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Hypersensitivity reactions in the small intestine

2 Effects of allograft rejection on mucosal architecture and lymphoid cell infiltrate

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SUMMARY Small intestinal mucosa contains both thymus dependent and thymus independent lymphoid cells and thus has the capacity to act *via* humoral and cellular mechanisms as a site of local immunity and local hypersensitivity. Allograft rejection of mouse small intestine is a model of a local cell mediated reaction. The effects of this clearly defined, immunologically mediated damage on villi, crypts, enterocytes, and lymphoid cell infiltrate have been assessed by comparing the morphology of rejecting allografts with that of isografts and normal small intestine of the same age. In rejection there is infiltration of the lamina propria with lymphocytes, hyperplasia of the crypts of Lieberkuhn, and an eventual sloughing off of the mucosa. Usually, but not always, there is villous atrophy and increased numbers of intraepithelial lymphocytes. However, the morphology of individual enterocytes remains normal throughout rejection and neither plasma cells nor polymorphonuclear leucocytes infiltrate the lamina propria before mucosal ulceration. These results show unequivocally that a local T cell mediated immune response causes villous atrophy and crypt hyperplasia in this animal model, and since there is no evidence of local enterocyte cytotoxicity, a lymphokine may be the link between the activated T cell and the effects on mucosal architecture. We suggest that a local CMI reaction may be the cause of villous atrophy, crypt hyperplasia, and malabsorption in many clinical and experimental conditions, including coeliac disease, food allergy, and intestinal infections.

The immune response to an antigen may have an entirely protective function, in the classical sense of immunity to infection. However, an immune response may be the primary cause of disease and several types of these 'hypersensitivity' reactions can be differentiated according to the immune mechanisms of tissue damage (Gell and Coombs, 1969). Since animal experiments have shown that the small intestinal mucosa contains both thymus dependent and thymus independent lymphoid cells (Fichtelius *et al.*, 1968; Sprent and Miller, 1972; Ferguson and Parrott, 1973a; Guy-Grand *et al.*, 1974; Parrott and Ferguson, 1974) this tissue thus has the capacity to act via humoral and cellular mechanisms as the site of local immunity and local hypersensitivity.

The functions of local T cell-mediated immune reactions in the intestinal mucosa are, as yet, undefined. There is some evidence that local cell-mediated immunity (CMI) is important in immunity to helminth parasites (Jarrett and Urquhart, 1971; Dineen *et al.*, 1973; Ogilvie and Jones, 1973; Ogilvie and Love, 1974) and in resistance to local tumour spread (Calman, 1974). We have proposed that local CMI may also have a pathological role, as the cause of villous atrophy and crypt hyperplasia (Ferguson, 1974a, b; Ferguson and Jarrett, 1975). In a series of experiments in which thymus deprived rats were infected with the parasite *Nippostrongylus brasiliensis*, we found that not only were these rats immunodeficient, with delayed elimination of the parasite load, but also the worm associated partial villous atrophy failed to develop in 70% of experimental animals. This implied that the local enteropathy associated with parasite infection was due to the thymus dependent immune reaction—that is, was a local hypersensitivity reaction.

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Further support for the concept that local cell-mediated immune reactions may contribute to small bowel damage and disease have come from investigations of intestinal allograft rejection and graft versus host disease (Reilly and Kirsner, 1965; Holmes *et al.*, 1971; Ferguson, 1972; Ferguson and Parrott, 1973b). These produce partial or sub-total villous atrophy in affected areas of the small intestine and, in the case of graft-versus-host disease, the morphological changes have been shown to be accompanied by disaccharidase deficiency (Hedberg *et al.*, 1968), malabsorption (Palmer and Reilly, 1971) and a protein losing enteropathy (Cornelius, 1970).

We have used allograft rejection of grafts of foetal mouse small intestine (Ferguson and Parrott, 1973b) as a model of a cell-mediated immune reaction in the small intestinal mucosa. In the course of experiments with thymectomised, irradiated, and bone marrow reconstituted mice, we demonstrated that rejection of allografts in this system was a thymus dependent phenomenon (Ferguson and Parrott, 1973b) and, since rejection and the associated flat mucosa precede the appearance of serum antibody by several days (Elves and Ferguson, 1975), we have confirmed that the response is mediated by lymphocytes. In addition, since the transplanted tissue is foetal and has never been exposed to foods or micro-organisms, all pathological features observed can be attributed directly to the rejection process with no superimposed local immune reactions to foods or bacteria.

We have previously reported the histopathological appearances of rejection in these grafts (Ferguson, 1972; Ferguson and Parrott, 1972; Ferguson and Parrott, 1973b; Ferguson and MacDonald, 1975) and the most interesting finding was that for a few days before complete rejection, many tissues had a flat luminal surface with long crypts of Lieberkuhn, so that the morphology was remarkably similar to that of the small intestine in coeliac disease. The object of this work is to define more clearly the effects of this thymus dependent, cell-mediated rejection process on the villus and crypt architecture, the evolution of the changes observed, and also the effect on the individual enterocytes and the intestinal lymphoid cells. We report here the results of light microscopic examination; scanning and transmission electron microscopy of rejection are described in a separate paper (Carr *et al.*, 1976).

Methods

ANIMALS

Mice used were of the inbred strains CBA, C3H-Bi and Balb/c (histocompatibility antigens H-2^k, H-2^k and H-2^d respectively (Snell *et al.*, 1964)) maintained in the Department of Bacteriology and Immunology, University of Glasgow. The technique for grafting foetal mouse intestine has been described in detail elsewhere (Ferguson and Parrott, 1972; Ferguson, 1973) but is briefly as follows: pregnant mice were killed on the 18th or 19th day of gestation, the foetal small intestine was dissected out and cut into segments 5-10 mm in length, and pieces of small intestine were then implanted under the kidney capsules of adult recipient mice (two grafts per mouse).

Groups of recipient mice were killed by ether overdosage at intervals from one to 20 days after graft implantation. The grafts were easily recognisable as white swellings under the kidney capsules. These were dissected out, with a little underlying kidney tissue, and fixed in formol saline. Groups of normal CBA mice were killed by cervical dislocation at intervals from one to 20 days after birth and three pieces of small intestine were fixed in formol saline. Table 1 gives details of the number of specimens examined, including normal small intestine, isografts and allografts.

