Influence of Lymphocytes and of Cell-Mediated Immunity on the Epithelial Cell Kinetics in the Intestine

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Ontogeny of the intestinal immune system is relevant to infant nutrition in many ways. Not only are lymphocytes and lymphoid tissues integral components of the stomach, small intestine, and colon, but immune responses in these organs also influence the nature of the commensal gut flora, confer protective immunity against pathogenic microorganisms and parasites, and contribute, via hypersensitivity mechanisms, to intestinal diseases, including malabsorption. Studies of human neonatal intestinal immunology have not been performed, and, indeed, clinical investigation of the gastrointestinal lymphoid apparatus in man has been confined almost exclusively to studies of secretory antibodies and to counts of mucosal lymphoid cells. We have directed our attention to the lymphocytes of the small intestine, and to induction of cell-mediated immunity (CMI) in the intestinal mucosa. To date, most of their research has been performed in experimental animals. This chapter briefly reviews recent information on the nature of mucosal T-cells and their traffic and on the effects of a mucosal CMI reaction on the small intestinal epithelium. It also outlines a hypothesis of the various roles which mucosal T-cells may play in gastrointestinal physiology and pathology.

T-LYMPHOCYTES IN THE INTESTINES

There are T-lymphocytes in the organized gut-associated lymphoid tissues, such as Peyer's patches and mesenteric lymph nodes (MLNs) and many T-cells are scattered within the mucosae of the gastrointestinal tract.

Peyer's Patch Lymphocytes

The majority of Peyer's patch T-cells are present in the thymus-dependent areas under the epithelium and around the post-capillary venules. Studies of

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lymphocyte traffic, which involve tracing of isotope-labeled lymphocytes by autoradiography and liquid scintillation counting, show that there is a traffic of small T-lymphocytes from blood to the thymus-dependent areas of Peyer's patches, but that small T-lymphocytes do not enter the mucosa (21). It is still not known whether or not there are two distinct pools of recirculating small T-lymphocytes, intestinal and peripheral. Certainly, gut-associated immune reactions often occur independently of systemic immunity, but the cellular basis of this may be the separate traffic of intestinal and peripheral activated T lymphoblasts rather than of small T-cells.

Mucosal T-Cells

Lymphocytes, mainly medium-sized, are found within the epithelium (intraepithelial, IE lymphocytes) (4) as well as in the lamina propria of the gut (LP lymphocytes). Although the small-intestinal mucosa has been most extensively studied, similar IE and LP lymphocytes are present, although in smaller numbers, in gastric and colonic mucosae. The first evidence that many of these mucosal lymphocytes are thymus-dependent came from the findings of low IE lymphocyte counts in animals depleted of T-cells in various ways (7,9). Recent work, in rodents and in man, using antisera directed against T-cell antigenic determinants, has confirmed that most IE lymphocytes are T-cells (10,14,24). By using the OKT antiserum, the majority of IE T-lymphocytes have been found to be of the suppressor/cytotoxic phenotype (staining with OKT8 antiserum) (23).

Studies of lymphocyte traffic have shown that, just as in the case of B-cells, T-immunoblasts derived from lymph or from MLNs home to the gut mucosa, where most can later be identified as IE cells (10). Further, elegant experiments involving local irradiation of Peyer's patches or MLNs have confirmed that the route of T-cell traffic is from Peyer's patches via MLN and lymph back to the IE site in the small intestinal mucosa (14).

An important additional source of T-cells in the gut has been demonstrated by Rose and her colleagues (22) in their studies of T-immunoblast migration. They found that in normal animals mesenteric T-immunoblasts migrated back to the intestine, and peripheral T-immunoblasts migrated to inflamed peripheral tissues. However, peripheral T-immunoblasts were also found to home to the gut, but only if it was the site of inflammation, for example, as a result of infection with a nematode. Thus, it is likely that in situations of intestinal inflammation components of the systemic immune apparatus, such as serum proteins, may gain access to mucosal tissues, and local disease will also lead to recruitment of systemic T-lymphocytes into the gut.

ANIMAL MODELS OF INTESTINAL MUCOSAL CMI

The small-intestinal mucosa is a continuously changing organ, and, in addition to the considerable amounts of fluid which cross the epithelium in both
directions, the epithelial and connective tissue cells are regularly renewed by cell division in the crypts and exfoliation from the surface. Since CMI reactions take a day or more to evolve, it could be argued that conditions will rarely be appropriate for a CMI reaction to occur in this organ. However, the authors and others have successfully produced mucosal CMI reactions in a number of experimental situations, including graft-versus-host (GvH) disease, rejection of transplanted allografts of intestine, parasite infections (comparing normal and T-cell depleted hosts), and, recently, mucosal challenge with antigen in animals immunized orally or systemically.

