Immune-complex mediated colitis in rabbits

An experimental model

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SUMMARY  An experimental colitis in rabbits is described, following the intravenous injection of pre-formed immune complexes of human serum albumin (HSA) and anti-HSA into non-sensitised rabbits. Tissue damage was localised to the colon by the Auer technique of inducing local non-specific inflammation, by the rectal instillation of dilute formalin. Formalin alone gave transient changes that reverted to normal within 24 hours. In rabbits given intravenous immune complexes formed in antigen-excess, a severe colitis was initiated, with histological features including mucosal ulceration, mixed inflammatory cell infiltration in the lamina propria, and crypt abscess formation. It is possible that immune-complex damage may be one of the pathogenic mechanisms involved in human ulcerative colitis.

The pathogenesis of human ulcerative colitis and Crohn’s disease remains unknown. Immune complexes have been reported in the sera of patients with these diseases (Doe et al., 1973; Jewell and MacLennan, 1973), particularly if the disease is active or extra-intestinal manifestations are present (Hodgson et al., 1977). Such findings may be secondary to the presence of inflamed gut, but it is possible that immune complexes within the mucosa, by activating the complement sequence, may contribute to the tissue damage.

This study describes the histological features of an experimental colitis in rabbits caused by the deposition of soluble immune complexes from the circulation into the colonic mucosa. A single intravenous injection of pre-formed complexes was given to non-sensitised rabbits, and the complexes localised to the colon by initiating mild inflammation in the colon before the injection.

Methods

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

PREPARATION OF IMMUNE COMPLEXES

An antiserum to human serum albumin (HSA, Behringwerke) was raised in rabbits by repeated subcutaneous injection of HSA in Freund’s complete adjuvant. These sensitised rabbits were not used in the colitis experiments.

HSA-anti-HSA complexes were precipitated from the serum of these rabbits by HSA at a concentration of 0.7 mg/ml. This represented the equivalence point as determined by quantitative precipitation using trace-labelled $^{131}$I-HSA (Radiochemical Centre, Amersham). The precipitate of HSA-anti-HSA complexes was then partially redissolved in an HSA solution containing 12 times the concentration of HSA present at equivalence. The supernatant was used as ‘antigen-excess’ complexes.

Rabbit globulin was obtained from the serum of the immune rabbits by precipitation with an equal volume of saturated ammonium sulphate, washing the precipitate three times in half saturated ammonium sulphate, redissolving the precipitate in saline and dialysing against phosphate buffered saline pH 7.2. The final protein concentration was 20 mg/ml as measured by the method of Lowry et al., (1951). A precipitate of HSA-anti-HSA complexes obtained at equivalence was then partially redissolved in the globulin solution, yielding ‘antibody-excess’ complexes in the supernatant.
Sucrose density ultracentrifugation was used to confirm the presence of immune complexes in the 'antigen-excess' and 'antibody-excess' supernatants, and estimate their size. For this purpose, complexes were made using trace labelled 131I-HSA. Twenty-two millilitres sucrose density were prepared according to the method of Britten and Roberts (1960) with a range of 10-40% sucrose in phosphate buffer, 0.05M, pH 7.4. 0.5 ml of sample was layered on to each gradient using a Pasteur pipette and the tubes centrifuged at 29,000 rev/min at 4°C for 6 hours in a 3 x 25 ml swing-out rotor on an MSE Superspeed 65 Centrifuge (MSE Ltd, Crawley, Sussex). The contents of each tube were then removed by tube piercing and 10 drop fractions collected under gravity. The fractions were analysed for radioactivity on a LKB-Wallace 1280-1 autogamma counter and alternate fractions analysed for sucrose concentration using an Abbé refractometer. The sedimentation coefficients were then calculated by the method of Funding and Steensgaard (1971).

