Gut

Leading article

Gamma/delta T cells in the gut epithelium

Although the intraepithelial T cell population in the gut is one of the largest in the body, the life history and function of intraepithelial lymphocytes has remained largely unknown. In 1988 considerable excitement was generated by the publication of two papers which suggested that intraepithelial lymphocytes in mice predominantly use the gamma/delta form of the T cell receptor (TcR),^{1,2} in contrast to most T cells in other lymphoid tissues and blood which use the alpha/beta form of the receptor. It is well established that α/β TcR+ cells recognise antigens presented on class I or class II major histocompatibility complex products; however, the antigens recognised by γ/δ TcR+ T cells are still poorly understood. There is also good evidence that α/β TcR+ cells and γ/δ TcR+ cells are of separate lineages.³ In a commentary accompanying the original description of these cells in the mouse, Janeway cited unpublished observations (at the time) which also showed that γ/δ T cells were predominant in human and chicken intraepithelial lymphocytes.⁴ Recently it has become clear that the situation is not as clear cut as was suggested, and that there are major differences between humans and rodents in γ/δ T cell expression in these lymphocytes. The observations in mice, however, prompted studies in humans which have given new insights into intraepithelial lymphocytes in coeliac disease that might be of pathogenic importance.

Mice

In the study of Goodman and Lefrancois,¹ mouse intraepithelial lymphocytes were surface iodinated, the cell membranes were solubilised, and immunoprecipitation was carried out using anti-CD3 monoclonal antibody.⁵ The TcR is non-covalently linked with the CD3 complex on the T cell surface and remains associated when solubilisation of the T cell surface is carried out using mild detergents. Immunoprecipitation with anti-CD3 thus precipitates not only the CD3 complex but also the TcR linked to it. Molecules of relative MW 34-35k corresponding to the γ chain, and 46k corresponding to the δ chain were immunoprecipitated. Similar results were obtained by Bonneville *et al.*,² who also precipitated bands representing abundant γ/δ heterodimers from intraepithelial lymphocytes.

The expression of T cell markers and TcR usage in intraepithelial lymphocytes of normal and athymic Balb/c mice has also been studied by immunoperoxidase histochemistry on frozen sections and by FACS analysis of isolated cells.⁶ In normal and athymic mice virtually all intraepithelial lymphocytes are CD3+, CD45+, that is, T

cells. α/β TcR+ cells can be identified in large numbers in situ in the intestinal epithelium of normal mice using the monoclonal antibody H57.597,⁷ which recognises all murine α/β TcR, and which stains around 50% of intraepithelial lymphocytes. These cells are not present in athymic mice.⁶

Guy-Grand *et al.*,⁸ using FACS analysis, extended these findings and showed that there were two main populations of intraepithelial lymphocytes in mice. About half use an α/β TcR, and are thymus dependent. Most of the other cells express the γ/δ TcR and are thymus independent. Northern blot analysis of intraepithelial lymphocytes from normal mice showed the presence of messenger RNA for both α/β and γ/δ TcR, whereas athymic mice had no α/β intraepithelial lymphocytes but did have intraepithelial lymphocytes with messenger RNA for the γ/δ TcR.

There appears to be a paradox between the immunoprecipitation data which fails to detect α/β TcR in intraepithelial lymphocytes and the immunohistochemical and FACS data where α/β TcR+ cells can be clearly identified. In germ free and weanling mice there are very few intraepithelial lymphocytes, but most appear to be γ/δ +. ⁸ This is the reverse of the case in older normal mice where there are increased numbers of intraepithelial lymphocytes and many α/β + lymphocytes. Thus the frequency of α/β TcR+ cells in the epithelium depends on the degree of antigenic stimulation from the gut and the age of the mouse. Investigators using young mice or specified pathogen free mice would find fewer α/β TcR+ cells in intraepithelial lymphocytes than investigators using normal mice with a varied intestinal flora and concurrent infections. Nevertheless, even if γ/δ TcR+ T cells make up only 30-50% of intraepithelial lymphocytes, this is still a much higher level than found in other T cell compartments.

Other species

Intraepithelial lymphocytes in rat gut have also recently been studied. By immunoperoxidase histochemistry, 61.4% of intraepithelial lymphocytes are α/β + by staining with R73 antibody which recognises all rat α/β TcR.⁹ T cells bearing the γ/δ TcR are more numerous in the gut epithelium of the chicken than in the underlying lamina propria, but a number of the CD3+ cells in the epithelium bear the α/β TcR.¹⁰ Analysis of the published figures (Fig 3 in Bucy *et al.*¹⁰) shows that only approximately half of the CD3+ cells are γ/δ + T cells. It should also be noted that in the chicken, γ/δ + T cells are more abundant in the periphery and other lymphoid tissue than in mice or humans.¹⁰

γ/δ TcR+ T cells in the epithelium of normal and coeliac jejunum. γ/δ TcR+ cells are uncommon in normal jejunum (top) but are abundant in coeliac epithelium (bottom). Immunoperoxidase with the antibody TCR δ -1 ($\times 400$ original magnification).

