

Progress report

Intraepithelial lymphocytes of the small intestine

Part 1: Morphology and experimental immunology of intraepithelial lymphocytes

HISTORICAL ASPECTS

The epithelial lining of the small intestine contains within it many non-epithelial cells, the majority of which are lymphocytes. Small round cells within the epithelium of the small bowel were described in 1847 by Weber¹ who thought that these performed some function in nutrient absorption. Eberth² was the first to recognise these cells as leucocytes and before the end of the 19th century several detailed descriptions of the intraepithelial leucocytes in man and other vertebrates were published³⁻⁶. The early literature was reviewed in 1939 by Wolf-Heidegger⁷, in 1947 by Andrew and Sosa⁸, and recently by Otto⁹. Advances in the understanding of lymphocyte immunology have rendered obsolete all the earlier theories as to the derivations, fates, and functions of intraepithelial lymphocytes. However, although conventional light microscopy was the only investigative technique available to these early pathologists, their theories and hypotheses reveal remarkable ingenuity, imagination, and some insight as to the significance of this population of cells. Guieysse-Pellissier¹⁰ proposed that lymphocytes migrated into the epithelium to rejuvenate the ageing enterocyte nuclei by a process which he called 'caryoanabiosis'; this idea was developed further by Goldner¹¹ who proposed that the wandering mesenchymal cells were the only source of enterocyte nuclei. In the 1950s there was a theory that lymphocytes might act as trephocytes—that is, transfer nutrients to other cells by means of dialysis, cytoplasmic budding, or even by cytolysis. In their book on lymphocytes and mast cells, Kelsall and Crabb¹² illustrated the many features of intraepithelial lymphocytes which would make them suited to this trephocytic role.

Another hypothesis was that the gut was the graveyard of lymphocytes, the site of loss of the many millions of lymphocytes known to pour into the bloodstream each day from the thoracic duct¹³. However, this theory was refuted by Erf¹⁴ who showed that lymphocytes could disappear from the blood even when the entire small and large intestines had been resected. Now, of course, it is known that lymphocytes leave the bloodstream to recirculate from blood to lymph in the lymphoid tissues¹⁵.

In 1967 Fichtelius¹⁶ originated yet another theory of the functions of the intraepithelial lymphocytes which he called 'theliolymphocytes'. He had undertaken an extensive phylogenetic study of the lymphoepithelial organs¹⁷ and had found lymphocytes within the gut epithelium of all species studied. He proposed that the theliolymphocytes were a population of cells, still evolving in mammals as a 'bursa-equivalent', influencing and controlling

the maturation of B lymphocytes. The strength of this argument was reduced by his simultaneous publication of work suggesting that Peyer's patches, skin, salivary glands, bronchi, and mammary glands were also bursa equivalents¹⁷⁻²⁰. Nevertheless, despite the lack of evidence to support his theory, the basis of Fichtelius' ideas is still interesting. He points out that primitive antigen exposure was at body surfaces, skin, and gut. At first the epithelial cells were directly antigen-reactive, and capable of phagocytosis. Perhaps they have maintained this defensive role by instructing lymphocytes and antibody-forming-cells which evolved later. This is certainly true of the thymus and bursa of Fabricius, which are derived from epithelial surfaces; their presence certainly does not preclude a continuing direct instructive function of the epithelia.

NOMENCLATURE

A variety of names have been used to describe the lymphocytes which wander within the epithelium—*runde Zellen*, *Lymphocytenwanderung*, *Zellenwanderung*, epithelial lymphocytes, theliolymphocytes, intraepithelial lymphocytes, interepithelial lymphocytes. As these cells lie within the epithelial layer—that is, are between the basement membrane and the lumen—and are between the enterocytes, their precise description is 'intraepithelial' but 'interenterocytic'. In this review the term intraepithelial, abbreviated to IE, will be used.

MORPHOLOGY

Light and electron microscopic appearances of the IE lymphocytes of man, mouse, and the rat have been described in detail²¹⁻²⁷. They are rarely round, and often have very irregular contours, nuclei are homogeneous and densely stained, and the cytoplasm is pale and relatively featureless to light microscopy. Many IE lymphocytes contain tiny metachromatic granules^{23,26,28}, and the cytoplasm of the large IE lymphocytes may be pyroninophilic^{24,26}. Electron microscopy has shown that the IE lymphocytes resemble closely the lymphocytes of the lamina propria²⁵. A typical IE lymphocyte has dense nucleus, granular cytoplasm with some rough endoplasmic reticulum, ribosomes, lysosomes, mitochondriae, and a well-developed Golgi apparatus. All authors agree that most IE lymphocytes are larger in volume than the typical small lymphocytes of blood. However, in semi-thin sections, a variety of sizes and forms of IE lymphocytes can be distinguished^{23,25,26}. In mice Röpke and Everett²⁶ classified 49% of the IE lymphocytes as small (with diameter in section of 3.2 μm) and 51% large (average cell diameter 5.7 μm). Marsh²⁵ has reported that the majority of IE lymphocytes are medium sized with diameters 5 to 9 μm , and the remaining 10% of cells are large, classified as immunoblasts. Collan²³ used photography of serial sections, 0.5 μm thick, to define the volume of each of 37 intraepithelial lymphocytes in rat ileum. He found that one was a typical small lymphocyte, with the volume of the cell being 112 μm^3 and of the nucleus 64 μm^3 . He found one large lymphocyte with cellular volume 296 μm^3 ; 22 lymphocytes with granules were detected, mean volume of these cells being 183 μm^3 ; seven basophilic lymphocytes with condensed nuclear chromatin and a mean cell volume of 273 μm^3 ; one globule leucocyte, two eosinophils, two mitotic cells (one with granules and one without), one necrotic cell and the pseudopodium of a macrophage (the body of this cell being situated in the lamina propria).