HISTOLOGY

The fixed tissues were paraffin embedded, 5μ thick

Experimental group	*Total number of grafts implanted*	*Histological preparations suitable for analysis* (*no.*)	*Age of tissue after implantation (days)*			
			0-5	*6-10*	*11-15*	*16-20*
Normal CBA small intestine	29	29	7	8	7	7
Isografts of small intestine						
CBA → CBA	35	27	4	6	4	13
Balb/c → Balb/c	36	31	7	9	4	11
Allografts of small intestine						
CBA → Balb/c	67	56	23	21	8	4
Balb/c → CBA	79	63	28	34	1	—
CBA → C3H	35	35	9	10	8	8
C3H → CBA	22	22	3	1	6	12
Total number of specimens	303	263				

Table 1 *Details of number and nature of tissues used to study the effect of allograft rejection on mucosal morphology and ymphoid cell infiltrate*

sections were cut at three levels and stained with haematoxylin and eosin and with methyl green pyronin. Sections were coded, mixed, and examined on three occasions for morphological grading of the stage of rejection, for measurements of the lengths of crypts and villi and of epithelial cell height, and for lymphoid cell quantitation.

MORPHOLOGICAL GRADING OF REJECTION

This was done by using the criteria described in our earlier paper (Ferguson and Parrott, 1973b). The gradings were as follows:

Normal for age, with villi normal, crypts short, and virtually no plasma cells or lymphocytes in the lamina propria.

L+ lymphocyte infiltration of the lamina propria, with many lymphocytes and pyroninophilic blast cells in lymphatics.

L+ + dense lymphocyte infiltration throughout the full thickness of the graft, but villi still recognisable.

Flat Villi very short or absent although epithelium and crypts still present—that is, the appearance of partial or sub-total villous atrophy (Shiner and Doniach, 1960).

Submucosa The epithelium and lamina propria destroyed so that the graft consists of smooth muscle heavily infiltrated with lymphocytes and plasma cells.

MEASUREMENTS OF VILLI, CRYPTS, AND EPITHELIAL CELLS

An eyepiece micrometer was used to measure the lengths of villi and crypts and epithelial cell height, in well-orientated sections. Where possible, 10 measurements of enterocyte height/graft were made and the mean value calculated. Villus height and crypt depth were calculated as the mean of five measurements per graft. In the smallest grafts (up to about 10 days after implantation), it was rarely possible to make accurate measurements of the villi, but measurements of crypts and enterocyte height were possible in most specimens.

QUANTITATION OF LYMPHOID CELLS

The lymphocytes within the epithelium (intra-epithelial, IE, lymphocytes) and in the lamina propria were examined separately. IE lymphocytes were investigated in two ways. In normal gut, in isografts and in the allografts between CBA and C3H mice it was possible to carry out differential counts of epithelial cells and IE lymphocytes as previously described (Ferguson and Murray, 1971) and thus to obtain counts of the number of lymphocytes per 100 epithelial cells (a total of 500 cells counted).

In the other strain combination between CBA and Balb/c mice, the presence or absence of IE lymphocytes was noted (presence being defined as at least one lymphocyte per 200 epithelial cells).

Lamina propria lymphoid cells were assessed as absent, scanty infiltrate, or dense infiltrate. Plasma cells in the lamina propria were best observed in the methyl green pyronin stained sections.

STATISTICS

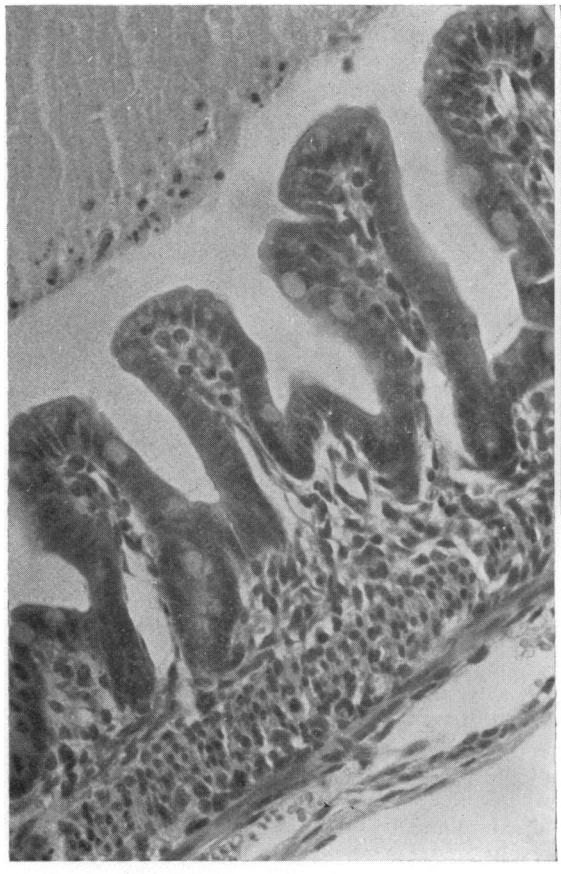
Results of the measurements and counts in the groups of tissues were compared in two ways—by grouping specimens according to age and also according to the stage of rejection. Student's *t* test was used to assess the significance of differences between the various groups of measurements, with the exception of intraepithelial lymphocyte counts in the tissues where these were defined as being simply present or absent. Yates's χ^2 test was used in analysis of these results.

