Histopathology of cell mediated immune reaction in mouse colon—allograft rejection

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SUMMARY Grafts of mouse fetal colon, implanted beneath the renal capsule of adult hosts, have been used to study the growth and development of colonic isografts and the rejection of colonic allografts. Isografts grew normally and maintained a structure similar to normal colon. Grafts between strains with H2 histocompatibility differences were rejected by 13 days after transplantation. Early progressive infiltration of the grafts by lymphoid cells was followed by increasing damage to, and subsequent loss of, the epithelial cell layer and destruction of the underlying muscle, changes which parallel those seen in rejection of skin and small bowel. The increase in survival time which is seen in allografts between strains with H2 identity was longer in the colon than has been seen in the skin or small bowel; none of the allografts of colon were completely rejected before 30 days, and some remained viable at 50 days. Comparison of the appearances of rejection in the colon with those of ulcerative colitis and colonic Crohn's disease does not show the striking similarity which is seen between small bowel rejection and coeliac disease. Many of the individual features of these diseases are, however, present in the course of colonic rejection.

Within the lumen of mammalian small bowel and colon large amounts of food and microbial antigens confront the intestinal populations of lymphocytes and plasma cells. It has been postulated that local immune reactions involving these antigens may cause damage to the bowel wall—for example, in inflammatory bowel disease (Shorter *et al.*, 1972).

Studies of animal models of local hypersensitivity can give an indication of the effects of antibody and cell mediated hypersensitivity and to date most studies in bowel have concentrated on the small intestine (Holmes *et al.*, 1971; Ferguson and Parrott, 1973). Canine and mouse small intestine undergoing rejection show features of villous atrophy and crypt hyperplasia which are similar to the lesions seen in human coeliac disease (Holmes *et al.*, 1971; Ferguson and Parrott, 1973). The association of a T-cell mediated immune response and villous atrophy is also seen in animals with graft versus host disease (Reilly and Kirsner, 1965) and in the response of the intestine to parasitic infestation (Ferguson and Jarrett, 1975).

T-cell mediated mechanisms have also been

postulated as important in causing disease in the colon, and, in an animal model in swine, contact hypersensitivity to DNCB in the colon has produced changes similar to those of ulcerative colitis (Bicks *et al.*, 1965; Bicks *et al.*, 1967).

Preliminary studies in mice had shown that fetal colon could be transplanted in a similar way to small intestine, and the growth of colonic isografts has been studied in more detail here. Allografts between mouse strains with the same and with different H2 histocompatibility antigens have been used to study the microscopic features and course of rejection in mouse colon. These changes have been compared with the features of small bowel rejection and with the typical features seen in the colon in Crohn's disease and ulcerative colitis.

Methods

Mice used were of three strains, CBA (H2k), C3H (H2k), and Balbc (H2d), maintained in the Department of Bacteriology and Immunology. The grafts were implanted using a previously described technique (Ferguson and Parrott, 1972). Pregnant mice were killed a day before they were due to litter, the fetuses removed, and the fetal colons dissected free. A piece of fetal colon 0.5-1.0 cm long was then

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implanted beneath each renal capsule of an adult host mouse. A total of 242 grafts were implanted and the strain combinations are shown in Table 1.

Mice with isografts were killed by ether overdosage at weekly intervals up to eight weeks after transplantation. Allografts between CBA and Balbc mice were examined daily from one to 16 days, and also at 20 and 25 days. Allografts between CBA and C3H mice were examined between four and 50 days after transplantation. For comparison, pieces of normally situated colon were taken from mice aged four to 55 days.

All tissues were fixed in formol saline, embedded in wax, and 5 μ histological sections were cut. These were stained with haematoxylin and eosin (H/E) and methyl green pyronin (MGP). The overall histological appearances were assessed in the normal bowel, in isografts, and in rejecting grafts. Particular attention was paid to the graft contents and to the details of mucosal structure and the cell types present. The temporal development of the inflammatory cell infiltrate in the lamina propria and submucosa was studied and the appearances of the muscle coat and serosa were noted. When the pattern of homograft rejection had been defined the sections were once more assessed, blindly, and graded into one of five grades to allow comparison of the time course of rejection between strains. To allow statistical comparison between stages of development in normal colon, isograft, and homograft, and to allow these groups to be compared with each other, measurements of the epithelial cell height and of crypt length were made. These measurements were made using a micrometer eye piece at a magnification of $\times 400$. Columnar surface epithelial cells with basal nuclei and present as a single layer were used for the measurement of cell height. Within older isografts there were often cystic areas lined by flattened epithelial cells. In such grafts only areas of normal crypt-forming mucosa were used for the cell height measurements. Crypts which had been sectioned longitudinally with their bases abutting on to the muscularis mucosa were used for the measurement of crypt length. This measure is a reflection of the thickness of mucosal layer. The groups used for