LOCATION WITHIN THE EPITHELIUM

For a century there has been dispute as to whether the IE lymphocytes are all extracellular (lying between the enterocytes) or whether some or all are present in vacuoles within the epithelial cells. This question has been resolved by the several electron microscopic studies²¹⁻²⁷. Although the IE lymphocytes may indent adjacent enterocytes, they are unequivocally intercellular in position. They are in close contact with adjacent epithelial cells, but there are no desmosomes or other adhesion specialisations between lymphocytes and enterocytes. There is no evidence of adhesion or contact with the basal lamina, so it is likely that the lymphocytes are swept along the sides of the villi as the sheet of epithelial cells moves from crypt to lumen.

Many lymphocytes can be seen by light or electron microscopy to cross the basal lamina between lamina propria and epithelium. It is very likely that cells move in both directions²¹. Lymphocytes in transit are constricted at the level of the interface between epithelium and lamina propria, and the ruptured edge of the basal lamina can be seen close to the margin of the lymphocyte²².

IE lymphocytes are concentrated in the basal part of the epithelium, in the layer between the level of the epithelial cell nuclei and the basal lamina. By counting supranuclear, perinuclear, and infranuclear lymphocytes, three groups of workers agree that between 95% and 98% of the IE lymphocytes are in the basal region of the epithelium^{21,29,30}. The absence of IE lymphocytes near the brush border shows that they are not migrating across the epithelium into the lumen of the small intestine.

PROLIFERATION KINETICS AND LIFESPAN

The heterogeneous nature of the IE lymphocyte population has been discussed above. This creates problems in the investigation of cell proliferation kinetics, as different categories of cells may have quite different mitotic rates and lifespans.

Mitosis of IE lymphocytes

Very occasionally, an IE lymphocyte in mitosis can be seen in a conventional histological preparation. Darlington and Rogers reported that, of 1600 IE lymphocytes examined, three were in mitosis³⁰. An estimate of the rate of entry of cells into mitosis in a population under study can be made by using colchicine to block mitosis in metaphase. Values obtained using this technique are one entry into metaphase per 100 IE lymphocytes per hour³¹ and 2.3 colchicine blocked metaphases per hour (when the population studied was confined to the large IE lymphocytes)²⁶. Thus some nine new lymphocytes will appear on the average mouse villus per hour³¹.

DNA synthesis by IE lymphocytes

Cells in DNA synthesis can be identified by using ³H thymidine, which is incorporated into newly synthesised DNA so that the cells containing ³H may later be identified by autoradiography. The first study of this type, by Darlington and Rogers³⁰, showed that DNA synthesis by small bowel epithelial cells was confined to the crypts. However, IE lymphocytes scattered all over the villus surface were found to be labelled. Label was taken up without relationship to the location of the IE lymphocytes on the villus and

without relationship to the location of the column of labelled epithelial cells which moved slowly up from crypt base to villus tip during the two to three days after thymidine injection.

An hour after injection of ^3H thymidine in mice, some 10% of the IE lymphocytes are labelled³². Values obtained at 24 hours vary from 4%³⁰ to 20%³². Even 10 days after a single bolus injection of ^3H thymidine, there are still substantial numbers of labelled cells in the epithelium. This must indicate that the cells have been in DNA synthesis at a site outside the epithelial layer and have migrated into the epithelium during the 10 days concerned, as the epithelial surface will have been renewed several times in that period³⁰.

The presence of blast-like intraepithelial lymphocytes, and high rate of DNA synthesis in this cell population, might be thought to result from antigenic stimulation by the antigens of the intestinal lumen²⁵. However, the kinetics of intraepithelial lymphocytes of germ free mice are similar to those of conventionally reared animals³³. IE lymphocytes in DNA synthesis can also be found in numbers similar to those in normally sited intestine³⁴, in completely antigen free isografts of small intestine.