Humans

In humans there are several monoclonal antibodies available which recognise α/β TcR and γ/δ TcR. In 1987, Cerf-Bensussan *et al*¹¹ showed that 80–90% of human intraepithelial lymphocytes were WT31+ (at the time considered to be a marker of α/β T cells). It is now clear, however, that in some cases WT31 will also bind to γ/δ T cells, and so these results have been re-evaluated using the large number of anti- δ antibodies available. Five recent immunohistochemical studies on γ/δ intraepithelial lymphocytes in humans have all reached the same conclusion.^{12–16} In human intraepithelial lymphocytes about 10% of the CD3+ cells are γ/δ +, approximately the same proportion as in blood. It should be pointed out that there is wide variation between individuals, between 3% and 38% in a sample of 16 patients.¹⁶ The antibody BMA031 recognises the α/β TcR and stains most

human intraepithelial lymphocytes (92%).¹⁵ Small intestine from patients with coeliac disease, tropical sprue, cows' milk protein intolerance, and Crohn's disease have also been studied.¹⁶ Only in coeliac disease is the proportion of γ/δ T cells raised to over 30% of the CD3+ intraepithelial lymphocytes (Figure). Of great interest is the observation that γ/δ TcR+ intraepithelial lymphocytes remain increased in treated coeliac disease, even after the patient has been on a gluten free diet and has normal mucosal morphology and intraepithelial lymphocyte levels (E Savilahti, personal communication).¹⁶

There are, however, differences between γ/δ TcR+ intraepithelial lymphocytes and peripheral blood γ/δ T cells. In blood most γ/δ TcR+ cells use the disulphide linked form of the receptor and are recognised by the monoclonal antibody BB3, which identifies cells predominantly using the V γ 9,

V δ 2 gene rearrangements.¹⁷ In the gut, cells recognised by BB3 are relatively uncommon, whereas cells recognised by another monoclonal antibody, δ TCS-1 (which recognises the cells using the V γ 1, V δ 1 rearrangement) predominate.¹⁸ This is especially prominent in untreated coeliac disease where the increase in γ/δ + intraepithelial lymphocytes is independent of the BB3+ population and is largely accounted for by δ TCS-1+ cells.¹⁸

Do γ/δ TcR+ cells show epithelial tropism?

In the mouse it is clear that there is a population of Thy1+, γ/δ T cells which migrate from the thymus to the skin epithelium.¹⁹ Taken together with the results which showed that many mouse intraepithelial lymphocytes are γ/δ TcR+,¹² it has been considered that γ/δ TcR+ T cells may have a tropism for epithelia.⁴ The analogy between intraepithelial lymphocytes and skin γ/δ TcR+ T cells is incomplete, however, since most of the γ/δ intraepithelial lymphocytes in mice are not thymus derived.^{8, 20, 21} There is also good evidence that these cells migrate directly from the bone marrow to the epithelium, without the need for thymus processing.⁸

It is not clear whether γ/δ T cells undergo productive rearrangements of their T cell receptor genes in the marrow or elsewhere and then migrate to the epithelium, or whether precursors (perhaps also precommitted to use the γ/δ TcR) migrate to the epithelium and rearrange in situ. It is important to distinguish between these two possibilities for the former would indicate a tropism of γ/δ TcR+ cells for the gut epithelium, whereas the latter could reflect random migration to all tissues, but only maturation and differentiation within the epithelium. It would be difficult to preclude the possibility that a precursor of γ/δ + cells also selectively migrated to the gut epithelium in the absence of a marker for such a cell.

Immunohistochemical studies in chickens and humans have shown that in the gut γ/δ + cells are more common in the epithelium than the lamina propria.^{10, 14, 16, 18} T cells expressing the α/β TcR may transit rapidly through the lamina propria and leave the gut via the lacteals, whereas γ/δ TcR+ cells, or their precursors, might remain in the lamina propria. There is some evidence for this in that in mice γ/δ TcR+ T cells are not found in thoracic duct lymph, which mostly comprises gut derived cells.⁸ Once in the lamina propria, however, γ/δ TcR+ T cells must move into the epithelium relatively quickly in order to explain their relative sparsity in the lamina propria. It is also likely that once in the epithelium the γ/δ T cells do not re-enter the lamina propria, for if that was the case, then one would expect to see γ/δ + cells either side of the epithelial basement membrane, and a more equal distribution between the epithelium and lamina propria. In recent studies it has been shown that δ TCS-1+ peripheral blood γ/δ T cell clones which use the C γ 2 constant region and bear a non-disulphide linked TcR exhibit extensive motility in vitro.²² BB3+ clones, using the C γ 1 constant region and bearing disulphide linked TcR are not motile. Most of the γ/δ T cells in the epithelium are δ TCS-1+,¹⁸ which might be related to their increased migratory properties.