Results

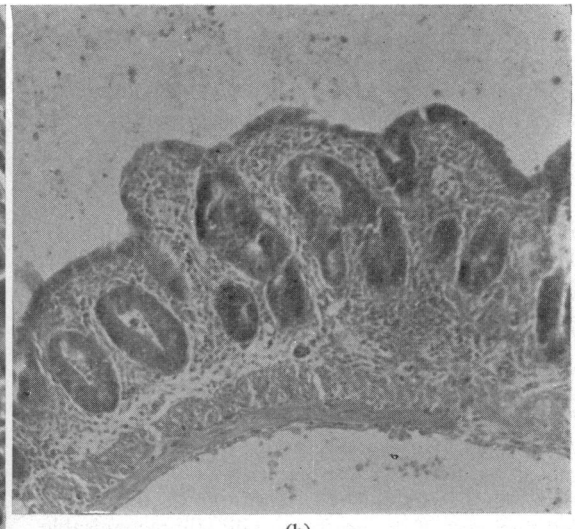
In these experiments the time course of rejection and the morphological appearances of small intestinal allografts were as expected from our previous work, and tissues suitable for histological assessment were obtained in 234 of 274 experiments. The effect of strain combination on the time course of rejection is illustrated by the results of morphological grading

Experimental group	*Grade of rejection*	*Number of allografts at each grade, when examined at intervals after transplantation (days)*					
		1-3	*4-5*	*6-7*	*8-10*	*11-15*	*16-20*
Allografts	Normal	10	—	—	—	—	—
CBA ⇌ Balb/c	L+	3	3	1	—	—	—
	L++	5	15	15	5	—	—
(H-2 incompatible)	Flat	5	8	9	5	—	—
	Submucosa	1	1	9	13	7	4
Allografts	Normal	7		2		1	—
CBA ⇌ C3H	L+	2		4		2	—
	L++	2		2		4	2
(H-2 compatible)	Flat	1		3		6	13
	Submucosa	—		—		1	7

Table 2 *Morphological grading of rejection in allografts of mouse small intestine*



(a)



(b)

Fig. 1 (a) *Isograft of mouse small intestine, nine days after implantation of a fragment of CBA foetal intestine under the kidney capsule of an adult CBA mouse. Villi and crypts appear normal, and there are few lymphoid cells in the lamina propria and within the epithelium. H and E,* × *120.* (b) *Allograft of mouse small intestine, nine days after implantation of a fragment of CBA foetal intestine under the kidney capsule of an adult Balb/c mouse. The mucosa appears flat, with villous atrophy, the crypts of Lieberkuhn are long and the lamina propria is extensively infiltrated with lymphocytes. H and E,* × *120.*

of allografts, summarised in Table 2. The sequence of events which leads to rejection is identical in all strain combinations studied but rejection between the H-2 identical strains CBA and C3H takes around 20 days, whereas rejection of the allografts between the unrelated strains CBA and Balb/c is completed by 10 days after transplantation. Figures 1a and 1b illustrate the typical appearances of a normal isograft and a partly rejected allograft.

INFLUENCE OF REJECTION ON CRYPTS OF LIEBERKUHN

In newborn mice, the crypts are very short and may occasionally be impossible to define; however, they increase in length during the first week of life and by the age of 9 days have a mean length of 25·6 μm (standard error, SE 4·6 μm). Isograft crypts also enlarge during this period and have a mean length of 32 μm (SE 2·6 μm) by day 9 after implantation. This value is not significantly different from the mean crypt length of normal mice of the same age.

However, objective measurement of well-orientated allograft crypts has shown that they are consistently longer than crypts of isografts and of normal small intestine (Fig. 2). From the fourth day, the crypts of rejecting grafts are always longer than those of isografts and normal intestine of the same age, this difference being significant ($P < 0.001$) in every comparison made. This finding of abnormally long crypts—crypt hyperplasia—has been the most consistent feature of rejection in these experiments, and forces us to modify previous subjective statements (Ferguson and Parrott, 1973a) about the 'normal' appearance of some crypts in rejection. Although some variation in crypt length does occur during rejection, our previous finding was probably due to observation of crypts which were not properly orientated.

The relation between crypt depth and the general morphological grading of rejection is shown in Fig. 3. By using this type of analysis, the differences in the time course of rejection between different strains are circumvented, and it is clear that the effect of rejection on crypt length is similar in slow and fast rejection. Significant lengthening of the crypts has

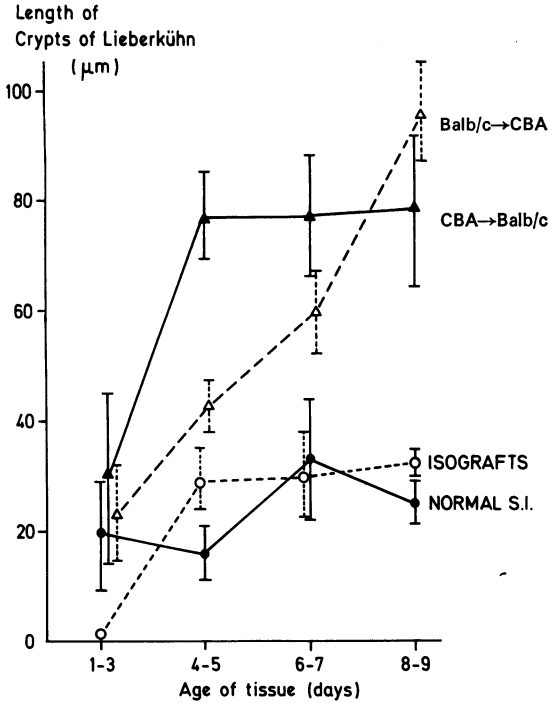


Fig. 2 *The influence of allograft rejection on the lengths of the crypts of Lieberkuhn in mouse small intestine. Means ± SE of crypt length in groups of specimens of normal small intestine, isografts of small intestine in CBA and Balb/c mice, and allografts of small intestine between the strains CBA and Balb/c. With this strain combination rejection starts around the third day after graft implantation and is completed by 10 days. Specimens are grouped according to tissue age—that is, days after birth or implantation. At least five grafts per point for grafted intestine and four mice per point for normally sited intestine.*