statistical comparison were of five grafts or more, and comparisons were made using Student's *t* test.

Results

Of the 242 grafts which were implanted, histological assessment was possible in 202 (Table 1). The other 40 grafts, which were scattered among the experimental groups, were either not found at post-mortem examination or yielded insufficient material for histological purposes. Not all of the 202 grafts assessed histologically could be used to measure cell height or crypt length because some had been sectioned tangentially.

GROWTH OF NORMAL MOUSE COLON

During the first two weeks of life mouse colon had a simple glandular structure with short crypts and few lymphocytes in the lamina propria or submucosa. As mice grew, the mucosa became thicker with progressive lengthening of the crypts. In haematoxylin and eosin preparations, the epithelium comprised columnar absorptive cells on the surface and mucus cells lining the crypts. However, it is likely that the cells in the lower part of the crypt are not true mucus cells but vacuolated precursor cells of the columnar cell line (Chang and LeBlond, 1971). A lymphoid cell infiltrate began to appear at about two weeks and lymphocytes and some plasma cells were both scattered through the lamina propria and aggregated into lymphoid follicles. The number of infiltrating lymphoid cells increased with age and lymphoid follicles grew in number and prominence. The muscle layers, at first thin, became thicker as the colon grew and the bowel was covered by a thin layer of serosa.

During the study the height of the epithelial cells did not differ from the height of normal adult cells (Table 2). Crypt length increased almost threefold (Fig. 1) and each period showed an increase over the preceding period (weekly to 28 days $P < 0.001$; 29-42 and 43-56 days $P < 0.05$). At eight weeks crypts had reached adult length.

Table 1 Numbers of isografts and allografts studied

Strain		Grafts implanted	Suitable histology
Donor	Recipient		
CBA	CBA	32	28
Balbc	Balbc	28	26
Blabc	CBA	60	52
CBA	Balbc	50	30
CBA	C ₃ H	72	66
Total		242	202

Table 2 Height of epithelial cells in normal colon and isografts at intervals during development (mean $\pm$ SD)

Age (days)	Cell heights (μ)	
	Normal Colon	Isografts
0-7		26.2 $\pm$ 0.8
8-14		27.9 $\pm$ 0.73
0-14	27.5 $\pm$ 0.4	27.0 $\pm$ 0.6
15-28	26.8 $\pm$ 0.27	28.4 $\pm$ 0.48
29-42	27.1 $\pm$ 0.25	28.1 $\pm$ 0.63
43-56	27.4 $\pm$ 0.23	29.2 $\pm$ 0.7
130	27.0 $\pm$ 0.23

Fig. 1 Mean depth ($\pm$ SEM) of crypts in normal colon after birth and in isografts and allografts after transplantation. ●—● normal colon. x—x isograft. ▲—▲ CBA + C₃H homograft. ■—■ CBA + Balbc homograft.

ISOGRAFT GROWTH

Isografts survived and grew up to the end of the experimental period, when the grafts were large and often obscured the underlying kidney. The kidneys and other abdominal organs were normal and the host animal remained healthy. The overall histological appearances of the isografts were similar to normal colon; the lumens of the grafts were filled with mucus and some shed epithelial cells. Columnar epithelium with a normal complement of mucus cells lined the grafts and formed normal crypts (Fig. 2). Grafts aged more than 3 to 4 weeks usually had in

their structure areas of cystic swelling (Fig. 3) which were lined by flatter epithelium. The lamina propria was featureless for the first two weeks. After this, scattered lymphoid cells and lymphoid aggregate began to appear, increased in number as grafts aged but were always less prominent than in normal colon of similar age. The muscle layers appeared normal and the underlying kidney showed no reaction to the graft. Measurements of cell height were similar to those in normal colon, though values tended to be higher in isografts. At first isograft crypts lengthened at a rate similar to those of normal colon (Fig. 1) but

Fig. 2 Normal appearance of 21 day colonic isograft. H and E, $\times 300$.