Are intraepithelial lymphocytes an end stage population?

There is some limited evidence that the gut epithelium represents an end stage tissue for lymphocytes. In mice the monoclonal antibody M371 only stains intraepithelial lymphocytes.²³ T cells elsewhere in the body do not bear this marker. Interestingly, M371 predominantly stains γ/δ T cells in the epithelium.²³ M371 cells are never seen in the lamina propria. If cells are induced to express M371 when they enter the epithelium then it would be difficult to imagine that if the

cell migrated back to the lamina propria it would immediately lose M371.

Lymphocytes in the epithelium, lying basally between the epithelial cells, are also surrounded by epithelial cells constantly migrating up the sides of the villi to be extruded from the villus tips. Autoradiographic studies in mice indicate that intraepithelial lymphocytes do not divide in the crypts and migrate up the sides of the villi, but travel back and forth across the basement membrane from the lamina propria.²⁴ The cells migrating back into the lamina propria have not been categorised in terms of TcR, nor had it been determined if more cells enter the epithelium than re-enter the lamina propria.

If most γ/δ TcR+ intraepithelial lymphocytes do not re-enter the lamina propria then they must eventually enter the lumen or else the epithelium would soon become filled with lymphocytes. The movement of lymphocytes within the epithelium is not known. They may be carried towards the villus tips by the epithelial cells, they may remain static and allow the epithelial cells to flow around them, or they may be able to migrate laterally between the epithelial cells to retain their position on the villus. Heyworth and his colleagues have shown that about 30 000 viable lymphocytes can be collected in a single short lavage of mouse intestine.²⁵ Cell death in the lumen and the fact that the lavage will probably not sample cells in the mucus coat make it likely that 30 000 is a minimum estimate of the number of intraepithelial lymphocytes being lost into the gut. In addition, a single lavage gives no estimate of the rate of intraepithelial lymphocyte loss into the lumen, which may be considerable. It would be of interest to identify the T cell receptor usage of lymphocytes recovered from the gut lumen. Alternatively, intraepithelial lymphocytes may die in situ, though detailed electron microscope studies fail to substantiate this notion.²⁶

Importance of increased number of γ/δ TcR+ cells in coeliac disease

It has been recognised for many years that lymphocyte density is increased in the epithelium in untreated coeliac disease.^{27, 28} In untreated coeliac disease the percentage of CD3+, CD4-, CD8- (subset negative) T cells increases from 6% to over 30% of CD3+ intraepithelial lymphocytes.²⁹ Interestingly, the percentage remains high in patients on a gluten free diet.²⁹ The same occurs with γ/δ TcR+ intraepithelial lymphocytes which remain high in coeliac patients even on a gluten free diet when mucosal morphology has returned to normal (E Savilahti, personal communication).¹⁶ This would be expected since γ/δ cells are more common in the subset negative population than in CD4+ or CD8+ T cells.³⁰ The main function attributed to γ/δ cells is that of non-major histocompatibility complex restricted cytotoxicity,³¹ although there are more and more reports of γ/δ TcR+ cells with specificity for nominal antigens, classical alloantigens, and class Ib histocompatibility antigens such as CD1.³² In addition, in mice intraepithelial lymphocytes are potent cytotoxic effector cells.¹ It is possible that in coeliac disease γ/δ intraepithelial lymphocytes are cytotoxic for epithelial cells. These lymphocytes could be induced to become cytotoxic by specific recognition of gluten, presented by an epithelial cell, or non-specifically by interleukin-2 released by gluten reactive α/β T cells. In the absence of gluten, γ/δ TcR+ T cells, although present, would not be activated, and hence would not be cytotoxic.

On a gluten free diet, patients with coeliac disease still have raised γ/δ intraepithelial lymphocytes. As discussed above this might reflect increased tropism of these cells for the epithelium, or increased numbers lodging in the epithelium and undergoing productive rearrangements in situ. More γ/δ cells would produce a greater cytotoxic response when these

cells are activated, contributing to the surface enterocyte damage which is so much a feature of coeliac disease. This of course might still be an epiphenomenon unrelated to crypt hypertrophy and villus atrophy since there is now good evidence that a flat mucosa can be produced by activated lamina propria T cells³³ which do not bear the γ/δ TcR.³⁴ In addition, in patients with villus atrophy of uncertain aetiology, unresponsive to gluten withdrawal, the histological lesion is indistinguishable from untreated coeliac disease, but the proportion of γ/δ TcR+ cells in the epithelium is reduced.¹⁶

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