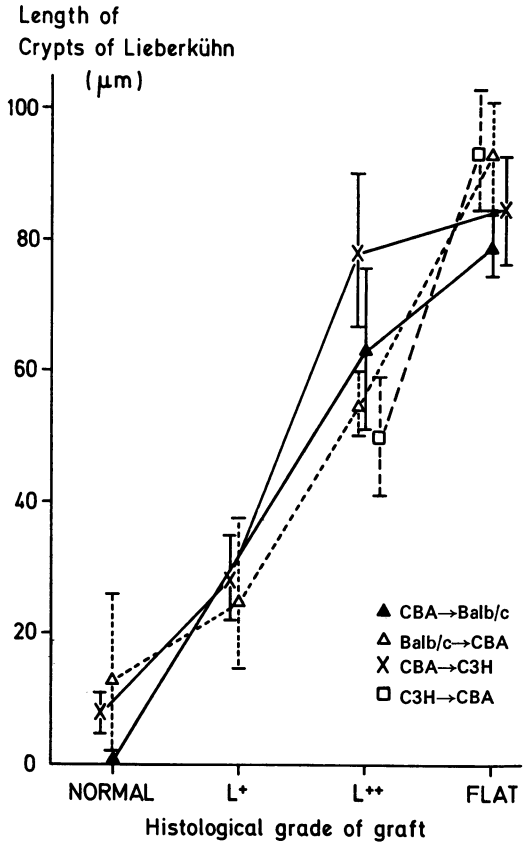


Fig. 3 *Correlation between histological grade of rejection and the length of the crypts of Lieberkuhn in allografts of foetal mouse small intestine (four strain combinations). Means ± SE of all groups of specimens classified according to histological grade, irrespective of tissue age. At least six grafts per point.*

been found at grades L+ and L++ in all of the groups studied, and this occurs before the grafts have developed atrophy of the villi.

INFLUENCE OF REJECTION ON VILLI

For the first few days after implantation, grafts are extremely small and it is rarely possible to find enough suitably cut areas to allow measurement of villous height. Thus, we did not obtain meaningful measurements of the villi during rejection of allografts between the CBA and Balb/c strains. The slower speed of rejection in the CBA-C3H combination allowed the grafts to grow to such a size that sequential measurements of villous height could be made and the results are illustrated in Fig. 4. In normal small intestine and in isografts, villi slowly increase in height for three weeks after birth. However, the villi are shorter in isografts. This difference from normal intestine is real, and not due to fixation or orientation artefacts. Possible reasons for the shorter villi in isografts are presently under investigation. However, in allografts undergoing rejection, villi are consistently shorter than in isografts of the same age ($P < 0.01$ for all groups after day 6). This difference is at maximum in specimens examined between 16 and 20 days after implantation; the mean villous height for normal intestine of this age is 390 μm; for isografts the mean villous height is 217 μm; and for allografts between CBA and C3H strains the mean villous height is only 12 μm.

INFLUENCE OF REJECTION ON EPITHELIAL CELL HEIGHT

Measurement of enterocyte height did not reveal any

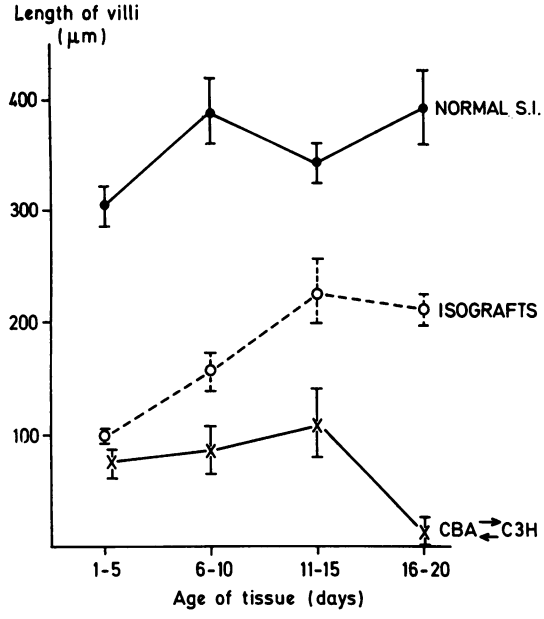


Fig. 4 *Influence of allograft rejection on the length of villi in mouse small intestine. Means ± SE of villus length in groups of specimens of normal small intestine, isografts of small intestine in CBA and Balb/c strains, and allografts of small intestine between strains CBA and C3H. With this strain combination rejection starts about five days after implantation and in most grafts is completed by 25 days. At least six grafts or normally sited small intestine per point.*

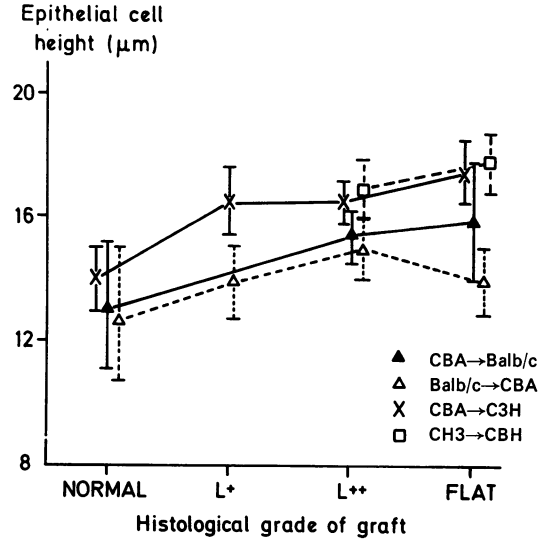


Fig. 5 *Lack of influence of allograft rejection on the height of small intestinal villous epithelial cells. Means ± SE of epithelial cell height in allografts of mouse small intestine (four strain combinations). Specimens are grouped according to histological grade of rejection. At least five grafts per point.*

significant differences between normal intestine, isografts and allografts (Fig. 5). Enterocyte height was in the range 13-18 μm in all groups studied. The enterocytes appeared to have essentially normal morphology as assessed by light microscopy. However, electron microscopic studies have shown some changes in the brush border and these findings are discussed in another paper (Carr *et al.*, 1976).

INFLUENCE OF REJECTION ON LYMPHOID CELLS OF LAMINA PROPRIA

Lamina propria lymphoid cells are not present in neonatal mouse intestinal mucosa (Ferguson and Parrott, 1973a; Mattioli and Tomasi, 1973) and by our definition the earliest sign of rejection is the appearance of mucosal lymphocytes. These are at first confined to the draining lymphatics where pyroninophilic blast cells are obvious. As rejection proceeds, the lymphocytes infiltrate the lamina propria and in established rejection there is dense lymphocyte infiltration of the mucosa and submucosa with the lymphatic vessels packed with lymphocytes (Fig. 6). Plasma cells are not found in the mucosa in early rejection but at the 'submucosa' stage—that is, when the mucosa has been destroyed, they are present and infiltrate the residual muscle layers.