**Colitis Experiments**

Preliminary experiments were performed to determine the effect of dilute formalin on rabbit rectal mucosa. One millilitre of 1% formalin was chosen as a concentration which gave mild, transient changes in the histological appearances of mucosal biopsy specimens.

Proctoscopy and biopsy were performed on each animal at the beginning of each experiment and at intervals up to three months. The exact time intervals are detailed in the Table. Biopsies were taken between 5 and 8 cm from the anal verge, and, if visible, previous sites of biopsy were avoided. Four groups of animals were studied as follows:

### Table: Mucosal changes in group 2 rabbits

<table>
<thead>
<tr>
<th>Time</th>
<th>Surface epithelium</th>
<th>Lamina propria</th>
<th>Crypt abscesses</th>
<th>Vessels</th>
<th>Goblet cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Normal</td>
<td>Normal</td>
<td>None</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>2 h after formalin</td>
<td>Subnuclear vacuolation</td>
<td>Normal</td>
<td>None</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>1 hour after injection of complexes</td>
<td>Subnuclear vacuolation</td>
<td>Oedema</td>
<td>None</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>3 h</td>
<td>Patchy ulceration</td>
<td>Increased plasma cells, Polymorphs†</td>
<td>Increased polymorphs, None</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>22 h</td>
<td>Superficial ulceration, fibropurulent exudate</td>
<td>Striking increase in cells † † †</td>
<td>Present</td>
<td>Dilated, Migration of polymorphs</td>
<td>Depletion</td>
</tr>
<tr>
<td>4-5 d</td>
<td>Severe mucosal ulceration</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>7-8 d</td>
<td>Severe mucosal ulceration</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>6 w</td>
<td>Normal</td>
<td>Normal</td>
<td>None</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>3 m</td>
<td>Normal</td>
<td>Normal</td>
<td>None</td>
<td>Normal</td>
<td>Normal</td>
</tr>
</tbody>
</table>

**Group 1: controls (six rabbits)**

One millilitre of formalin was instilled rectally and two hours later, after a further rectal biopsy, an intravenous injection of 1 ml saline (two rabbits), or 1 ml HSA solution (11 mg/ml) (two rabbits), or 1 ml rabbit globulin (20 mg/ml) (two rabbits), was given into an ear vein.

**Group 2: antigen-excess (four rabbits)**

Two hours after intrarectal formalin, 1 ml of antigen-excess complexes were injected intravenously.

**Group 3: antibody-excess (two rabbits)**

Two hours after intrarectal formalin, 1 ml antibody-excess complexes were injected intravenously.

**Group 4: (two rabbits)**

No formalin was instilled, but 1 ml antigen-excess complexes was injected intravenously.

The biopsies were fixed in formalin, and sections were stained with haematoxylin and eosin. The histological appearances were assessed by one of us (J.S.) without knowledge of the experimental procedure in each animal or the timing of the biopsies.

**Results**

Rabbit colonic mucosa is similar to that of humans, differing only in that the superficial epithelium is composed mainly of simple columnar cells with few goblet cells, and the cellular population of the lamina propria is less.

**Group 1: Controls**

In five rabbits the changes caused by 1% formalin were minimal, amounting to occasional subnuclear vacuolation in the surface epithelial cells (Fig. 1).
These changes occurred within two hours of the formalin instillation and had disappeared within 48 hours. One rabbit, receiving intrarectal formalin and intravenous saline, showed more marked changes. These were seen two hours after formalin and were maximal at three hours—that is, one hour after intravenous saline. In this animal there was superficial epithelial cell loss, dilatation of capillaries in the superficial layer of the lamina propria, and a mucopurulent cap. There was no crypt distortion, no oedema, and no dilatation of the deeper capillaries. By 22 hours after the injection, these changes had reverted to normal (Fig. 2).

Fig. 1  Rabbit rectal biopsy two hours after instillation of 1% formalin. There are surface epithelial abnormalities. H and E × 176 (original magnification).