Fig. 3 Area of cystic dilatation in isograft aged 35 days. Crypts are shorter and epithelial cells flattened. H and E, $\times 100$.

after 28 days this growth ceased and at each subsequent period crypts in isografts were significantly shorter than in normal colon (28-42 days and 43-56 days $P < 0.005$).

REJECTION OF ALLOGRAFTS

Host animals bearing allografts remained healthy. The time course of rejection between C3H and CBA mice was longer than between Balbc and CBA mice; the features of rejection were otherwise similar. In both groups the overall appearances could be graded into five grades. Grade I showed essentially normal appearances, grades 2, 3, and 4 increasing degrees of lymphocytic infiltration and increasing mucosal damage, and grade 5 grafts consisted of heavily infiltrated muscle remnants devoid of mucosa, this appearance being taken as the end point of rejection.

Grafts between CBA and Balbc mice could be identified macroscopically until 12 or 13 days after transplantation. It was impossible to identify the graft site after 16 days. Histological examination of the grafts showed normal structure for the first day or two with some haemorrhage, related to the operation, in and around the graft. After the first two days the lumens of the grafts contained increasing numbers of shed epithelial cells and by 10 days after transplantation only small islands of epithelium lined the grafts, which were filled with much cellular debris. On days 2 and 3 the epithelium of the grafts was normal but

lymphocytic infiltration had begun. This infiltrate was of small lymphocytes with occasional large pyrinophilic blast cells and was in the lamina propria, the submucosa, and the renal bed of the graft. These were grade 2 changes (Fig. 4). Grafts examined 5, 6 and 7 days showed grade 3 changes (Fig. 5) with evidence of mucosal damage. Mucosal detail remained clear but mucus cell numbers were decreased, the cells became smaller and there were fewer crypts. The lymphocytic infiltrate had increased to involve all layers of the graft wall and the graft bed. Eight or nine days after grafting, mucosal detail became more indefinite and individual cells were difficult to identify. Few crypts were seen and these grade 4 (Fig. 6) changes progressed, with loss of the remaining epithelium, to grade 5 (Fig. 7) by day 11 or 12. The graft then remained as a recognisable but heavily infiltrated framework of muscle covered by thickened renal capsule, and these remnants were destroyed over the next two or three days. In this strain combination rejection was rapid and all grafts examined 13 or more days after transplantation had been completely rejected (Fig. 8). Cell height was reduced during rejection (Table 3) when compared with isografts, but the reduction, clear at the end of the first week, was not progressive. Cell height between 0 and three days of rejection was greater than between four and six days ($P < 0.001$) but there was no change after six days. Crypt lengths over the first week of rejection did not differ from isografts at

Fig. 4 Colonic allograft, grade II changes, increased cellular infiltrate with normal epithelial structure. H and E, $\times 300$.

Fig. 5 Colonic allograft grade III. Mucous cell depletion and increasing cellular infiltration. H and E, $\times 300$.

the same period but, as there was no change in crypt length in the periods 0—three, four—six, and seven—nine days after transplantation; the expansion of the crypts seen during the first two weeks in isografts was not occurring in the allografts.

Grafts between C3H and CBA mice survived much longer and could still be easily identified up to 50 days after transplantation. The overall appearances of rejection were similar and the same grading could be used. The lymphocytic infiltrate in this strain combination tended to be more dense but there was some suggestion that it lessened in some grafts after three weeks. The course of rejection was

more prolonged and more variable when compared with Balbc and CBA allografts (Fig. 9). No grafts were rejected until 30 days and of 24 grafts examined between 30 and 50 days after transplantation 10 had relatively normal appearances. The slower pace of rejection was reflected in the longer maintenance of normal cell heights and crypt lengths (Table 3; Fig. 1). A difference in cell height did not emerge until the period eight—14 days when the cells were shorter than in isografts of similar age ($P < 0.01$), a difference which then persisted up to 50 days. As in CBA and Balbc grafts this decrease was not progressive; there was a difference between allografts of 0-14 and 15-28

Fig. 6 Colonic allograft grade IV. Short crypts, reduced in number with poor epithelial cell detail. *H and E*, $\times 300$.

