Chronic immune colitis in rabbits

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SUMMARY A chronic colitis has been induced in rabbits having many of the histological features of human ulcerative colitis. Animals were first immunised with the common enterobacterial antigen of Kunin and haemagglutinating antibodies demonstrated in high titre. An immune complex colitis was then established by the injection of soluble immune complexes following mild irritation of the rectum with dilute formalin as previously described. The rabbits developed an acute colitis within the first week but, in contrast with unsensitised rabbits, the inflammation persisted and was still present at six months as assessed by proctoscopy and rectal biopsy. Kunin-sensitised rabbits receiving intravenous saline, antigen, or antibody alone did not develop a chronic colitis. It is suggested that hypersensitivity to colonic bacterial antigens may be one mechanism whereby an acute colitis becomes chronic.

The pathogenesis of human ulcerative colitis may involve antigen-antibody reactions with the fixation of complement leading to tissue damage (Hodgson *et al.*, 1977a, b). Some support for this hypothesis has been obtained by the demonstration that immune complexes can cause a colitis in rabbits which has many of the histological features of the human disease (Hodgson *et al.*, 1978). However, the experimentally induced colitis was acute and a chronic lesion typical of human ulcerative colitis did not develop. The present series of experiments was designed to investigate whether prior sensitisation to colonic bacterial antigens influenced the course of the experimentally induced colitis.

Methods

Young New Zealand white rabbits of either sex, weight 1.5-3.5 kg, on a normal laboratory diet were used. Colonic appearances were assessed by proctoscopy using an infant proctoscope, and rectal biopsy was performed.

SENSITISATION

All rabbits were immunised with the common enterobacterial antigen of Kunin by repeated subcutaneous injections of 500 µg of antigen in complete Freund's adjuvant. Antibody titres were determined by tanned red cell haemagglutination (Boyden, 1951).

PREPARATION OF IMMUNE COMPLEXES

Complexes of human serum albumin (HSA) and anti-HSA were prepared according to the method previously described (Hodgson *et al.*, 1978).

COLITIS EXPERIMENTS

At the beginning of each experiment proctoscopy and rectal biopsy was performed on each animal. One millilitre of 1% formalin was then instilled rectally and a biopsy obtained two hours later, taking care to avoid the initial biopsy site. The animals were then divided into four groups: group I was injected intravenously with 1 ml of antigen-antibody complexes made in antigen excess (four animals); group II was injected with 1 ml of isotonic saline (two animals); group III was injected with 1 ml of HSA solution (10 mg/ml, two animals); group IV was injected with 1 ml of rabbit anti-HSA globulin (20 mg/ml, two animals).

After the injections serial rectal biopsies were obtained at intervals up to six months. Biopsies were fixed in formalin and sections were stained with haematoxylin and eosin. The histological appearances were evaluated by one observer (J. McL.) without knowledge of the experimental procedure.

Results

The antibody titres to Kunin antigen in the rabbits used were greater than 1/256 in all rabbits. No histological change was seen in the rectal biopsies of Kunin-sensitised animals before formalin instillation.

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After the formalin and before the intravenous injections there was a mild inflammatory infiltrate but no evidence of glandular or epithelial distortion.

GROUP I

One rabbit unfortunately died 48 hours after the injection of immune complexes and autolysis prevented adequate postmortem assessment. The remaining three rabbits developed an acute inflammatory infiltrate of the lamina propria consisting of polymorphonuclear neutrophil cells, plasma cells, and polymorphonuclear eosinophils. There was dilatation of the mucosal capillaries, some of which showed paving of polymorphonuclear cells. By the end of the first week there was distortion and destruction of the glands with early crypt abscess formation. The surface epithelium tended to be flattened and irregular, although no frank ulceration occurred. Similar changes occurred at six weeks. By six months the histological appearances were those of a chronic inflammatory process. These changes included a plasma cell and lymphocyte infiltrate, occasional crypt abscesses, surface epithelial distortion, and bifid glands which did not reach to the muscularis mucosae (Figs. 1-3). The histological changes of these rabbits are summarised in the Table.

GROUPS II-IV

Rabbits in these control groups developed an acute inflammatory infiltrate after the injections of saline, HSA, or anti-HSA. This was present at one week after the injection but the mucosa had returned to normal by six weeks. Even during the first week no

changes in glandular or epithelial architecture were observed. At six months one rabbit which had received saline showed very minimal changes but the remaining rabbits had a healthy rectal mucosa (Fig. 4). The changes are summarised in the Table.

