four hours, as well as fasting gastric volume, are significant determinants of symptoms.^{6,7} To misquote a recent statement in a US Court of law: "When the glove *does* fit, you should *not* acquit."

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T cells

A ticket to the gut for thymic T cells

W Falk

The intestinal epithelium is colonised by, among other cells, normal T helper and cytotoxic T cells which obtain their antigen experience in the gut associated lymphatics after migration from the thymus. New evidence suggests a CD8 positive T cell that directly migrates to the intestinal epithelium increasing the role of the thymus in shaping intestinal immunity

A normal splenic T cell would be abhorred should it get close to the intestinal epithelium. Instead of its usual rather sterile environment, it would be bathed in an ocean of usually non-pathogenic microbial and food derived antigens never seen during its passage through the educating thymus. It would get ready to become very excited. However, although the number of T cells in the mouse small intestine is approximately 50% of those residing in all lymphoid organs,^{1,2} there is peace. This peace is the result of a balanced presence of activated protecting T cells and activated controlling T cells.

The gut represents a unique dilemma for the immune system. It has the largest surface of the body harbouring numerous species of microorganisms. It is thus challenged with the task to be a barrier to the outside world and also to be the organ for most effective absorptive and secretory functions. To be able to accomplish this task, mammals have evolved a special highly regulated protective immune system comprising T cells which are on constant alert, as is evident from their activated phenotype. Broadly, the gut harbours three categories of T cells. Those in the gut associated lymphoid tissues are very similar to T cells found in peripheral lymphoid organs. Another population of T cells is scattered between other

different cell types in the lamina propria. Their education follows the standard path of meeting their antigens presented by antigen presenting cells in the mesenteric lymph nodes and trafficking back to the lamina propria.³

The third category is a very peculiar one, intraepithelial lymphocytes (IEL). They sit on the luminal side of the basement membrane, singly scattered between and protected by intestinal epithelial cells, and are presumably the first T cells to encounter antigens. IEL comprise T cells carrying the $\alpha\beta$ T cell receptor (TCR) or the $\gamma\delta$ TCR, with their ratio depending on the species. Further subdivision may be made between conventional T cells characterised by a $\alpha\beta$ TCR and coexpression of CD4 or CD8 $\alpha\beta$ and non-conventional T cells which express either $\alpha\beta$ TCRs or $\gamma\delta$ TCRs but no CD4 or CD8 $\alpha\beta$ (double negative (DN)).⁴ Such cells are residents of the intestinal epithelium. A proportion of these cells express the CD8 $\alpha\alpha$ homodimer which, however, can also be detected on double positive conventional IEL together with CD4 or CD8 $\alpha\beta$. Non-conventional T cells are rarely found in tissues other than the intestinal epithelial layer and they are also characterised by a surprisingly high degree of autoreactivity. It was reported, using transgenic models, that CD8 $\alpha\alpha$ $\alpha\beta$ TCR IEL can be found in animals that

efficiently delete their CD8 $\alpha\beta$ cells in the thymus by negative selection.² This and other findings have led to the search for extrathymic sites allowing differentiation into gut specific T cells. In mice, small aggregations of haematopoietic cells were found in the lamina propria close to crypts which were named cryptopatches (CP).⁵ In elegant studies it was shown however that CP are most likely not the sites for extrathymic sources of IEL and that most, if not all, gut IEL originate from the thymus. Some recombinase activating gene activity was found in the absence of a functional thymus^{6,7} indicating that, under special conditions, extrathymic maturation of T cells can still occur in the mesenteric lymph nodes or Peyer's patches which is heavily skewed to generation of $\gamma\delta$ TCR T cells.

These $\gamma\delta$ TCR T cells do not recognise peptide antigens and leave the thymus at a DN stage to later colonise predominantly epithelial surfaces. They can be rapidly activated by some non-peptide antigens and self stress antigens, such as the human MICA and MICB molecules.^{8,9} Their capacity to secrete growth factors like KGF has also led to the assumption that they might be involved in repair of damaged tissue.¹⁰ Another fascinating feature was recently shown. Human V δ 2 expressing cells through activation were able to present peptide antigens to naive $\alpha\beta$ TCR T cells, thus bridging innate and acquired immunity.¹¹ Whether a similar function is also true for IEL $\gamma\delta$ TCR T cells remains to be shown.