The primary objective of our series of experiments in animals has been to establish the features of intestinal pathology and/or changes in function which mark the presence of a local CMI reaction, and which can allow measurement of the magnitude of a CMI reaction in experimental conditions and in man.

Effects of Allograft Rejection on Small-Intestinal Mucosa

The models which we have studied most thoroughly have been rejection of hetrotransplanted grafts of fetal intestine in mice, and GvH disease in adult and neonatal F₁ mice. Experiments with mice which have been thymectomized, irradiated, and bone marrow reconstituted (8) and studies of the humoral immune response to allografts of fetal small intestine (3) confirm that the tissue damage in allograft rejection is thymus-dependent and cell-mediated. The effects of allograft rejection on the small-intestinal mucosa have been studied by conventional histopathology with subjective grading of the abnormality, by morphometry of paraffin sections, and by counts of IE lymphocytes. We have also used a stathmokinetic technique to measure crypt cell production rate and scanning and transmission electron microscopy to study epithelial changes (5,8,11,12).

The sequence of events observed in small-intestinal allograft rejection are infiltration of the LP by lymphocytes, which are also found within the epithelium; greatly increased mitotic activity in the epithelial cells of the crypts of Lieberkuhn with, later, flattening of the villi, exfoliation of surface enterocytes, and, finally, ulceration and destruction of the mucosa. The pathology of allograft rejection is very similar to that of the jejunal mucosa in celiac disease, and in helminth parasite infections of man and animals.

By using various strain combinations, and neonatal and adult hosts, a spectrum of intestinal pathology (as assessed subjectively) has been demonstrated, ranging from apparently completely normal histology to subtotal villous atrophy with crypt hyperplasia (Table 1). However, studies of the cytokinetics of the absorptive epithelium (the technique used involved injection of colchicine to block mitosis in metaphase, microdissection, measurements of villi and crypts, and counts of crypt metaphases) (12) have shown that even when conventional histological appearances are normal, there is an increase in ep-
**TABLE 1. Subjective grading of small-intestinal pathology in animal models of CMI in the small-intestinal mucosa**

<table>
<thead>
<tr>
<th>Model</th>
<th>Strains</th>
<th>Pathology of small intestine&lt;sup&gt;a&lt;/sup&gt; (1 week)</th>
<th>Pathology of small intestine&lt;sup&gt;a&lt;/sup&gt; (2 weeks)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rejection of heterotopically transplanted allograft</td>
<td>CBA–BALB/c</td>
<td>SVA</td>
<td>Destroyed</td>
</tr>
<tr>
<td>Rejection of heterotopically transplanted allograft</td>
<td>CBA–C3H</td>
<td>Normal</td>
<td>PVA</td>
</tr>
<tr>
<td>GvH disease (neonatal host)</td>
<td>CBA–C3H</td>
<td>PVA</td>
<td>Normal</td>
</tr>
<tr>
<td>GvH disease (adult host)</td>
<td>CBA–BALB/c</td>
<td>Normal</td>
<td></td>
</tr>
<tr>
<td>GvH disease (adult host)</td>
<td>CBA–C3H</td>
<td>Normal</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> PVA, partial villous atrophy with crypt hyperplasia; SVA, subtotal villous atrophy with crypt hyperplasia.

Epithelial cell proliferation rate, accompanied by increased lymphocyte infiltrate of the tissue, the two features which are found in the early stages of allograft rejection.