Fig. 7 Allograft rejection grade V. Complete mucosal destruction with infiltrated muscle skeleton remaining. *CBA $\times$ Balbc*. 12 days. *H and E*, $\times 75$.

days ($P < 0.001$), but no further change. Allograft crypts grew in the first two weeks, but in the second week were shorter than equivalent isografts suggesting that this growth was less than optimal. After two weeks crypt growth was not maintained and the crypts remained shorter than those of isografts of the same age.

Discussion

The preparation of isografts and allografts of mouse colon has proved simple and reliable. A high propor-

tion of the grafts were recovered at post-mortem and yielded satisfactory histological preparations. Isografts of colon grew and developed with a structure similar to normal colon and allografts showed changes during rejection similar to those seen in rejecting skin and small bowel. Because intraluminal antigens are absent the morphological changes observed can be attributed to the rejection process alone.

Normally situated colon grew for eight weeks and had then normal adult structure. During this time the colonic crypts lengthened by almost threefold to

Fig. 8 Graft grades after transplantation in CBA $\times$ Balbc allografts.

reach adult depth, as described in a previous report (Martin, 1961) and in this laboratory. There was no infiltrate in the lamina propria at birth and lymphocytes and plasma cells began to appear at 12–15 days, about the time of weaning. These cells increased in number over the next six weeks, as did the number of lymphoid follicles which appeared at about the same time.

When fetal mouse colon was transplanted into syngenic hosts the isografts survived and grew normally. This corresponds with observations made on small bowel using the same technique (Ferguson and Parrott, 1972), but details of colonic morphology did not exactly match that of normally developing bowel. Epithelial cells were similar in size with a tendency to be larger, a difference reaching signifi-

ficance only at the 15–28 day period. Crypts grew for four weeks but then growth stopped. This arrest of growth occurred as grafts became distended with mucus. Continued secretion of mucus into the closed grafts might be expected to raise the pressure within the grafts and, if pressure affects cell kinetics, to result in shorter crypts. The other major difference between grafts and normal colon is the lack of antigen within the graft lumen for there is no faecal residue, and in this system the graft remains sterile. Intraluminal antigen is probably important in controlling the rate of cell turnover and its lack will add to any effects of pressure within the graft.

Several studies have demonstrated the importance of genetic differences on the course of rejection. In the mouse the rate at which skin grafts are rejected is influenced by antigens represented at the H2 locus; more rapid rejection occurs when there is H2 incompatibility (Billingham *et al.*, 1954; Counce *et al.*, 1956; McKhann, 1962; Medawar, 1963). Rejection of mouse small bowel also shows a doubling of survival time when grafts between strains with identical H2 antigens are compared with strains with different H2 antigens (Ferguson and Parrott 1973; MacDonald and Ferguson, 1976). When colon was grafted between Balbc and CBA mice, which differ at the H2 locus, all the grafts were rejected within 13 days and the histological appearances closely resembled the changes seen during rejection of both skin and small bowel. When CBA and C3H mice, which have the same H2 locus, were used for grafting a different pattern was seen. Recognisable graft elements were easily identified up to 50 days after transplantation and almost half the grafts examined between 30 and 50 days had reasonably normal structure. It seems that colon is much less vigorously rejected than skin or small bowel if histo-incompatibility represented by the H2 locus is absent.

Mucosal changes in the colon during rejection differ from those in small bowel, although the presence of villi in the small bowel makes direct comparison difficult. The essential changes in the small bowel are villous atrophy and crypt hyperplasia,

Table 3 Height of epithelial cells after transplantation in allografts in CBA $\times$ Balbc and CBA $\times$ C₃H

Cell heights (μ)			Cell heights (μ)		
Age (days)	CBA $\times$ Balbc	Isograft comparison	Age (days)	CBA $\times$ C ₃ H	Isograft comparison
0-3	25 $\pm$ 0.58	(0-7)—NS $P < 0.01$	0-7	25.6 $\pm$ 0.24	NS
4-6	20.2 $\pm$ 1.2		8-14	25.0 $\pm$ 0.51	<0.01
0-7	22.6 $\pm$ 0.78		15-28	21.1 $\pm$ 1.1	<0.001
7-9	20.4 $\pm$ 1.0		30-50	21.8 $\pm$ 1.2	<0.001

Comparison with isografts by Student's *t* test.