Short and long-lived IE lymphocytes

If ^3H thymidine is given by continuous infusion, or at frequent intervals for several days, then those lymphocytes which remain unlabelled can be considered as fairly long-lived. Several such studies have been carried out in mice, showing that around 70% of the IE lymphocytes are short-lived and 30% long-lived^{26,35}. When small and large IE lymphocytes are considered separately, it is found that 90% of the large lymphocytes are labelled by 10 days, although the number never reaches 100%; and only 5% of the small lymphocytes are labelled after three days of regular injections, although this slowly increases to 80% at about 10 days²⁶. If the number of grains of silver overlying each labelled nucleus is counted, information as to the number of times the cell has divided since the administration of ^3H thymidine can be obtained. All the workers who have studied the IE lymphocytes agree that these lymphocytes tend to remain heavily labelled, even after the passage of several weeks. Thus 'short-lived' cells which in the main populate the intraepithelial site are at a stage in their maturation or differentiation when DNA synthesis and mitosis are likely to take place. However, the daughter cells of a divided IE lymphocyte do not appear to retain this proliferative capacity. Röpke and Everett²⁶ suggest that the most likely kinetic model for the majority of IE lymphocytes is that B and T lymphoblasts invade the epithelium and undergo mitosis. They suggest that the B lymphocytes give rise predominantly to plasma cells and T lymphoblasts give rise to small lymphocytes—probably long-lived—which re-enter the circulation.

T AND B POPULATIONS

The cells of the lymphoid series can be broadly divided into T, thymus dependent, and B, bursa dependent or bone marrow derived lymphocytes. Within each of these broad categories there are long- and short-lived cells, and lymphocytes may be small, medium, or large. Furthermore, a population of non-T non-B lymphocytes exists (null cells).

The first evidence that many of the IE lymphocytes are thymus-dependent T cells came from examination of their numbers in animals which had been

depleted of T cells in various ways. In all thymus-deprived animals examined, low counts of IE lymphocytes have been found^{33,36,37,38}. Yet even in the congenitally athymic nude mice, small numbers of IE lymphocytes persist. The nature of these remaining cells, whether B or null, has not yet been established^{33,38}.

Antisera which react with T cell markers can be labelled with fluorescein and thus used to identify T cells in tissue sections by immunofluorescence. With this method the majority of IE lymphocytes in rat small intestine were found to be T cells³⁹. Meuwissen⁴⁰ and his colleagues made similar observations in human intestinal biopsies, finding that the majority of the IE lymphocytes were also T cells, although Strickland⁴¹ and his colleagues reported both T and B IE lymphocytes in two specimens of normal intestine and two from Crohn's patients. Further evidence that the majority of IE lymphocytes are T cells is that isotope labelled T immunoblasts from rat lymph have been found to home to the intraepithelial site, as described below³⁹.

Many workers have tried to prepare suspensions of lymphoid cells from the small intestine, with limited success. However, rabbit and human gut mucosal lymphocyte suspensions can be prepared, thus allowing studies of membrane properties and *in vitro* culture studies of these cells^{28,42}. Some 35% of human mucosal lymphocytes formed rosettes with sheep red cells⁴³ and 11% of rabbit gut mucosal cells reacted with antiserum to T antigens in a lymphocytotoxicity test⁴²; mucosal lymphocytes have a modest blast response to T cell mitogens such as phytohaemagglutinin^{28,43}. Gut lymphocytes were found to act both as stimulating cells and responding cells in a mixed lymphocyte culture system⁴⁴, evidence that, as well as having T cell markers, the mucosal lymphocyte population contains functional T cells.

LYMPHOCYTE TRAFFIC INTO THE EPITHELIUM

Most cells of the lymphoid system are constantly moving between blood, the extravascular spaces, lymphoid organs, and the lymph. This traffic can best be examined by using isotope labelled cells, traced by autoradiography or by liquid scintillation counting.

In the classical report by Gowans and Knight on the route of recirculation of lymphocytes in the rat¹⁵, it was reported that small lymphocytes recirculate between blood and lymph *via* the post-capillary venules of lymph nodes. In the same paper it was pointed out that small lymphocytes did not home to the gut mucosa, but that large lymphocytes from the lymph, labelled with isotope and then reinjected into the bloodstream, were subsequently found in the lamina propria of the gut. These authors did not mention IE lymphocytes at all, and in the further studies of immunoblast homing by Hall and others⁴⁵⁻⁴⁸, only in one instance is it mentioned that cells derived from immunoblasts may be found in the epithelium⁴⁸. Parrott and Ferguson examined this point in detail in their studies of mouse lymphoid cell traffic to the gut⁴⁹ and found no labelled IE lymphocytes after infusion of different types of donor cells. However, Guy Grand and her colleagues³⁹ have found T immunoblasts from rat lymph to home to the intraepithelial site where they can be later identified as IE lymphocytes. Parrott and her colleagues⁵⁰ have also found that immunoblasts will home to the intraepithelial site, and in their experiments the donor cells were contained in whole spleen transplants.