Fig. 2  Rabbit rectal biopsy 22 hours after instillation of 1% formalin, followed by intravenous saline. The surface mucosa has returned to normal. H and E × 250 (original magnification).
GROUP 2: ANTIGEN-EXCESS COMPLEXES
AFTER FORMALIN
The size of the immune complexes obtained in antigen-excess was estimated, using sucrose-density ultracentrifugation. Figure 3 shows the distribution of 131I-HSA within the sucrose gradient. It will be seen that a variety of complexes were obtained with sedimentation characteristics from 9-30 S, although the predominant species was 14-17 S.

Forty-five minutes after the injection of these complexes there was, in all four rabbits, oedema of the lamina propria and dilatation of the deeper capillaries of the mucosa. The dilated capillaries showed margination of the polymorphonuclear cells and were surrounded by plasma cells and polymorphonuclear cells (Fig. 4). The surface epithelium showed only a mild degree of subnuclear vacuolation. By three hours, large numbers of polymorphonuclear cells were seen passing through the capillary walls and there was a marked infiltrate of these cells and of plasma cells throughout the lamina propria.

By 22 hours, the changes were well-developed (Fig. 5). There was surface epithelial ulceration and, in places, there was a fibrinopurulent cap. The lamina propria infiltrate had increased. There was distortion of crypt architecture and patchy loss of goblet cells from the crypts; other crypts were dilated showing early crypt abscess formation and retention of mucus. The submucosa, however, was virtually unaffected although the biopsy specimens included only small amounts of this tissue. By 48 hours, the changes remained much the same but by five days the damage to the mucosa was at its most extreme with the formation of ulcers, severe disruption of crypts, and the formation of crypt abscesses (Fig. 6).

There were similar findings at seven days, but by six weeks the inflammatory changes were minimal, amounting to a slight increase of plasma cells in the lamina propria. The crypt architecture remained distorted with short, narrow, or branched crypts, and these changes persisted at three months (Fig. 7).

The Table summarises the time-course and extent of these changes.

GROUP 3: ANTIBODY-EXCESS COMPLEXES
AFTER FORMALIN
Changes similar to those seen in group 1 were found in all rabbits after the instillation of formalin. Further changes after the injection of the complexes were slight in comparison with those noted in group 2. By 22 hours there was an increase in plasma cells and polymorphonuclear cells in the lamina propria, most noticeable around capillaries. The polymorphonuclear cells did not penetrate the crypts or superficial epithelium, and the crypt architecture was undisturbed. The infiltration was less by 48 hours and the mucosa had reverted to normal by five days.

GROUP 4: NO FORMALIN. ANTIGEN-EXCESS COMPLEXES
Mild changes, comparable in severity to those seen in group 3, were seen in the lamina propria 24 hours after the injection of complexes. There was oedema of the lamina propria, with some capillary dilatation and perivascular infiltration, but these changes were slight compared with those seen in group 2. An increase in cell numbers in the lamina propria persisted for seven days, but had reverted to normal by four weeks.
Immune-complex mediated colitis in rabbits

Fig. 4 Rabbit rectal biopsy 45 minutes after intravenous injection of pre-formed complexes formed in antigen-excess, 24 hours after rectal formalin. There is an early inflammatory infiltrate in the deep layers of the lamina propria. H and E × 250 (original magnification).

Fig. 5 Rabbit rectal biopsy 22 hours after injection of antigen-excess complexes. There is mucosal ulceration, loss of goblet cells from the glands, and inflammation throughout the lamina propria. H and E × 176 (original) magnification.)
Fig. 6  Rabbit rectal biopsy five days after injection of antigen-excess complexes. There are dilated glands with crypt abscess formation, widespread inflammation in the mucosa and surface ulceration. H and E × 176 (original magnification).