Fig. 9 Graft grades after transplantation in CBA $\times$ C₃H allografts.

features which mirror the changes of human coeliac disease (Ferguson and Parrott, 1973; MacDonald and Ferguson, 1976). Cell size and, as assessed by light microscopy, cell morphology are normal. These features are not seen in the colon. In grafts between CBA and Balbc mice and between CBA and C3H mice there was an early reduction in cell size and, in both groups, crypts were shorter than in isografts, although this took two weeks to appear in the CBA and C3H grafts. At no time was crypt hyperplasia seen. However, both in small bowel and colon the appearance could be due to the same mechanism—increased rate of cell turnover.

In small bowel, crypts lengthen three-fold and villi disappear; the mucosa becomes thinner (MacDonald and Ferguson, 1976). It is suggested that this appearance of villous atrophy is due to imbalance between cell loss and cell production (Pink *et al.*, 1970; Wright *et al.*, 1973). In early rejection of the colon crypt length keeps pace with that in isografts; the accumulation of increasing numbers of shed epithelial cells in the lumens of the grafts suggests that cell loss is increased and that cell production will be raised. Studies using a metaphase-

blocking technique support this (Holden, unpublished observations). As cell loss outstrips cell production crypt lengths fall behind those of equivalent age and, as in small bowel, the mucosa is thinner than normal. Of necessity, the crypts are shorter than normal.

Because the appearance of rejecting small bowel resembles that in human coeliac disease, comparison of the changes seen in rejecting colon—a T-cell mediated immune response in the large bowel—with the features of ulcerative colitis and Crohn's disease is pertinent. In these diseases immune mechanism may be important and abnormalities of humoral immunity (Bregman and Kirsner, 1960; Perlmann *et al.*, 1967) and cellular immunity (Bendixen, 1967; Shorter *et al.*, 1969; Perlmann and Broberger 1973; Richens *et al.*, 1974) have been described. Immune complexes have been found in the serum (Jewell and MacLennan, 1973) and in the bowel wall (Koffler *et al.*, 1967). A hypothesis that cell mediated immune reactions to bacterial antigens in the bowel wall are the underlying cause of ulcerative colitis and Crohn's disease has been proposed (Shorter *et al.*, 1972). The histological appearances of ulcerative colitis and Crohn's disease usually allow separation of the two

diseases (Morson and Dawson, 1972; Whitehead, 1973) and in ulcerative colitis the lesion is confined to the superficial layers of the mucosa with evidence of mucosal damage, crypt abscesses, mucus cell depletion, and destruction of some crypts. Attempts at repair occur and cell turnover in the crypts is increased (Eastwood and Trier, 1973). By contrast, the mucosal architecture in Crohn's disease is often well preserved but there is transmucosal inflammation involving the submucosa, muscle layers, and serosa. The bowel wall is oedematous, with widening of the submucosa and typical features are fissuring ulceration and granuloma formation. There is no doubt that rejection is accompanied by mucosal damage with decrease in epithelial cell size, decrease in mucus cell numbers, and changes in the crypts which lessen in number and fail to grow normally. This change, however, is associated with thinning of the mucosa and a lymphocytic infiltrate which extends through the graft wall. Plasma cells which form a major part of the infiltrate of both colitis and Crohn's disease and neutrophil leucocytes are absent; so too are the eosinophils so often present during an attack of colitis (Wright and Truelove, 1966; Korelitz and Sommers, 1974). Fissuring ulcerations and granulomas have not been seen and the striking similarity of the appearances of rejecting small bowel to coeliac disease is not paralleled when rejection in mouse colon is compared with the typical appearances of ulcerative colitis and Crohn's disease. However, individual features of these diseases do occur during rejection and this comparison cannot refute the suggestion that cell-mediated immune reactions may be important early in the genesis of non-specific inflammatory disease of the colon.

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