Just as has been discussed above in relation to DNA synthesis by IE lymphocytes, it would seem logical to assume that homing of immunoblasts to the gut is controlled by the presence of antigen within the gut lumen. However, considerable efforts to prove this point have not met with success. Indeed, there is evidence from simple morphology and also from cell traffic studies that lymphocytes can home to the small bowel in the complete absence of antigen^{37,39,46,47,49}. Attempts to attract lymphocytes to the gut by using parasite infected segments have produced a modest increase in the number of homing cells⁵¹. The cell types which home to the gut, T and B immunoblasts, are similar to those which home to sites of non-specific inflammation. However, by using donor blast cells stimulated either by the enteric route or by dermal sensitisation, Parrott and her colleagues have shown convincingly that the gut is not regarded by activated T blasts as merely a non-specific inflammatory site. T blasts derived from mesenteric nodes have a capacity to home to the gut in a non-specific way, but this is not the case for T blasts from peripheral nodes⁵².

Lymphocytes destined to enter the epithelial site probably leave the bloodstream *via* the capillaries in the villi. The route by which the lymphocytes leave the epithelium is, however, still a matter for debate. It was originally thought that the gut was a site of extrusion of lymphocytes from the body¹³. Small numbers of lymphocytes can be found among the exfoliated epithelial cells in the lumen⁵³, but these do not remain viable⁵⁴. It is likely, although not proven, that the IE lymphocytes re-enter the lamina propria and leave the villi in the lymphatics. Evidence which tends to support this route of exit includes the similar morphology of IE lymphocytes and intralymphatic lymphocytes²⁵, the absence of lymphocytes within the lumen of isolated grafts of small intestine³⁴, and the absence of lymphocytes in the extruded epithelial cells of small intestine grown in organ culture⁵⁵.

There are as yet no reports of the traffic of a population of lymphocytes separated from the gut mucosa and subsequently traced by autoradiography or other means.

FACTORS WHICH INFLUENCE THE NUMBERS OF IE LYMPHOCYTES

The number of IE lymphocytes can be counted either by using a length of epithelium or an entire villus as the reference point, or by carrying out a differential count of types of cells within the epithelium and expressing the result as lymphocytes per 100 epithelial cells. A variety of experimental manipulations have been performed in attempts to influence the numbers of the IE lymphocytes and thus to obtain information as to their possible functions, and also, in some instances, to integrate findings in animal models with those of human diseases.

One of the most consistent ways of reducing the number of IE lymphocytes has already been discussed above—namely, by depleting an animal of T cells.

Age

The effect of age on IE lymphocyte counts has been examined in detail in the mouse. This animal is unusual in that IE lymphocytes are absent at birth and do not appear in the epithelium until the third week of life. Thereafter counts rise exponentially to plateau after the age of 6 or 7 weeks³⁴. Lymphocytes appear within the epithelium at approximately the same time as plasma

cells are first found in the lamina propria⁵⁶, and almost coincident with other changes in the enzyme and cell kinetic properties of the developing gut⁵⁷. The timing of the infiltration of IE lymphocytes is not, however, produced by changes in the intestinal contents around the time of weaning, for when grafts of foetal intestine are transplanted to mice of the same strain, lymphocytes (present at the time of transplantation in the bloodstream) do not infiltrate the epithelium until the third week after transplantation³⁷. Thus some factor in the graft itself or in its stroma attracts lymphocytes from the bloodstream into the epithelium and lamina propria.

Microbial antigens

In germ-free animals many lymphoid organs are small and there are few lamina propria plasma cells^{58,59}. IE lymphocyte counts are also low in germ-free animals^{33,60-64}. When germ-free animals are also T-cell depleted, counts of IE lymphocytes drop even further^{33,64}. However, the removal of both microbial and food antigens from the lumen of the gut, in the case of antigen free isografts, did not further deplete the numbers of IE lymphocytes³⁷. There is no information on IE lymphocyte counts in germ-free animals who have been reared on antigen-free diets.

Food antigens

An early study of the effect of removal of food antigens on IE lymphocyte counts was the examination of the intestines of hibernating squirrels. These had a slightly higher IE lymphocyte count than did controls⁶⁵. When animals reared on an elemental diet (containing no dietary antigens) were compared with animals reared on a conventional diet, no differences in IE lymphocyte counts were found⁶¹. Thus, at least in healthy, conventionally reared animals, the antigens provided by food seem to play little part in influencing the numbers of IE lymphocytes.

Parasite infections

Mice which are chronically infected with the protozoal parasite *Giardia muris* have higher counts of IE lymphocytes than uninfected mice⁶⁶. During the course of infection with the rat parasite *Nippostrongylus brasiliensis*, considerable changes in the numbers and types of lamina propria lymphoid cells are found. IE lymphocyte counts tend to drop rather than rise as the mucosa becomes flatter at the height of the infection with the parasite⁶⁷.

Irradiation

After 300 r gamma irradiation, a slight drop in IE lymphocyte count at 24 hours was followed by a rise in the proportion of cells in DNA synthesis over the next few days⁶⁸. Higher doses of neutron or gamma irradiation, 800 r to 900 r, produce a consistent and significant drop in the numbers of IE lymphocytes at three days after irradiation⁶⁹.