INFLUENCE OF REJECTION ON IE LYMPHOCYTES

Intraepithelial lymphocyte counts have been carried out in normal intestine, in isografts, and in allografts between the strains CBA and C3H. The results of these counts are summarised in Fig. 7. Whereas IE lymphocytes are infrequent or absent in neonatal mouse intestine and in isografts, many are present in the allografts from day 6 ($P < 0.01$ for all comparisons); and the numbers of IE lymphocytes increase progressively with time after implantation. Figures 8a and 8b highlight the absence and presence of IE lymphocytes within the epithelium of an isograft and an allograft.

Only a limited assessment of IE lymphocyte infiltration of grafts between CBA and Balb/c has been possible and Table 3 illustrates the numbers of grafts in these combinations in which IE lymphocytes were assessed to be present or absent (they were considered absent if no lymphocytes were found among 200 epithelial cells examined). As in slow rejection, this semiquantitative assessment of IE lymphocyte levels in acute rejection shows that as rejection proceeds there is an influx of lymphocytes into the epithelium.

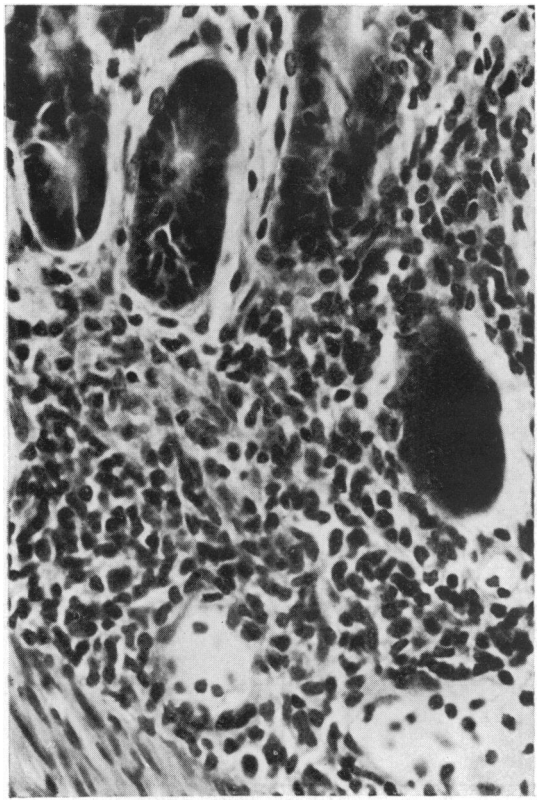


Fig. 6 *Lymphocyte infiltration of the lamina propria in an allograft of CBA foetal small intestine 17 days after implantation in a C3H host mouse. H and E, × 300.*

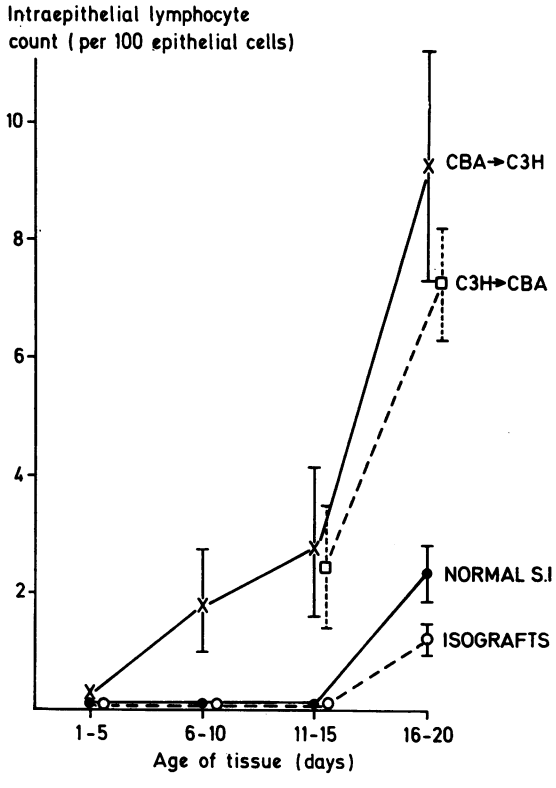


Fig. 7 *Influence of allograft rejection on the intraepithelial lymphocytes of the small intestine. Means ± SE of IE lymphocyte counts (per 100 epithelial cells) in normal small intestine, isograft of small intestine in CBA and Balb/c mice, and allografts between CBA and C3H strains. Specimens are grouped according to tissue age. At least five grafts or normally sited intestines per point.*

Table 4 illustrates intraepithelial lymphocyte levels and villus/crypt ratio during rejection (obtained by dividing the mean villus height by the mean crypt depth at each time interval). During rejection, villus height diminishes and crypt length increases, thus producing as rejection progresses a decrease in the value of the v/c ratio. At the same time there is a striking increase in the intraepithelial lymphocyte levels. In isografts, however, villus height is maintained during the time studied, the v/c ratio remains high, and there is only a slight increase in IE lymphocyte levels.

The association between the morphological changes accompanying rejection or the lamina propria lymphocyte infiltration and the presence of intraepithelial lymphocytes is, however, not complete, as is illustrated by Table 5. It can be seen that, even in late rejection, in all of the strain combinations combined, almost one quarter of the grafts with a 'flat' appearance, and thus a low v/c ratio and dense lamina propria lymphocyte infiltrate, have no IE lymphocytes present.