**Evolution of Intestinal Mucosal Changes in GvH Reaction**

A series of experiments have been conducted in order to define the speed of onset of effects on epithelial cell kinetics in the graft–versus–host reaction (GvHR) and, further, to examine the hypothesis that mucosal changes occur by direct effect of T-cells on the crypts rather than via preliminary damage to villus cells (6,16,18). A GvHR was induced in neonatal F<sub>1</sub> hybrid mice (CBA × BALB/c)F<sub>1</sub>, by intraperitoneal injection of spleen cells from adult CBA strain mice. Control littermates were injected with either F<sub>1</sub> spleen cells or medium alone. GvHR was induced between 5 and 7 days after birth, and, at intervals after transfer of parental cells, recipient mice were killed, weighed, their spleens removed and weighed, and the magnitude of the GvHR measured by a spleen index, being mean relative spleen weight (mg/10 g body weight) in GvHR mice divided by mean relative spleen weight in controls (Fig. 1). Measurements of jejunal villi and crypts were performed in Feulgen-stained preparations, by a microdissection technique, and crypt cell production rate by a stathmokinetic technique using colchicine. Tissue was also processed for routine histological examination and IE lymphocyte counts carried out on H & E stained sections; mucosal mast cell counts were performed in Carnoy-fixed, astra blue safranin-stained sections. Groups of animals were studied between one and 62 days after induction of GvHR.

There was no reduction in villus length in animals with GvHR; however, both crypt length (Fig. 2) and crypt cell production rate increased significantly within 3 days of induction of the GvHR, and there was significant correlation between crypt length and spleen index ($r = 0.76, p < 0.01$) and between cell production rate and spleen index ($r = 0.74, p < 0.01$). IE lymphocyte count
FIG. 1. Progress of the GvHR in neonatal CBA × BALB/c F₁ mice. Spleen indices in mice with GvHR, and growth rates of GvHR and control mice. Bars represent mean ± 1 SD. The lower panel gives details of the number of mice per group and age at time of death. (From Mowat et al., ref. 18, with permission.)

FIG. 2. Crypt lengths in neonatal CBA × BALB/c F₁ mice with GvHR and in littermate controls, at intervals after transfer of parental cells. Mean ± 1 SD for each age group. (From Mowat et al., ref. 18, with permission.)
(Fig. 3) was also significantly correlated with spleen index \((r = 0.86, p < 0.01)\) but the mucosal mast cell count, although it did rise, did not parallel the GvHR.

**Phases of Intestinal Mucosal CMI Reactions**

The experiments reported above confirm our previous hypothesis that both crypt cell production rate and the IE lymphocyte count provide evidence, in animal experiments, of a local CMI reaction. We have suggested that these features, when found in a jejunal biopsy from a patient with intestinal infection or inflammatory disease, may indicate that T-cell-mediated responses are taking place within the mucosa.

It is clear that the effects of local CMI reactions on epithelial cell kinetics occur in two stages (6):

*Phase 1* appears normal to conventional histopathological examination, for the villi are of normal length, but there is an increase in mitotic activity of the crypts, and, when compared with normals, the enterocytes move more rapidly up the sides of the villi and have a shorter lifespan.

*Phase 2* crypt hyperplasia persists with a high mitotic rate, but villi are short or absent, and, on the basis of electron microscopic studies of the enterocytes (5), it seems likely that this effect is due, not to direct damage of villus entero-
cytes, but to an effect of the underlying immune reaction on the adhesion of epithelial cells to one another and to the underlying tissues.

**Mechanism of T-Cell-Mediated Damage to the Intestine**

The traffic of activated T-cells from Peyer’s patches via lymph back to the intestinal mucosa has been discussed above. Thus, within a few days of the ingestion of a new antigen, or infection of the intestinal mucosa with a newly encountered agent, the potential for any of a variety of T-cell-mediated immune reactions will be presented within the mucosa. As illustrated in Fig. 4, there are a number of ways in which the presence of antigen, together with the presence of a population of antigen-specific T-cells, could lead to mucosal damage. These include the spectrum of antibody-mediated immune reactions (including reaginic hypersensitivity and immune complex hypersensitivity) via helper T-cell effects; direct cytotoxicity due to cytotoxic T-cells in the LP or epithelium; and/or the effect of various humoral factors secreted by activated T-lymphocytes, lymphokines. Although we by no means exclude cytotoxic T-cell effects, and effects of humoral antibody, we consider that there is now good evidence that the features of intestinal mucosal damage described above are the result of secretion of lymphokines by activated T-cells, rather than by direct cytotoxicity. We initially made these proposals in view of the absence of evidence of cytotoxicity by the IE lymphocytes, as assessed by electron microscopy. However, support for the existence of humoral enteropathic factors in GvH disease has been provided by the work of Elson et al. (2). These workers produced GvH disease in mice, the animals previously having had implants of fetal small intestine. The objective of their experiments was to determine whether the intestine was injured in the GvHR as a direct antigenic target of immunocompetent cells or as an “innocent bystander” to the donor—