Immunosuppressive drugs

Cortisone, when given at a dose sufficient to produce depletion of thymic lymphocytes and runting syndrome, has no effect on the IE lymphocyte count of mice⁶⁹, but cyclophosphamide, 100 mg/kg, causes the IE lymphocyte count of rats to drop some 30% within 24 hours⁶³.

Local contact hypersensitivity

In mice which had previously been sensitised to the contact sensitising agent, oxazolone, mucosal challenge (by feeding oxazolone) had no effect on IE lymphocyte counts⁷⁰.

Rejection of allografts

Heterotopically transplanted allografts of intestine are rejected by a local T-cell mediated immune reaction which probably acts *via* enteropathic lymphokines⁷¹. The histopathology of allograft rejection of small intestine has been examined in detail by using foetal small intestinal grafts in mice⁷². Measurements of the effects of rejection on mucosal architecture have shown crypt hyperplasia with villous atrophy^{73,74} but, although counts of IE lymphocytes are higher in rejecting allografts than in isografts or normal intestine of the same age⁷³, the lymphocyte counts are only of the same order of magnitude as are found in the normal intestine of adult mice. In one series of experiments 20% of the rejecting grafts examined with a completely flat mucosal surface had no intraepithelial lymphocytes⁷³.

CURRENT THEORIES OF THE FUNCTION OF IE LYMPHOCYTES

Although many observations have now been made on the morphology of IE lymphocytes, and on the effects of various experimental systems on their numbers and some of their properties, the function of these cells is still unknown. A number of the current theories are listed and briefly discussed below.

1. The IE lymphocytes are attracted to the gut by the presence of antigens within the gut lumen, and are stimulated to blast transformation by contact with appropriate antigens. The studies on completely antigen-free intestine, described above, show that this theory is incorrect. The size and proliferative capacity of the IE lymphocytes is a reflection of their parent cell population's properties, and is not produced by the exposure to antigens at the epithelial surface.

2. The epithelium is the site where antigens from the gut first make contact with cells of the lymphoid system, and IE lymphocytes are important in making this first antigen contact. This is possibly so of the IE lymphocytes which overlie Peyer's patches⁷⁵ but there is now evidence that the important site of penetration of intraluminal antigens into the body is via specialised 'microfold' cells in the Peyer's patches⁷⁶.

3. The gut epithelium is a bursa-equivalent—a central lymphoid organ responsible for instruction and maturation of B cells. The absence of IE lymphocytes in the gut of young mice and the preponderance of T cells in this location make this theory most unlikely.

4. The epithelium is an important site on the migration pathways of young T cells, for reasons as yet unknown but perhaps because of the large amounts of antigen which may be present in this location. This is not really a theory with regard to the function of the IE lymphocytes, but is likely to be nearer the truth than most of the other theories discussed here.

5. IE lymphocytes are a marker of a local cell mediated immune reaction. The basis for this proposal was the correlation between villous atrophy with crypt hyperplasia and high IE lymphocyte count in coeliac disease⁷⁷. However, this theory must now be held to be untenable because in the most

profound tissue damage produced by a cell-mediated immune reaction (allograft rejection) IE lymphocytes may be absent.

6. IE lymphocytes are in fact K cells (killer, cytotoxic, non-T non-B cells)⁷⁸. The only evidence to support this theory is that most IE lymphocytes do appear to be null cells and lack the conventional membrane properties of small T and B lymphocytes. Electron microscopy has shown no evidence of cytotoxic properties of IE lymphocytes.

7. IE lymphocytes are a type of mast cell. This idea arose as a result of the observation that many IE lymphocytes contained metachromatic granules. IE lymphocytes do contain histamine, although at only about 10% of the content of an equivalent number of basophils. However, whereas basophils, passively sensitised with reaginic serum and then challenged, will release their histamine, this is not the case for intestinal lymphocytes⁷⁹.

Attempts to define the function of lymphocytes at a body surface may be doomed to failure if the only functions examined are those which have already been recognised in lymphocytes of the bloodstream or of the solid lymphoid organs. This was so in the case of investigations into the properties of IgA, which does not have and almost certainly does not need many of the functions of other immunoglobulins such as IgG. The most important property of IgA is that it is capable of combining with antigens and then doing little else; in particular, it does not initiate local hypersensitivity reaction with associated tissue damage and interference with organ functions. A similar, very limited immune capacity is recognised in the main reticuloendothelial organ of the gastrointestinal tract—the Kupffer cells. They trap and ingest antigens and reduce the immunogenicity of such ingested antigens, in contrast with most other cells of the reticuloendothelial system which will process antigen, render it highly immunogenic, and initiate humoral and cell mediated immune responses.