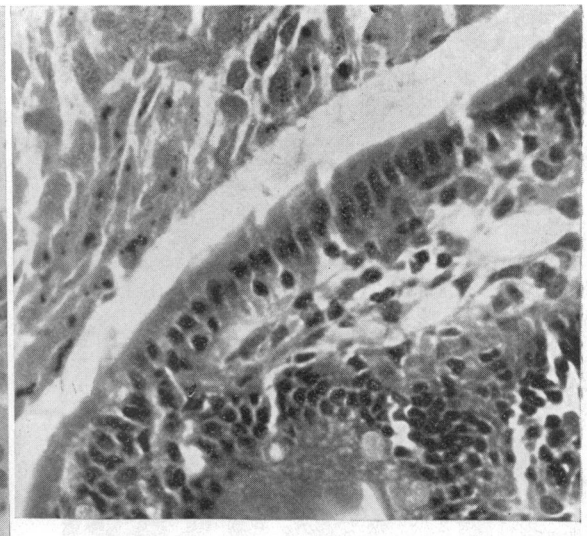
Experimental group	*Days after implantation of graft*	*Grafts examined (no.)*	*Grafts with IE lymphocytes present (at least 1 lymphocyte per 200 epithelial cells (no.)*
Isografts	1-3	7	0
	4-5	2	1
CBA → CBA	6-7	2	2
Balb/c → Balb/c	8-9	4	0
Allografts	1-3	20	4
CBA ⇌ Balb/c	4-5	27	18
	6-7	25	23
	8-9	9	7

Table 3 *Presence or absence of intraepithelial lymphocytes in isografts and allografts of mouse small intestine during acute rejection*

Discussion

Allograft rejection of small intestine is a cell-mediated reaction directed against histocompatibility

(a)



(b)

Fig. 8 *Isograft of small intestine in a CBA mouse 18 days after implantation. Note the absence of intraepithelial lymphocytes. H and E, × 315.* (b) *Allograft of CBA small intestine implanted in a C3H host 17 days previously. Note the presence of basally situated intraepithelial lymphocytes between columnar enterocytes. H and E, × 315.*

Age of tissue (days)	*Isografts* CBA → CBA Balb/c → Balb/c		*Allografts* CBA ⇌ C3H	
	v/c ratio	*IE lymph/100 epithelial cells*	*v/c ratio*	*IE lymph/100 epithelial cells*
1- 5	—	0·2	5·1	0·5
6-10	4·7	0·2	1·8	1·7
11-15	3·2	0·2	1·7	2·5
16-20	3·5	1·4	0·2	8·3

Table 4 *Villus/crypt ratio (v/c) and IE lymphocyte numbers at different times during slow rejection compared with isografts of same age*

Morphological grading of graft	*Grafts examined (no.)*	*Rejecting grafts with no IE lymphocytes present (no.)*
Normal	20	19
L+	15	12
L++	50	12
Flat	50	12

Table 5 *Presence or absence of intraepithelial lymphocytes in allografts of mouse small intestine at different grades of rejection*

antigens which differ in the cells in the small intestinal mucosa of the different strains of mice used (Ferguson and Parrott, 1973b; Elves and Ferguson, 1975). We have now shown that constant morphological features of this reaction are infiltration of the lamina propria with lymphocytes, hyperplasia of the crypts of Lieberkuhn, and ulceration of the mucosa. Usually, but not always, there is also villous atrophy and increased numbers of intraepithelial lymphocytes. The morphology of individual enterocytes seems to remain normal throughout rejection, these cells being columnar, with basal nuclei and an easily distinguishable brush border even when the general architecture of the specimen is of sub-total villous atrophy. Plasma cells have not been found in the lamina propria during early rejection. These infiltrate the debris of the allografts which remains after ulceration of the mucosa. Polymorphonuclear leucocytes have rarely been found in rejecting allografts in our experiments.

The striking changes which occur in villus-crypt architecture in the absence of morphological damage to individual enterocytes are important and interesting. Indeed, we have previously reported that sheets of apparently normal enterocytes are extruded into the lumen of rejecting grafts (Ferguson and Parrott, 1973b). This exfoliation of the epithelium cannot be attributed entirely to a cytotoxic effect of the infiltrating intraepithelial lymphocytes, for,

although these cells are in intimate contact with the enterocytes and are usually present during rejection, this is not always the case. A more constant observation has been the presence of many lymphocytes in the lamina propria below the basement membrane. It seems possible that the local immune response may influence adhesion of enterocytes to the underlying connective tissue rather than have a directly cytotoxic effect on these epithelial cells. Enterocyte adhesion could be impaired by an effect of the local cell-mediated immune reaction on local blood flow or on the connective tissue of the basement membrane; alternatively, the effect could be produced by direct damage to the cell membranes which would then influence the adhesion of the enterocytes to the basement membrane and to one another.

If the cell height is a valid measurement of injury to mucosal cells, the absence of any decrease in cell height during rejection would seem to indicate that it is the stroma of the transplanted tissue which is bearing the brunt of the reaction rather than the epithelial cell itself. The link between the histological picture of villous atrophy and crypt hyperplasia and the local cell mediated immune response may be by one or more humoral factors produced as a result of the interaction between sensitized lymphocytes and the histocompatibility antigens of the allograft. Lymphokines have been shown to be mitogenic and cytotoxic for other cell types (Remold, 1972) and we have suggested that such an enteropathic lymphokine may cause enterocyte exfoliation, which would cause villous atrophy and compensatory crypt hyperplasia, not only in allograft rejection but also in other situations where a local CMI reaction takes place in the vicinity of the small intestinal mucosa (Ferguson, 1974b; Ferguson and Jarrett, 1975; Ferguson and MacDonald, 1975; Ferguson *et al.*, 1975). A humoral factor which causes migration inhibition of human peripheral leucocytes has been observed after gluten challenge of intestinal biopsies from patients with coeliac disease (Ferguson *et al.*, 1975) and a factor which inhibits the migration of mouse peritoneal macrophages has recently been found to be produced during allograft rejection of foetal intestine, but not in isografts of the same age (MacDonald and Russell, 1976). Further investigations into the role of lymphokines in rejection are at present being carried out.

Hyperplasia of the crypts of Lieberkuhn occurs early in rejection (at four to five days) and this may be due to a direct effect of the rejection process on the crypt cells or a compensatory hyperplasia after an increased rate of enterocyte loss. An early interaction between sensitised lymphocytes and the tissue antigens might produce a mitogenic lymphokine which would cause increased cell division in the crypts; however, the same interaction might produce an enteropathic lymphokine which would cause increased cell loss and compensatory hyperplasia. But, as crypt hyperplasia is occurring without any demonstrable loss of villus height, we favour the concept of a direct mitogenic effect of rejection upon the crypt cells themselves. In late rejection, however, when villus height dramatically drops, compensatory crypt hyperplasia should occur.

