fully differentiated and functional cytolytic T cells. As mentioned above, this subset is refractory to nominal antigen, superantigen, and anti-TCR-mediated activation. The exclusive assay in which responsiveness of this subset has been demonstrated is antibody-dependent cell-mediated cytotoxicity (ADCC) (19). While there is no reason to assume that this is not a physiologically relevant function, two points merit consideration in this context. The first is that ADCC function is retained in T cells from ζ -deficient mice, indicating that functional TCR/CD3 complexes are not required for this process (13). Second, the inference from the Guy-Grand group is that CD8 α ⁺ β ⁻ TCR- α/β ⁺ iIEL have evolved as a functionally specialized T cell subset. It is perhaps inappropriate to draw this conclusion since CD4⁺ helper T cells can also perform efficiently in ADCC assays (20).

While it remains unclear why the various iIEL subsets develop differentially in two strains of ζ -deficient mice (13, 15), it is noteworthy that phenotypically normal TCR- γ/δ ⁺ and TCR- α/β ⁺ iIEL are present in another circumstance in which thymic T cell development is severely impaired. Intrathymic T cell development in mice lacking the protein tyrosine kinase p56^{lck} is profoundly blocked at the double negative stage, indicating the central role of this kinase for the maturation of thymus-derived T cells (21). Yet, the iIEL compartment within these same animals is unaffected (21). Although it is as yet unclear whether the TCR/CD3 complexes expressed by these iIEL are functional, this result indicates that either all iIEL subsets develop in situ, or that the rare CD4⁺ and CD8 β ⁺ peripheral T cells in these animals can migrate to the intestinal epithelium and accumulate at this site.

The TCR- α/β Repertoire of iIEL Is Oligoclonal. In contrast to TCR- γ/δ ⁺ cells found in other epithelia in the

mouse and human, the TCR repertoire of those present in the intestinal epithelium is diverse (22, 23). The first evidence in support of a distinct TCR repertoire in TCR- α/β^+ iIEL came from studies in the human (24–26). Specifically, it was shown that most iIEL are derived from the expansion of a few T cell clones as evidenced by (a) the preferential expression of certain V α s and V β s, and (b) the presence of identical VDJ sequences in a large proportion of clones isolated from an individual. In one of these studies a comparison of the TCR repertoire of TCR- α/β^+ iIEL and peripheral blood T cells (PBL) was performed. It was found that the latter were polyclonal and that the predominant clones of iIEL from the same individual were not present in the PBL repertoire. It is important to note that the predominant TCR variable regions expressed among individuals can be different, indicating that the putative expansion of select clones could result from their distinct specificities for nominal antigen in combination with individual MHC (26). In this context it has been demonstrated that the CD1 family of MHC class I-like antigens may function as a ligand for CD8 $^+$ iIEL in the human (25).

A new piece of information is provided by Gross et al. (27) in this issue. These authors analyzed the TCR- α/β repertoires of iIEL isolated from distinct segments of the small intestine and/or the colon from the same individual. This study confirms the previously described oligoclonality of TCR- α/β^+ iIEL within each segment of the intestine, but more importantly, provides evidence for the presence of the same clones in proximal and distal sections from the same individual (27). This indicates that either TCR- α/β expressing iIEL migrate from one site to another along the intestinal mucosa, or that precursors of iIEL, which rearrange TCR gene segments at an as yet unidentified site, expand before seeding the length of the intestine. In the mouse it has been suggested that iIEL developing within the intestinal epithelium can migrate to the lamina propria (7). In this context another study in the human has demonstrated that the TCR repertoire of lamina propria T cells (LPT) is also oligoclonal, and moreover some of these LPT clones were also represented among iIEL of the same individual (26). Thus, it is possible that iIEL can cross the basement membrane separating the intestinal epithelium and the lamina propria. In the context of the results presented in the study of Gross et al. (27), these same cells may be able to return to the intestinal epithelium, providing a mechanism for the expression of identical clones along the gut mucosa. In support of this mechanism, mouse lymphocytes have been observed crossing the basement membrane of the gut epithelium (28).