I suggest that studies of the properties and functions of the IE lymphocytes should bear in mind the great value to mucosal surfaces of an immunological apparatus which is able to combine with antigen, block its absorption across the mucosa, and prevent the interaction of antigen with potent but potentially harmful immunological systems such as IgG and small T cells. The IE lymphocytes may be a disarmed, dedifferentiated category of T cells capable of combining with antigen but with few or no other properties (Figure), forming part of the complex mechanisms which have developed to modulate immune responses and to localise hypersensitivity reactions at sites remote from the mucosal surfaces.

Part 2: Clinical application of intraepithelial lymphocyte counts

Intraepithelial lymphocytes are recognised, mentioned, and illustrated in many reports of jejunal pathology in the 1960s and the first attempt to estimate their numbers in man was reported in 1971⁸⁰. The technique used was a differential count of the cell types within the villus epithelium, thus measuring the IE lymphocyte count per 100 villus epithelial cells. This technique has been strongly criticised by Skinner and Whitehead⁸¹, as the reference value for the measurement is the epithelial cell—a component of the gut which may vary in size, shape, and numbers in disease processes. Skinner and Whitehead recommend that the reference value be taken as the

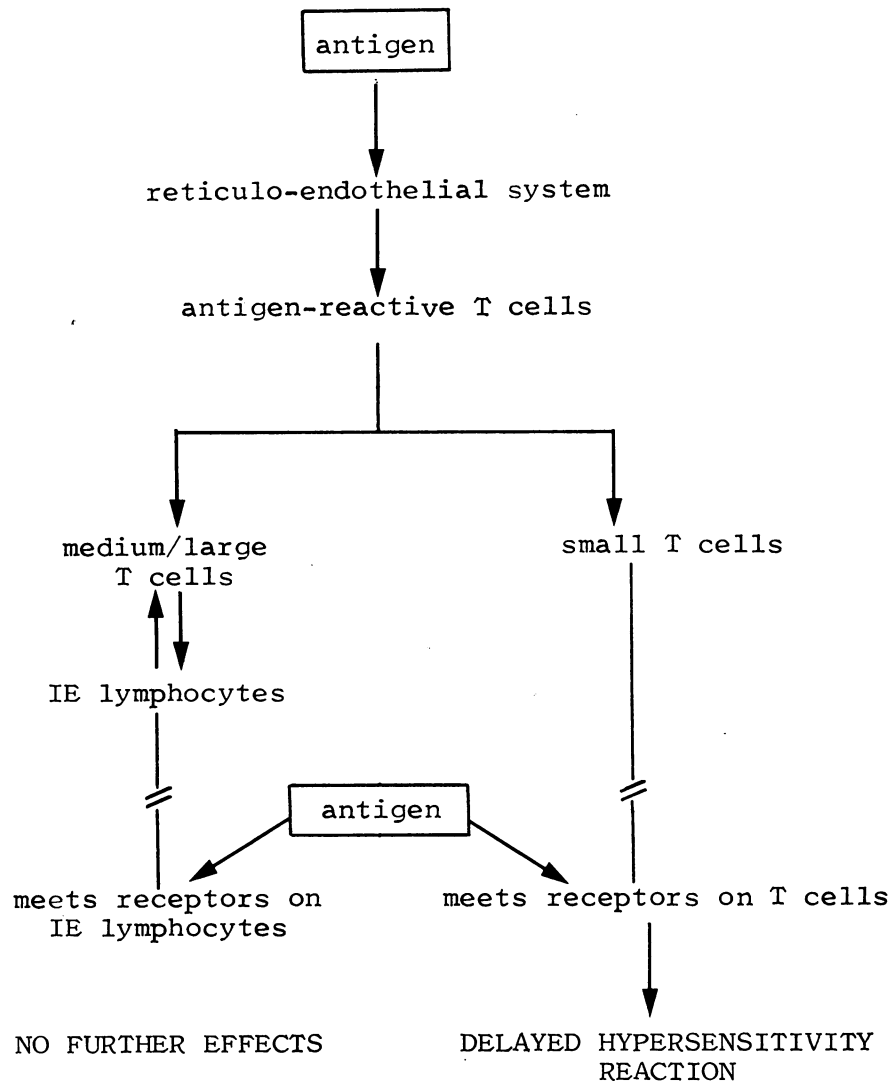


Figure Hypothetical role of intraepithelial lymphocytes after antigen challenge. It is proposed that this category of T cells will combine with antigen but have few or no other properties. Thus, whereas, as shown in the lower right corner of the diagram, small T lymphocytes combine with antigen release lymphokines and produce tissue damage; intraepithelial lymphocytes (on the lower left of the diagram) will not initiate and may even block local hypersensitivity reactions.

muscularis mucosa, and that the number of IE lymphocytes per length of muscularis mucosa be counted. This criticism is valid if the immunological or clinical significance of IE lymphocytes depends on their total number per unit length or area of intestine, or the total number in the entire organ. However, it is equally possible that the relevance of the IE lymphocytes relates to the microenvironment in a single villus or at an even smaller scale, in the villus epithelium. IE lymphocyte counts per epithelial cell have the

advantage that they can be carried out in virtually any routinely processed biopsy, and if one bears in mind that this provides a measurement of one facet of pathological change in the intestine, simple quantitation remains a useful technique to the gastroenterologist. The method has now been widely applied in a variety of research projects in adults and children and may be of value in routine diagnostic pathology.