![FIG. 4. Possible mechanisms for T-cell-mediated intestinal mucosal damage—including direct cytotoxicity, enteropathic lymphokines, and antibody-mediated hypersensitivity, indirectly induced via helper T-cells.](image)
<table>
<thead>
<tr>
<th>Experimental group</th>
<th>Tissue</th>
<th>Villus height (µm)</th>
<th>Crypt mitoses (per hr; mean)</th>
<th>IEL count (per 100 enterocytes; mean)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (F&lt;sub&gt;1&lt;/sub&gt; cells)</td>
<td>Host jejunum</td>
<td>484.5</td>
<td>6.1</td>
<td>9.9</td>
</tr>
<tr>
<td>GvH (CBA cells)</td>
<td>Host jejunum</td>
<td>536.1</td>
<td>10.6 (p &lt; 0.02)</td>
<td>16.0 (p &lt; 0.001)</td>
</tr>
<tr>
<td>Control (F&lt;sub&gt;1&lt;/sub&gt; cells)</td>
<td>Grafted small intestine</td>
<td>288.5</td>
<td>3.1</td>
<td>3.7</td>
</tr>
<tr>
<td>GvH (CBA cells)</td>
<td>F&lt;sub&gt;1&lt;/sub&gt; graft</td>
<td>284.7</td>
<td>8.6 (p &lt; 0.01)</td>
<td>6.9 (p &lt; 0.001)</td>
</tr>
<tr>
<td></td>
<td>CBA graft</td>
<td>290.3</td>
<td>6.2 (p &lt; 0.05)</td>
<td>7.2 (p &lt; 0.001)</td>
</tr>
</tbody>
</table>
host lymphoid interaction. Results of their experiments supported the latter explanation.

We tested our hypothesis that mitotic activity of the crypts of Lieberkuhn and infiltration of the tissue with lymphocytes were markers of the presence of CMI in the intestinal mucosa by using Elson’s graft-versus-host model (16). The protocol is outlined in Table 2. Both CBA × C3H and CBA × BALB/c strain combinations were examined, and similar results were obtained for both. Heterotopic grafts of fetal small intestine were transplanted under the kidney capsules of adult F₁ hosts. In half of the animals, the transplanted tissue was F₁ fetal gut, and, in the others, transplanted tissue was of the parental strain CBA. Four weeks later GvHR was induced in the hosts by the intraperitoneal injection of 60 × 10⁶ CBA spleen cells. Two weeks later the animals were injected with colchicine, killed at intervals over the next 2 hr, and measurements made of mucosal structure and epithelial cell proliferation in the crypts of Lieberkuhn, together with counts of IE lymphocytes. Measurements of spleen weight confirmed the presence of GvH disease in the adult F₁ hosts. Results for the CBA × BALB/c strain combination are summarized in Table 2. Subjective examination of intestinal histology in the host intestine showed no difference between controls and GvH animals. Objective measurements, however, confirmed that, although villus height was similar in the two groups, crypt cell production rate and IE lymphocyte count were both significantly higher in the GvH animals when compared with the controls.

Studies of the grafts showed that, as in previous experiments, villus height and IE lymphocyte counts were lower in isografts than in normally sited intestine, but, in both types of grafts implanted in animals with GvH reaction, crypt cell production rate and IE lymphocyte count were significantly higher than for isografts. Thus, in a GvH reaction induced in CBA × BALB/c F₁ hosts by the injection of CBA cells, crypt hyperplasia and IE lymphocyte infiltration have been produced in a graft of CBA fetal intestine. These results confirm the “innocent bystander” phenomenon in this GvH disease model and support the hypothesis that crypt cell production and IE lymphocyte counts are markers of this local CMI reaction.

INDUCTION OF INTESTINAL CMI TO A FED ANTIGEN

Tolerance and Suppressor Mechanisms

Feeding of antigen induces a secretory antibody response, but little is known of the factors which will induce CMI to fed antigens. We failed in a number of previous attempts to induce CMI to fed antigens using a variety of antigen dosage, regimes, and routes of administration. In retrospect, these failures can be explained by recent evidence that oral administration of antigen induces the specific immune reaction of tolerance for CMI as well as humoral immunity (15). The fact that feeding of protein antigen to mice results in reduced humoral
TABLE 3. Effects of mucosal immune reaction to ovalbumin on intestinal tissues