Certain aspects of the TCR- β repertoire of mouse iIEL parallel observations made in humans, whereas others distinguish the two species, as characterized by the study of Regnault et al. (29) published in this issue. These authors demonstrate that as in humans, mouse iIEL expressing TCR- α/β are oligoclonal, and identical clones are present along the length of the intestinal epithelium. However, in contrast to human iIEL, the array of TCR V β s expressed by iIEL in the mouse is not skewed relative to that expressed within lymph node T cells of the same animal. Rather, the authors demonstrate

that in iIEL there is preferential recombination of a given V β with a given J β segment in individual mice. It is important to note that this is the first analysis that compares the repertoire of CD8 $\alpha^+\beta^-$ iIEL and CD8 $\alpha^+\beta^+$ iIEL. Strikingly, both subsets are oligoclonal, and furthermore, the repertoires are for the most part nonoverlapping. It is important to note that identical clones of CD8 $\alpha^+\beta^-$ and CD8 $\alpha^+\beta^+$ iIEL were found at different sites along the small intestine which is difficult to reconcile with the above suggested mechanism for traffick of iIEL. Whereas CD8 $\alpha^+\beta^+$ T cells are found in both the intestinal epithelium and the LP, CD8 $\alpha^+\beta^-$ are found exclusively in the intestinal epithelium. Thus, if iIEL do migrate along the length of the gut mucosa, there must be a route of circulation distinct from the lamina propria for some iIEL subsets.

The advantage of mouse studies is that it is possible to compare repertoires among genetically identical animals exposed to the same environment. In these circumstances, the results presented by Regnault et al. demonstrate that the oligoclonal repertoires of the two iIEL subsets analyzed differ among individual animals. These results are not easily reconciled with antigen driven oligoclonal expansion unless one assumes that different V β DJ β combinations encode receptors of similar antigen specificity. Of course the V α J α repertoire need be considered in this context, but was not characterized in this study. Another difficulty with antigen driven selection is the fact that intestinal flora differ along the bowel. This raises an alternative explanation for the basis of TCR oligoclonality of iIEL which involves selection/expansion of particular clones in response to the recognition of "self" antigen.

There is evidence in support of self-antigen/MHC skewing the repertoires of CD8 $\alpha^+\beta^-$ and CD8 $\alpha^+\beta^+$ iIEL. We (3), and others (30), have recently demonstrated that most CD8 $\alpha^+\beta^-$ iIEL in H-2 b male mice transgenic for an HY/H-2D b -specific TCR- α/β express the autoreactive transgenic TCR, whereas in female mice of the same haplotype, they do not. Importantly, the CD8 $\alpha^+\beta^+$ iIEL expressing the transgenic TCR are depleted in H-2 b males, whereas they are present in high frequencies in H-2 b females (3). The inference drawn by Regnault et al. is that the nonoverlapping repertoires of CD8 $\alpha^+\beta^-$ and CD8 $\alpha^+\beta^+$ iIEL rule out their lineage relationship. However, preferential selection/expansion of individual clones during maturation has not been excluded.

In closing, within the context of these new insights regarding the TCR/CD3 structure and repertoire of iIEL, it is appropriate to consider the recent data analyzing the potential influence of MHC molecules expressed in the gut. The absolute number of CD4 $^+8^+$ iIEL expressing TCR- α/β is profoundly reduced in MHC class I-deficient animals (31). In contrast, the phenotype of iIEL in MHC class II-deficient animals appears unaffected (2). It has been recently demonstrated that MHC class II molecules expressed by gut epithelial cells are distinct from those expressed at other sites within the same animal. Specifically, while no differences could be detected at the level of mRNAs for class II molecules and invariant chain (32), gut epithelial cells (enterocytes) do not

express invariant chain protein (32). This may explain the observation that most class II-specific mAbs fail to recognize MHC class II molecules expressed on enterocytes (32). Furthermore, recent evidence demonstrates that enterocytes and conventional APCs differ in their capacity to process and present protein antigens (33). Moreover, LPTs, some of which

may be derived from iIEL, respond to alloantigen expressed by enterocytes but fail to respond to the "same" alloantigen expressed on conventional APC (34).

Fundamental insights await the characterization of lineage relationships among iIEL subsets and understanding the role of iIEL and LPTs in the immune response.

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