NORMAL VALUES FOR IE LYMPHOCYTE COUNTS IN JEJUNAL BIOPSIES

Ferguson and Murray⁸⁰ reported that values for IE lymphocyte counts in jejunal biopsies from a group of 40 adult controls were in the range of 9 to 39 lymphocytes per 100 epithelial cells with a mean value of 21.1 and standard deviation of 7.5. They proposed that the working values for the normal range be taken as 6-40. In the Table are summarised the values from the literature for IE lymphocyte counts in children and adult controls of other series. In the majority, mean values are between 15 and 25 with the exception of the paper by Otto⁸³ who found a mean value for 87 adults of only 6.3 lymphocytes per 100 epithelial cells. Thus for most purposes 6 and 40 can be taken as the lower and upper limits of the normal range, although in most normal biopsies values are between 10 and 30.

Holmes, Ferguson, and their colleagues from Birmingham have used IE lymphocyte counts per millimetre of epithelium in their work⁹⁴⁻⁹⁶. With this technique results will vary according to the amount of shrinkage in a biopsy specimen, but in their hands the method has proved reliable and values for coeliac disease and inflammatory bowel disease have shown trends similar to those obtained by the differential count technique. Normal values for IE lymphocyte counts appear to be similar in children and adults, although there is no information on newborn or very young human infants.

Table 'Normal' values for intraepithelial lymphocyte counts

Authors and reference nos.	Diagnosis	Number of subjects	IEL count per 100 epithelial cells			
			Mean	SD	SE	Range
Ferguson and Murray ⁸⁰	No GI disease present	40 adults	21.1	7.5	—	9.6-39.3
Fry <i>et al.</i> ⁸¹	Skin diseases or gastric surgery	11 adults	13.9	—	1.4	6.5-23.5
Otto ⁸³	No GI disease	87 adults	6.3	1.7	—	—
Marks ⁸⁴	Skin diseases	11 adults	—	—	—	9.48
Fry <i>et al.</i> ⁸⁵			15.9	—	1.3	6.9-28.5
Montgomery and Shearer ⁸⁶	Normal subjects	20 adults	—	—	—	7-22
Lancaster-Smith <i>et al.</i> ⁸⁷	No GI disease present		23	—	3.0	—
Stevens <i>et al.</i> ⁸⁸	Not stated	Not stated	—	—	—	13.2-45.0
A. Ferguson <i>et al.</i> ⁸⁹	No GI disease	7 children	—	—	—	14-25
Lancaster-Smith <i>et al.</i> ⁹⁰	Coeliac disease excluded	10 children	30	—	2.6	—
Mavromichalis <i>et al.</i> ⁹¹	No GI disease	17 children	15	4	—	8-27
	Coeliac disease excluded	12 children	16	4	—	11-26
	Normal siblings of coeliacs	34 children	17	8	—	7-40
McNicholl <i>et al.</i> ⁹²	Normal siblings of coeliacs	25 children	25.8	6.7	—	—
Schaad <i>et al.</i> ⁹³	Miscellaneous GI diseases (excluding coeliacs)	26 children	24.4	6.6	—	14.2-38.0
			<i>IEL per millimetre length of epithelium</i>			
Holmes <i>et al.</i> ⁹⁴	No GI disease	12 adults	42	12	—	—
R. Ferguson <i>et al.</i> ⁹⁵	Healthy controls	15 adults	44	—	3	—
R. Ferguson <i>et al.</i> ⁹⁶	Obesity	20 adults	53	—	5	—

IE LYMPHOCYTE COUNTS IN COELIAC DISEASE AND DERMATITIS HERPETIFORMIS

All the published reports on this subject agree that IE lymphocyte counts are high in patients with untreated coeliac disease and also in those patients with dermatitis herpetiformis who have partial or sub-total villous atrophy while taking a normal diet^{77,80,82,83,85,86,87,89,91,93,94,97}. It is also agreed that IE lymphocyte counts drop towards normal in the months or years after withdrawal of gluten from the diet^{77,80,89,91,93,94,95,97}. IE lymphocyte counts can be very high in untreated coeliac disease—an occasional patient will have more lymphocytes than enterocytes within the epithelium⁸⁰. In contrast with the normally basal location within the epithelium in normal subjects, IE lymphocytes are found at the basal, perinuclear, and supranuclear regions of the epithelium in the sub-total villous atrophy of coeliac disease or dermatitis herpetiformis^{77,85}. When partial villous atrophy is present there is an uneven distribution of lymphocytes, with more IE lymphocytes at the tops of the villi facing onto the intestinal lumen than along the sides of the villi. Jejunal biopsies are usually taken after an overnight fast and therefore there is no gluten in the lumen at the time of biopsy. However, this lumenopetal distribution does suggest that the contents of the gut influence IE lymphocyte distribution in some way.