Discussion

The acute inflammatory lesion seen in Kunin-sensitised rabbits in response to intravenously administered soluble immune complexes was similar, although less severe, to that previously induced in non-immunised rabbits (Hodgson *et al.*, 1978). However, prior sensitisation with Kunin antigen resulted in the development of a chronic colitis in those animals receiving immune complexes. These long term changes were not seen in the control animals. The number of rabbits in the experiment is too few to allow any comment to be made on the relationship between the antibody titres and the risk of developing a severe colitis.

The mechanisms whereby sensitisation to colonic bacterial antigens induced a chronic inflammatory response to immune complexes could not be elucidated from the data obtained in this experiment. However, it is suggested that the acute colitis initially induced by immune complexes deposited in formalin damaged mucosa allowed the increased absorption of bacterial antigens into the colonic mucosa. In the immunised animals this would lead to local antigen-antibody reactions and subsequent tissue damage. In this way the inflammatory response could be perpetuated with the formation of a chronic

Fig. 1 Rabbit rectal biopsy from a sensitised animal six months after injection of complexes. Dilated blood vessels are seen and there is a chronic inflammatory infiltrate extending to the surface epithelium. H and E $\times 140$.

Fig. 2 Rabbit rectal biopsy from a sensitised animal six months after injection of complexes. The surface epithelium is distorted and a crypt abscess is seen. The lamina propria contains a chronic inflammatory infiltrate. H and E $\times 140$.

Fig. 3 Rabbit rectal biopsy from a sensitised animal six months after injection of complexes. Glands are well separated and do not reach the muscularis mucosae. Bifid glands are seen. H and E $\times 140$.

lesion. It would appear that the initial acute colitis has to be relatively severe for a chronic lesion to develop, as chronicity was not seen after the mild inflammation induced by formalin and intravenous saline. Nevertheless, initial immune complex deposition may not be an essential prerequisite for a chronic colitis if the acute lesion is severe enough to allow adequate absorption of gut associated antigens. Our results confirm previous observations that sensitisation to bacterial antigens alone does not lead to colonic inflammation (Cooke *et al.*, 1968).

Similar events may occur in the colonic mucosa of patients with inflammatory bowel disease. It is known that these patients may have cellular hyper-

sensitivity and antibodies to gut associated antigens and that these antibodies may cross-react with colonic mucosa (Perlmann *et al.*, 1965; Lagercrantz *et al.*, 1968; Bull and Ignaczak, 1973; Bartnik *et al.*, 1974). At least some of the antibody produced against colonic bacteria is known to be synthesised locally within the lamina propria (Monteiro *et al.*, 1971).

Although none of these effector mechanisms has been shown to be correlated individually with any of the clinical features of the disease, it seems possible that they might lead to a perpetuation of the chronic inflammation akin to that seen in this rabbit model.

Fig. 4 Rabbit rectal biopsy from a control animal six months after injection of human serum albumin alone. Glands extend from the muscularis mucosae to the surface and the lamina propria contains few inflammatory cells. H and E $\times$ 140.

Table Histological changes

Rabbit	2 hours post-formalin before injection			1 week after injection			6 weeks after injection			6 months after injection		
	Cell infiltrate	Gland and epithelial distortion	Atrophy	Cell infiltrate	Gland and epithelial distortion	Atrophy	Cell infiltrate	Gland and epithelial distortion	Atrophy	Cell infiltrate	Gland and epithelial distortion	Atrophy
I/V complexes												
1	++	+	0	+	+	+	++	+-	+-	+	0	+
2 (died)												
3	++	+	0	++	+	0	++	+	0	++	++	+
4	+	+	0	++	+	+	++	+-	0	++	+	+
I/V saline												
9	+	0	0	+	0	0	0	0	0	*	*	*
10	+	0	0	+	0	0	0	0	0	+-	+-	+-
I/V HSA												
11	++	0	0	++	0	0	0	0	0	+-	0	0
12	++	+	0	++	0	0	+-	0	0	0	0	0
I/V α -HSA												
13	+	0	0	+	0	0	0	0	0	0	0	0
14	0	0	0	++	+-	0	0	0	0	0	0	0

*Rabbit 9 died at five months.

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