<table>
<thead>
<tr>
<th>Induction</th>
<th>Challenge</th>
<th>Migration index for MLN cells*</th>
<th>Villus height (µm)</th>
<th>Crypt mitoses (per hr)</th>
<th>IEL count (per 100 enterocytes)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyclophosphamide</td>
<td>—</td>
<td>1.12</td>
<td>680.7</td>
<td>6.6</td>
<td>15.8</td>
</tr>
<tr>
<td>Ovalbumin, 2 mg</td>
<td>Ovalbumin</td>
<td>1.00</td>
<td>638.8</td>
<td>8.4</td>
<td>14.5</td>
</tr>
<tr>
<td>0.1 mg (×10)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cyclophosphamide</td>
<td>Ovalbumin</td>
<td>0.63</td>
<td>686.5</td>
<td>12.0*</td>
<td>25.9*</td>
</tr>
<tr>
<td>0.1 mg (×10)</td>
<td>(p &lt; 0.01)</td>
<td></td>
<td></td>
<td>(p = 0.025)</td>
<td>(p &lt; 0.005)</td>
</tr>
</tbody>
</table>

* Cultured in presence of 1 mg/ml ovalbumin. As positive control popliteal and auxiliary LN cells from animals immunized by footpad injection were similarly studied; these gave migration index of 0.70.
and CMI responses when that antigen is subsequently presented is probably associated with the ability of the gut-associated lymphoid tissues to generate suppressor T-cells in response to orally administered antigen (13,20).

We have, by using BALB/c mice, confirmed the results of others that prefeeding of mice with ovalbumin subsequently renders the mice tolerant to induction both of humoral and CMI responses to ovalbumin when given parenterally. We reasoned that if we treated animals with an agent which reduced or eliminated suppressor cell activity, it might be possible to alter the nature of the immune response to fed antigen, and thereby induce CMI. Administration of cyclophosphamide to mice in the dose of 100 mg/kg enhances CMI reactions via suppressor cell inhibition without an appreciable effect on antibody synthesis (1), so we used the following regime. BALB/c mice were treated with cyclophosphamide alone (100 mg/kg), oral ovalbumin alone (2 mg), or cyclophosphamide followed by oral ovalbumin. Four weeks later they were challenged with ovalbumin in drinking water at a dose of 0.1 mg daily for 10 days. At the end of this time the animals were killed and, in some, evidence of gut-associated CMI to ovalbumin was sought by using direct migration inhibition of MLN cells in the presence of ovalbumin (19), whereas in others crypt cell production rate and IE lymphocyte counts were made (17) to measure the mucosal CMI reaction. Results are summarized in Table 3. It can be seen that, with the migration inhibition technique, the positive control of draining lymph nodes from mouse footpad showed significant migration inhibition; animals which had been fed ovalbumin alone, or which had been given cyclophosphamide alone, had no significant migration inhibition, but the combination of cyclophosphamide and ovalbumin resulted in the presence of lymphoid cells, positive in the test described, in the MLNs. Furthermore, significant changes in crypt cell production rate and IE lymphocyte counts were obtained only in the cyclophosphamide/ovalbumin group.

These preliminary experiments indicate that there is probably a spectrum in the CMI reaction to dietary antigens which ranges from the induction of a population of sensitized T-cells, on the one hand, to the induction of specific tolerance, associated with a population of suppressor cells, on the other. This and similar animal models should allow elucidation of the factors which influence mucosal CMI responses and may be of value in establishing the pathogenesis of food allergic diseases.

ACKNOWLEDGMENTS

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REFERENCES

DISCUSSION

Dr. Guesry: It seems that the permeability of the gut to food antigen is increased in premature babies—and it also seems that they don’t react as much as normal babies would. Do you think that this could be explained by a defect in the helper T-cells, or by some kind of immunological defect due to immaturity?

Dr. Ferguson: I am not as persuaded as you are of the evidence that there truly is increased permeability of the gut in the neonate. After all, the amount of antigen which reaches the intestine is profoundly influenced by nonimmunological factors, such as gastric acid. Animal experiments show that achlorhydria enormously increases the amount of antigen which reaches the small intestine. Furthermore, the mere existence of permeability to protein is not necessarily the cause of hypersensitivity diseases. We all experience continuous penetration of small amounts of protein across the intestinal epithelium to reach the immune system. The net effect of this on helper and suppressor mechanisms is likely to be influenced in part by the amount of protein, but also by its immunochmical nature. For example, the presence or absence of aggregation, whether the antigen is monovalent or multivalent. So the normal neonate may be protected from harmful effects of antigen absorption because it has not yet acquired helper T-cells for cell-mediated immunity. It does seem that in the first year of life suppressor mechanisms are likely to be very important in modulation of the immune response, so that perhaps relative deficiency of suppressor mechanisms is the explanation for milk protein sensitivity in infants. Older children and adults not only have a more mature digestive apparatus, but also are likely to have several suppressor mechanisms which regulate this important homeostatic property of the gut immune system.