After a period of treatment with a gluten-free diet, IE lymphocyte counts seem to be relatively independent of other manifestations of histological improvement in the jejunum. High counts may be found in patients in whom the villi have returned to normal after gluten withdrawal, and normal counts may be found in treated patients in whom the general architecture of the mucosa is still flat⁸⁰.

In several reports, correlation between IE lymphocyte counts and other facets of clinical state, pathology, or immunology has been reported in patients with coeliac disease or dermatitis herpetiformis. Low counts of blood T lymphocytes in untreated coeliac disease correlate with high IE lymphocyte counts in the same patients⁹⁸. Depletion of lamina propria lymphocytes occurs in parallel with increased intraepithelial lymphocytes^{87,90,94}. There is an association between intraepithelial lymphocyte counts and the presence of peripheral blood lymphocytes sensitised to gluten⁹⁹. The lymphocyte infiltrate correlates with malabsorption of xylose (reflecting enterocyte function)⁸⁸, and with the presence of serum antibodies to food antigens (reflecting the permeability of the mucosa)¹⁰⁰.

The effect of gluten challenge has been examined both in adults and in children. In children, previously thought to have coeliac disease but in whom subsequent gluten challenge did not produce clinical or histological relapse, IE lymphocyte counts were unaltered by the addition of gluten to the diet⁹¹. In contrast, high IE lymphocyte counts developed in all those children challenged with 10 or 20 g gluten for up to three months, in whom the diagnosis of coeliac disease was substantiated in other ways^{90,91}. Lymphocyte infiltration into the epithelium developed within 24 hours in all of five adults given a single challenge of 30 g gluten⁷⁷. Reintroducing gluten for a longer term also causes a rise in IE lymphocyte counts in adult coeliac patients⁸⁷.

There is dispute as to whether those patients who have dermatitis herpetiformis with normal intestinal histology have altered IE lymphocyte counts. Marks has reported that IE lymphocytes are absolutely normal in this group

of patients with dermatitis herpetiformis⁸⁴, whereas Fry and his colleagues have found abnormally high values^{82,85}.

IE lymphocyte counts are normal in the healthy relatives of coeliac patients in whom jejunal biopsies are otherwise normal in terms of architecture and enzyme content⁸⁸.

IE LYMPHOCYTE COUNTS IN OTHER GASTROINTESTINAL DISEASES

Jejunal biopsies from patients with a variety of diseases of the small and large bowel have been examined and reports of IE lymphocyte counts published. Only in the case of tropical sprue have the counts been of the same high magnitude as those found in coeliac patients⁸⁶. Unlike what obtains in coeliac disease, IE lymphocytes appear to rise with increasing duration of clinical illness in patients with tropical sprue⁸⁶.

High counts have been reported in some children with giardiasis or who have unexplained diarrhoea with failure to thrive^{89,91}, and occasional adult patients with high counts have been mentioned—hypogammaglobulinaemia⁸⁶, disseminated lupus erythematosus⁸⁰, blind loop syndrome⁸⁶, and irritable bowel syndrome⁸⁰. Values significantly higher than in control subjects were found in a small group of adults with post-infective malabsorption⁸⁶. IE lymphocyte counts have been normal in groups of children with marasmus¹⁰¹, kwashiorkor¹⁰¹, measles¹⁰², acute gastroenteritis⁸⁹, and post-gastroenteritis malabsorption¹⁰³. In both the reports of counts in children with enteropathies, no correlation has been detected between IE lymphocyte count and the severity of villous atrophy or of abnormality of the epithelial cells^{89,91}.

IE lymphocyte counts are normal in Crohn's disease and ulcerative colitis^{80,96}.

Information is scanty on children with intolerance to cows' milk protein. Values for such children may be normal or high, and it is likely that the IE lymphocyte counts rise after milk challenge^{103,104}.

INDICATIONS FOR IE LYMPHOCYTE COUNTS IN ROUTINE DIAGNOSTIC PATHOLOGY

A formal count of IE lymphocytes is unnecessary for routine jejunal biopsy examination, and adds nothing to the verbal report if a tissue is either absolutely normal or classically coeliac. However, this measurement is of value as an index of improvement in a coeliac biopsy after gluten withdrawal, and is an early and measurable change in the biopsy after gluten challenge in a previously treated coeliac patient. IE lymphocyte counts may help to identify sub-groups of children with unexplained diarrhoea, and of adults with vague gastrointestinal symptoms. When a child who has diarrhoea or failure to thrive is found to have partial villous atrophy, a normal IE lymphocyte count weighs heavily against a diagnosis of coeliac disease. The significance of the high IE lymphocyte count in some children and adults with gastrointestinal symptoms but an otherwise normal mucosa remains to be ascertained.

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Received for publication 11 May 1977

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