The homing of lymphoid cells to the mucosa of rejecting allografts differs from the usual homing of immunoblasts to the small intestine (Delorme *et al.*, 1969 in at least two respects. In rejection, the infiltrating cells are mainly lymphocytes, whereas in normal intestine there is a mixture of lymphocytes and plasma cells; furthermore, lymphocytes appear in the mucosa of allografts up to two weeks earlier than these cells infiltrate the intestine of normal mice. In this context, it is important to emphasize again that, in the small intestine of normal mice, within the age range used for these experiments, lymphocytes and plasma cells are almost never present outside the Peyer's patches (Ferguson and Parrott, 1973a; Mattioli and Tomasi, 1973). A specific homing of immunoblasts to the small intestine of the mouse begins in the third week of life and experiments in both mice and rats have shown that homing of plasma cell precursors to grafts of small intestine will occur in the complete absence of intraluminal antigens (Moore and Hall, 1972; Ferguson and Parrott, 1973a; Guy-Grande *et al.*, 1974; Parrott and Ferguson, 1974). In our work on the induction of antibodies to grafts of intestine, we found that the stimulus for production of the humoral response to an allograft was probably the debris produced by an early cell-mediated immune response (Elves and Ferguson, 1975). For this reason, the humoral antibody response to an allograft occurred several days after rejection was completely established. The late infiltration of grafts with plasma cells is in keeping with the time course of humoral antibody production and is likely, therefore, to be associated with the specific humoral immune response to an allograft rather than to a gut-directed homing mechanism. Further experiments on the traffic of lymphoid cells to allografts of small intestine will be required to confirm this.

There are scattered reports in the literature of the effects of experimental hypersensitivity reactions in the small intestinal mucosa, although these have usually been observed in the course of experiments designed for different purposes. For example, under appropriate conditions a local reaginic (type 1) immune response can produce oedema of the villi with exfoliation of the epithelium (Barth *et al.*, 1966).

A local Arthus (type III) reaction, with deposition of immune complexes within the mucosa, may cause local polymorph infiltration without obvious tissue damage (Bellamy and Nielsen, 1974). The morphological and functional changes associated with the initiation of, and recovery from, these hypersensitivity reactions have not been described so it remains to be defined whether or how an antibody mediated local immune reaction can influence the villus-crypt architecture, lymphoid cell infiltrate, and absorption. However, in the experiments on rejection described above, we have definitely shown that a local delayed hypersensitivity reaction affects villi, crypts, and the lymphoid cells of the intestine. If these changes are produced by lymphokines, they should occur in any situation where a cell-mediated immune response occurs in the vicinity of the intestinal mucosa, and not merely when the response is directed against the tissue itself. Indeed, there is a precedent for this concept of local tissue damage as an unavoidable effect of a protective immune reaction, for this is the case in the lesions of leprosy (Turk and Bryceson, 1971). A local cell-mediated immune response may be the common factor which causes villous atrophy, crypt hyperplasia, and mucosal ulceration in food allergy, coeliac disease, local parasitic infections, and gastroenteritis. When such a reaction occurs in an intestine already colonised by commensal flora and exposed daily to food, the ulceration, and perhaps also adjuvant effects mediated by lymphokines (Krejci *et al.*, 1973) could allow ingress of immunogenic food and microbial antigens, with immune reactions to these intestinal constituents superimposed upon and ultimately masking the primary disease process which is mainly cell mediated.

Nevertheless, local cell-mediated immunity cannot be considered in isolation. In the *Nippostrongylus brasiliensis* infection referred to in the introduction, there is evidence of IgE, IgG, mast cell, eosinophil and lymphocyte involvement in the local lesion (Jarrett and Urquhart, 1971; Dineen *et al.*, 1973; Ogilvie and Jones, 1973; Ogilvie and Love, 1974). When patients with coeliac disease are challenged with oral gluten, IgM, IgA, IgG and IgE are involved in the subsequent local immune reaction (Shiner, 1973; Lancaster-Smith *et al.*, 1974; *Lancet*, 1974). Recently, we have also reported evidence of a local cell-mediated immune response to gluten in coeliac disease (Ferguson *et al.*, 1975). Clearly, great caution must be exercised in attributing enteropathy and malabsorption to any one type of local hypersensitivity reaction. Nevertheless, by using a clearly defined experimental model such as allograft rejection, in which the extent and type of the local immune reaction can be controlled, interactions between local immunity, local hypersensitivity, the absorptive and the endocrine functions of the small intestine can now be thoroughly investigated.

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References

Barth, E. E. E., Jarrett, W. F. H., and Urquhart, G. M. (1966). Studies on the mechanism of the self-cure reaction in rats infected with *Nippostrongylus brasiliensis*. *Immunology*, **10**, 459-464.

Bellamy, J. E. C., and Nielsen, N. O. (1974). Immune-mediated emigration of neutrophils into the lumen of the small intestine. *Infection and Immunity*, **9**, 615-619.

Calman, K. C. (1974). Why are small bowel tumours rare? An experimental model. *Gut*, **15**, 552-554.

Carr, K. E., MacDonald, T. T., and Ferguson, A. (1976). Influence of allograft rejection on small intestinal mucosal architecture, assessed by scanning and transmission electron microscopy. (In preparation).

Cornelius, E. A. (1970). Protein-losing enteropathy in the graft-versus-host reaction. *Transplantation*, **9**, 247-252.

Delorme, E. J., Hodgett, H. J., Hall, J. G., and Alexander, P. (1969). The cellular immune response to primary sarcomata in rats: 1. The significance of large basophilic cells in the thoracic duct lymph following antigenic challenge. *Proceedings of the Royal Society of London (Biology)*, **174**, 229-236.

Dineen, J. K., Ogilvie, B. M., and Kelly, J. D. (1973). Expulsion of *Nippostrongylus brasiliensis* from the intestine of rats: collaboration between humoral and cellular components of the immune response. *Immunology*, **24**, 467-475.

Elves, M. W., and Ferguson, A. (1975). The humoral immune response to allografts of foetal small intestine in mice. *British Journal of Experimental Pathology*. **56**, 454-458.

Ferguson, A. (1972). Immunological roles of the gastrointestinal tract. *Scottish Medical Journal*, **17**, 111-118.