Dr. Arnaud-Battandier: Do you think that the secretion of soluble factors, lymphokines, is a specialized function of the IE lymphocytes, or is it a property of T-cells in general, those confined to the LP, or even those in Peyer’s patches?

Dr. Ferguson: I do not think IE lymphocytes are the cells responsible for the phenomena which we have shown. IE lymphocytes comprise at least two populations, and one group may not even be T-cells, for there is some evidence, including your own work, which shows them to be natural killer cells. Nevertheless, some IE lymphocytes have surface markers which suggest they are the suppressor/cytotoxic phenotype. In man, T-cells with the helper surface markers appear to be mainly in the LP, so that even within the intestinal mucosa there is a segregation of different T-cell subclasses. We think that the T-cells in the LP, clustered around the crypts, are likely to be those involved in T-cell hypersensitivity. As for the nature and function of cells within the epithelium, a great many groups are studying these. We have suggested that the function of IE lymphocytes may indeed be inertia, protecting the 30 μm deep layer lining the intestinal mucosa from harmful hypersensitivity reactions. [Mowat, A. M., and Ferguson, A. (1981): Induction and expression of mucosal cell-mediated immunity. In: Current Topics in Veterinary Medicine and Animal Science, edited by F. J. Bourne, pp. 107–129. Martinus Nijhoff, London.]

Dr. Arnaud-Battandier: I agree with you that probably many IE lymphocytes are not T-cells and that other populations are likely also to be present.
Dr. Sunshine: When you found crypt hyperplasia, was there also an increase in intestinal secretion? I have been bothered for a long time that in situations where there is crypt hyperplasia, there also seems to be an increase in the intestinal secretion going along with it.

Dr. Ferguson: I have not measured enzymes or transport function in these experimental models. Certainly we have confirmed our results by the stathmokinetic technique by limited experiments using autoradiography. Delphine Guy-Grand in Paris has similar results which confirm the existence of crypt hyperplasia in GvHR, with an accelerated rate of cell transit up the sides of the villi. Immature cells on the villi would be more likely to have impaired absorptive functions and even salt and water secretion. GvHR is an easy experimental model to reproduce, and would certainly be amenable to studies of the effects of intestinal CMI on absorptive and secretory functions.

Dr. Halikovski: Have you looked at vascular changes in the villi during the reaction you have described?

Dr. Ferguson: We used electron microscopy to study allograft rejection. This work was done with Dr. Carr in Glasgow, and, in the main, we concentrated on epithelial cells. We noticed that the epithelium seemed to strip readily from the LP and concluded that there were likely to be changes in the basal lamina and in LP, but our anatomical techniques did not detect any. So I am afraid I cannot answer your question.

Dr. Auricchio: Please comment on the absence of villous atrophy in these experimental models you use.

Dr. Ferguson: We are very interested in the state of crypt hyperplasia in the absence of villous atrophy. I have selected only a few of our experiments to illustrate the phenomena, but we have found a complete spectrum of appearances, from normal villi to mild, partial villous atrophy, and, in allograft rejection, a completely flat mucosa. I think that there are probably two separate effects of T-cell-secreted lymphokines in the small intestine. One is to stimulate crypt mitosis—and, since the LP is still there in normal amounts, villi remain of normal height, and cells merely move more rapidly up the sides of the villi. The second factor, which may exert its effect only when there is a vigorous CMI reaction, or may require a higher concentration, is truly toxic. We think that the villous atrophy occurs because the LP crumples, not because there is direct toxicity to epithelial cells. After all, even in celiac disease, for example, in patients undergoing gluten reintroduction, one often finds a state where there is crypt hyperplasia with high counts of crypt mitosis, but with villi remaining of normal length. I am convinced that, because of the dynamic nature of the gut epithelium, there is a stage where there is relative compensation for the early effects of intestinal CMI. Of course, sophisticated studies of function of rapidly proliferating enterocytes may well demonstrate subtle abnormalities in their functions.