Non-conventional $\alpha\beta$ TCR CD8 $\alpha\alpha$ T cells are likely to be a consequence of "agonist" selection by self antigens in the thymus (reviewed and discussed by Cheroutre¹²). It is proposed that these T cells interact very strongly with self antigens at a DN stage and so acquire the activated phenotype. These cells can then express the mucosal integrin $\alpha_E\beta_7$ and in this way be guided to the intestinal epithelium where the binding partner E-cadherin is abundantly expressed. Such cells do not damage

the epithelium¹³ but, in contrast, have a protective capacity, as was shown in the CD45RB^{high} transfer model of colitis.¹⁴

This leaves us with the conventional $\alpha\beta$ TCR T cells which normally undergo positive and negative selection due to intermediate strength interactions with self antigens presented by self MHC molecules in the thymus. They leave the thymus as either CD4 or CD8 $\alpha\beta$ mature T cells to follow the well trodden path of being activated by professional antigen presenting cells to become effector and memory T cells which then settle in defined locations. There is evidence that the more chancy moment of antigen encounter in the intestinal lymphoid structures imprints the cells to specifically home to the intestinal epithelium.^{15–16} This is the current thinking, but in this issue of *Gut*, Annunziato and colleagues¹⁷ challenge this view (see page 961). The thymus seems more sophisticated than just pouring out mostly conventional single positive T cells. The authors found a subset of mature thymocytes which in many respects closely resemble a subpopulation of conventional IEL, thus suggesting that the thymus produces these cells specifically targeted to the intestinal epithelium. Although their specific local function is still unknown, these cells may not be so "conventional".

A hallmark of all IEL is their constitutively activated phenotype. Accordingly, the thymus cell population identified by Annunziato and colleagues¹⁷ produces perforin and granzyme A, qualifying them as potential cytotoxic cells. They also produce cytokines such as interleukin 2 and interferon γ on polyclonal stimulation which support inflammation, and they express activation markers such as CD69 and CD45RO. All of this makes them look different from naïve mature T cells that await activation in peripheral lymphoid structures.

The current literature supports the notion that the gut TCR repertoire of conventional T cells is shaped by repeated encounter with gut specific antigens in the gut associated lymphoid structures, including Peyer's patches and mesenteric lymph nodes.³ This progressive selection leads to the typical oligoclonality normally found for gut associated T cells. The V β repertoire of this new subpopulation of T cells did not reveal any enrichment, ruling out the standard activation pathway. Support for this notion stems from the observation that these cells still have comparatively long telomers. Repeated stimulation results in frequent cell divisions which reduces the length of telomers.¹⁸ One would expect long telomers in a cell just leaving the thymus

but not in antigen experienced cells, as usually found in the gut. Yet this population of conventional gut T cells shows long telomers, supporting the conclusion that they just recently colonised the epithelium without having undergone multiple divisions.

How are the cells lured to the intestinal epithelial layer? The thymus micro-environment seems to provide a ticket and a guide. T cells are characterised by expression of the chemokine receptor CXCR3 and the presence of CD103, representing the α_E of the $\alpha_E\beta_7$ integrin. This is a sticky ticket because epithelial cells display E-cadherin and this interaction was shown to be essential for IEL localisation.^{19–20} Attraction is also provided by epithelial cells which can produce large amounts of two of the three ligands for CXCR3, CXCL9, and CXCL10.²¹ Support for the functional importance of CXCR3 is given by the authors by the use of CXCR3 deficient mice which show clear underrepresentation of CD8 $\alpha\beta$ T cells only in the gut, compared with wild-type mice, which was normalised within 24 hours after adoptive transfer of wild-type cells. In addition, it was shown recently that the chemokine receptor CCR7 is needed for T cell exit from peripheral tissue to enter afferent lymph.²² These cells specifically lack CCR7 so that they may not be trapped in lymphatic tissue. However, this equipment also means that cells are meant to stay in the intestinal epithelium.

There is no information in the report about a specific function of these interesting cells or about the signals in the thymus that are required to generate them. Provided the surface markers do not change, they will not be able to meet antigens in the lymphatics of the gut as the conventional cells are supposed to do. But there are other options. Firstly, their nearest neighbours, the intestinal epithelial cells, can perform efficient antigen presentation and produce cytokines and chemokines.^{23–24} Also, some dendritic cells, which can send their protrusions between epithelial cells to sample the intestinal lumen, are likely candidates.²⁵ Due to their polyclonal TCR, this subpopulation of T cells might be the firstline of defence to quickly combat antigens not normally present in the gut. Unveiling the role these cells play in intestinal homeostasis and disorders should be the next step.

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Colorectal cancer

Hedgehog Wnteraction in colorectal cancer

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The Hedgehog pathway was recently shown to antagonise constitutive activity of the Wnt pathway that drives proliferation of colorectal cancer cells. Studies in this issue of *Gut* refine our understanding of the underlying mechanism and provide evidence for such antagonism in colorectal cancers in vivo

The mucosa of the colon is covered by a single layer of epithelial cells that is replaced once every 3–7 days. New cells are produced by a pool of progenitor cells that lie at the base of small mucosal invaginations called crypts. It has been estimated that these progenitor cells generate approximately 10 billion new cells per day in the human colon.¹ Homeostasis in such a massive process of regeneration can only be achieved if the behaviour of cells in the system is not regulated at the level of the individual cell (intrinsic). Instead, the fate of individual epithelial cells must be determined by extracellular (extrinsic) signals that are generated at the population level. It is now becoming clear that these extrinsic signals are provided by morphogens, a class of molecules that has been identified by developmental biologists who studied patterning events during embryogenesis.² Morphogens are soluble proteins that form long range concentration gradients and act in a dose dependent manner to induce distinct cellular responses on their target cells. In this way, cellular fate is determined by the distance of a target cell from the source of the morphogen. Several morphogens have now been identified as regulators of cell fate in the adult colon.