Ferguson, A. (1973). Implantation of tissue under the kidney capsule. In *Handbook of Experimental Immunology*. p.A312-A313, Blackwell: Oxford.

Ferguson, A. (1974a). Lymphocytes in coeliac disease. In *Coeliac Disease*, pp. 265-276. Edited by W. T. J. M. Hekkens and A. S. Pena. Stenfert Kroese: Leiden.

Ferguson, A. (1974b). Thymus dependence of experimental villous atrophy. In *Coeliac Disease*, pp. 286-287. Edited by W. T. J. M. Hekkens and A. S. Pena. Stenfert Kroese: Leiden.

Ferguson, A., and Jarrett, E. E. E. (1975). Hypersensitivity reactions in the small intestine. 1. Thymus dependence of experimental 'partial villous atrophy'. *Gut*, **16**, 114-117.

Ferguson, A., and MacDonald, T. T. (1975). Effects of local hypersensitivity reactions on small intestinal morphology. *Behring Institute Research Communications*, **57**, 118-121.

Ferguson, A., and Murray, D. (1971). Quantitation of intraepithelial lymphocytes in human jejunum. *Gut*, **12**, 988-994.

Ferguson, A., MacDonald, T. T., McClure, J. P., and Holden, R. J. (1975). Cell-mediated immunity to gliadin within the small-intestinal mucosa in coeliac disease. *Lancet*, **1**, 895-897.

Ferguson, A., and Parrott, D. M. V. (1972). Growth and development of 'antigen-free' grafts of foetal mouse intestine. *Journal of Pathology*, **106**, 95-101.

Ferguson, A., and Parrott, D. M. V. (1973a). The effect of antigen deprivation on thymus-dependent and thymus-independent lymphocytes in the small intestine of the mouse. *Clinical and Experimental Immunology*, **12**, 477-488.

Ferguson, A., and Parrott, D. M. V. (1973b). Histopathology and time course of rejection of allografts of mouse small intestine. *Transplantation*, **15**, 546-554.

Fichtelius, K. E., Yunis, E. J., and Good, R. A. (1968). Occurrence of lymphocytes within the gut epithelium of normal and neonatally thymectomized mice. *Proceedings of the Society for Experimental Biology and Medicine*, **128**, 185-188.

Gell, P. G. H., and Coombs, R. R. A. (eds) (1969). *Clinical Aspects of Immunology*, pp. 575-596. Blackwell: Oxford.

Guy-Grand, D., Griscelli, C., and Vassalli, P. (1974). The gut-associated lymphoid system: nature and properties of the large dividing cells. *European Journal of Immunology*, **4**, 435-443.

Hedberg, C. A., Reiser, S., and Reilly, R. W. (1968). Intestinal phase of the runting syndrome in mice. 2. Observations on nutrient absorption and certain disaccharidase abnormalities. *Transplantation*, **6**, 104-110.

Holmes, J. T., Klein, M. S., Winawer, S. J., and Fortner, J. G. (1971). Morphological studies of rejection in canine jejunal allografts. *Gastroenterology*, **61**, 693-706.

Jarrett, E. E. E., and Urquhart, G. M. (1971). The immune response to nematode infections. *International Review of Tropical Medicine*, **4**, 53-96.

Krejci, J., Pekarek, J., Svejcar, J., and Johanovsky, J. (1973). The effect of lymphokines on the development of delayed hypersensitivity to an unrelated antigen. *Immunology*, **25**, 875-879.

Lancaster-Smith, M., Kumar, P. J., and Clark, M.L. (1974). Immunological phenomena following gluten challenge in the jejunum of patients with adult coeliac disease and dermatitis herpetiformis. In *Coeliac Disease*, pp. 173-174. Edited by W. T. J. M. Hekkens and A. S. Pena. Stenfert Kroese: Leiden.

Lancet (1974). The coeliac philosophy. (Editorial). *Lancet*, **2**, 501-502.

MacDonald, T. T., and Russell, R. J. (1976). The detection of lymphokines in rejecting allografts of foetal mouse intestine. (In preparation.)

Mattioli, C. A., and Tomasi, T. B. (1976). The lifespan of IgA plasma cells from the mouse intestine. *Journal of Experimental Medicine*, **138**, 452-460.

Moore, A. R., and Hall, J. G. (1972). Evidence for a primary association between immunoblasts and small gut. *Nature*, **239**, 161-162.

Ogilvie, B. M., and Jones, V. E. (1973). Immunity in the parasitic relationship between helminths and hosts. *Progress in Allergy*, **17**, 93-144.

Ogilvie, B. M., and Love, R. J. (1974). Co-operation between antibodies and cells in immunity to a nematode parasite. *Transplantation Reviews*, **19**, 147-168.

Palmer, R. H., and Reilly, R. W. (1971). Bile salt depletion in the runting syndrome. *Transplantation*, **12**, 479-483.

Parrott, D. M. V., and Ferguson, A. (1974). Selective migration of lymphocytes within the mouse small intestine. *Immunology*, **26**, 571-588.

Reilly, R. W., and Kirsner, J. B. (1965). Runt intestinal disease. *Laboratory Investigation*, **14**, 102-107.

Remold, H. G. (1972). Purification and characterization of lymphocyte mediators in cellular immunity. *Transplantation Reviews*, **10**, 152-176.

Shiner, M. (1973). Ultrastructural changes suggestive of immune reactions in the jejunal mucosa of coeliac children following gluten challenge. *Gut*, **14**, 1-12.

Shiner, M., and Doniach, I. (1960). Histopathologic studies in steatorrhoea. *Gastroenterology*, **38**, 419-440.

Snell, G. D., Hoecker, G., Amos, D. B. and Stimpfling, J. H. (1964). A revised nomenclature for the histocompatibility-2 locus of the mouse. *Transplantation*, **2**, 777-784.

Sprent, J., and Miller, J. F. A. P. (1972). Interaction of thymus lymphocytes with histoincompatible cells. II. Recirculating lymphocytes derived from antigen-activated thymus cells. *Cell Immunology*, **3**, 385-404.

Turk, J. L., and Bryceson, A. D. M. (1971). Immunological phenomena in leprosy and related diseases. *Advances in Immunology*, **13**, 209-266.