Fig. 7  Rabbit rectal biopsy three months after injection of complexes. There is no inflammation in the lamina propria, but distorted and bifid glands are present. H and E × 250 (original magnification).
**Discussion**

These experiments indicate that the intravenous injection of pre-formed immune complexes can lead to a severe colitis in rabbits, if the colonic mucosa has first been mildly inflamed. As the animals had not been sensitised to either of the components of the antigen-antibody complex, and severe damage was initiated before time for sensitisation had elapsed, it seems probable that the tissue damage was initiated by the immune complexes themselves. The histological features of the colitis in its early stages, with perivascular oedema and infiltration with polymorphonuclear cells, were compatible with those seen in classical immune-complex mediated lesions.

The control groups indicate that instillation of rectal formalin alone gave only transient inflammation, but this was required for severe damage to be initiated by the complexes. Antigen-excess complexes caused little damage if no formalin was administered. This was probably because the complexes injected were relatively small, mainly 14-17 S. Henson and Cochrane (1971) have shown that in experimental serum sickness small complexes, of less than about 19 S, do not initiate tissue damage, as they are incapable of increasing vascular permeability and leaving the circulation. Small complexes can however cause inflammation if injected directly into the tissues (Cochrane and Hawkins, 1968). In our experiments, when local inflammation had been initiated by the formalin, such small complexes could presumably leave the circulation. Auer (1920) demonstrated this effect of local inflammation localising systemic antigen-antibody responses. Even with such local inflammation antibody-excess complexes initiated little damage, as might be expected from their relative inefficiency in causing lesions in experimental serum sickness (Weigle, 1961).

There are two previously reported models of colitis in rabbits which are probably dependent upon immune-complex formation. In an Arthus-type reaction, Goldgraber and Kirsner (1959) induced severe local inflammation and haemorrhagic necrosis by mucosal injections of egg albumen into rabbits sensitised to that antigen. The second model involved the Auer reaction as in our experiments. Rabbits were sensitised to egg albumen and mild colonic inflammation was induced with dilute formalin. Intravenous injections of egg albumen led to mucosal oedema and ulceration, perivascular round-cell infiltration and a mixed inflammatory cell infiltrate in mucosa and submucosa. The antigen, though not specific antibody, was shown to be deposited in the affected areas (Kraft et al., 1963). However, as both these experimental models used sensitised animals, it seems likely that other immunological mechanisms of tissue damage, apart from immune complex formation, were operative.

The inflammatory lesion induced by immune complexes in rabbits shares histological features with human ulcerative colitis. The lesion was predominantly mucosal, with surface epithelial cell damage, loss of goblet cells, gland destruction, and crypt abscess formation. The cellular infiltrate in the lamina propria consisted of polymorphonuclear cells and plasma cells; there were also eosinophils, but the eosinophilia shown by rabbit polymorphonuclear cells made this feature difficult to assess. The gland dilatation in some biopsies was reminiscent of that seen sometimes in acute toxic dilatation occurring as a complication of human ulcerative colitis, but it is also seen in some cases of pseudomembranous colitis and amoebic colitis. The recovered rabbit mucosa showed some glandular distortion, such as may be seen sometimes in rectal biopsies of patients with ulcerative colitis in remission.

It is unlikely that human ulcerative colitis is caused solely by immune complex deposition. Cellular immune mechanisms directed against bacterial and colonic antigens have been described (Perlmann and Broberger, 1963; Shorter et al., 1968; Bull and Ignaczak, 1973), at least in vitro, and the possibility of an infective cause has not been excluded (Cave et al., 1976). However the animal model reported here suggests that, whatever the primary cause of colonic inflammation, further tissue damage may occur if immune complex deposition occurs. Such complexes could be formed locally, and there is considerable evidence of antibody formation against bacterial and colonic antigens in patients with ulcerative colitis (Lagerkrantz et al., 1966; Monteiro et al., 1971). This mechanism might explain the common clinical observation that ulcerative colitis may follow an infective colitis (Felsen and Wolarsky, 1953), and also provides an explanation for the chronicity of the condition.

**References**