The Wnt pathway (fig 1) was the first morphogenetic pathway that was implicated in colonic epithelial homeostasis when it was found that adenomatous polyposis coli (APC) acts as an important regulator of Wnt signalling.³ APC had initially been identified as the gene mutated in patients with familial adenomatous polyposis (FAP).^{4,5} Subsequently, mutations in APC have been found in the majority of sporadic

colorectal cancers.^{6,7} A central role in the Wnt pathway is played by β -catenin which can translocate from the cytoplasm to the nucleus to form a complex with Tcf transcription factors and regulate Wnt responsive genes. In the absence of Wnts, APC is required to hold together a protein complex that ensures continuous breakdown of β -catenin and prevents its nuclear translocation. Mutations in APC disrupt this complex and lead to uncontrolled stabilisation and nuclear accumulation of β -catenin.⁸ The probable reason for the link between unchecked activity of the Wnt pathway and intestinal tumorigenesis is the essential role of this pathway in the maintenance of intestinal precursor cell phenotype. Substantial evidence exists to show that canonical (β -catenin-Tcf mediated) Wnt signalling is required to drive intestinal precursor cell proliferation. Inactivating Wnt signalling by deletion of β -catenin,⁹ TCF4¹⁰ or expression of an inhibitor of soluble Wnts,¹¹ leads to loss of small intestinal precursor cells. Conversely, preventing inhibition of the Wnt pathway by inducible deletion of APC leads to rapid expansion of the small intestinal precursor cell compartment.¹² Although almost all experiments have focused on the small intestine, Wnt signalling probably plays a similar role in the colon as nuclear β -catenin is detected in the precursor cell compartment at the base of the crypts in the colon in vivo and Wnt signalling has been shown to maintain a precursor cell transcriptome in colon cancer cells in vitro.¹³ Given the importance of Wnt signalling in the maintenance of the precursor cell phenotype, it is understandable that APC mutant cells escape

the normal position dependent restraints on proliferation and are capable of clonal expansion even outside the crypt base.

Another morphogenetic pathway that regulates homeostasis of gastrointestinal epithelial cells is the hedgehog (Hh) pathway (fig 1).¹⁴ Similar to the Wnt pathway, the Hh pathway was initially identified in genetic screens for patterning genes in *Drosophila*.¹⁵ Three Hh genes have been identified in most vertebrates that have been named Sonic, Indian, and Desert hedgehog. Hhs act via a receptor system that consists of two different proteins, a Hh binding receptor patched (Ptc), expression of which is itself upregulated by Hh signalling, and a Hh signalling receptor smoothened (Smo). In the absence of Hh, Ptc inhibits Smo via a mechanism that remains to be resolved. When Hh binds Ptc, this inhibitory interaction is relieved and Smo signalling activated. Signalling downstream of Smo is still only partially understood but involves a complex of proteins that regulates the activity of downstream transcription factors Gli1–3. These Glis exist both in a full length activator and a truncated repressor form that is generated by proteolytic processing. Smo signalling inhibits the constitutive proteolytic processing of Glis leading to stabilisation and nuclear translocation of full length Glis and transcriptional activation of Hh target genes. Analysis of the phenotype of *Shh*, *Ihh*, and *Gli1-3* mutant mice suggest that Gli2 and Gli3 are the most important mediators of Hh signalling in the gut.¹⁶ Gli1 mutant mice do not seem to have a phenotype¹⁷ but Gli1 can rescue many aspects of loss of Gli2¹⁸ indicating that Gli1 function is similar but redundant. In the normal adult colon, Sonic hedgehog (Shh) is expressed by a few precursor cells at the base of the crypts¹⁹ whereas Indian hedgehog (Ihh) and Hh receptor Ptc are expressed by differentiated absorptive enterocytes at the luminal end of the crypt.^{20,21} Akiyoshi and colleagues²² now show in this issue of *Gut* that Gli1, both mediator and transcriptional target of Hh signalling, also localises to the differentiated colonic enterocytes at the top of the crypt (see page 991). These expression patterns are consistent with a major role of Hh signalling in cell fate regulation of colonic enterocytes at the top of the crypt. This is supported by the findings that Hh signalling regulates colonic enterocyte differentiation in HT29 colon