Hypothesis

A new hypothesis for the aetiology of Crohn's disease – evidence from lipid metabolism and intestinal tuberculosis

W.E.W. Roediger

University Department of Surgery, The Queen Elizabeth Hospital, Woodville, S.A. 5011, Australia

Summary: The stimulus for the immune response in Crohn's disease is unknown. In each of 19 cases of Crohn's disease evaluated by electron microscopy, epithelial cells of the ileum contained phagolysosomes with lamellar layers of lipid. These structures, now termed R or reactant bodies, are the proffered antigenic stimulus. They are proposed to be an amalgam of lipid (cholesterol esters, or phospholipids) and bacterial fragments (mycoplasma, mycobacteria or streptococci), which in combination are hypothesized to produce a powerful immunological response analogous to the adjuvant effect. For disease expression to occur, lipids and specific bacterial populations are needed in the bowel lumen. These factors may account for the success of elemental diets that are low in fat in the treatment of Crohn's disease and for the regional distribution of disease along the intestinal tract.

Introduction

Over the past three decades the incidence of Crohn's ileo-colitis has increased in Western societies. Search for an aetiological agent has devolved upon atypical mycobacteria¹ or cell-wall deficient organisms, but consistent observations have not been made. Because an inflammatory response is usually present in Crohn's disease (CD) an antigenic agent should be detectable in most, if not all, cases. A place for such an agent can be constructed from electron microscopic data and separate observations in clinical medicine, immunology, biochemistry and in microbiology.

Intestinal involvement with tuberculosis and Crohn's disease

A similarity between intestinal tuberculosis and Crohn's disease was mentioned in the classical descriptions of regional ileitis by Dalziel in 1913² and Crohn *et al.* in 1932.³ Caseation and detectable acid-fast bacilli distinguish tuberculosis from CD. Analysis of a series of patients with intestinal tuberculosis reported in 1985 showed that 64 of 72 cases (89%) had disease involving the ileocaecal region, amongst which were 20 cases of generalized peritonitis but none of miliary spread.⁴ In a more

recent study almost half of a group of patients with cavitating pulmonary tuberculosis were shown to have intestinal involvement, affecting either the ileo-caecum or ascending colon.⁵ The association of tuberculosis with the ileo-caecal region is similar to CD where 90% of cases will eventually have a disease focus.⁶ The similarity of distribution begs the question whether the two diseases have comparable pathogenetic mechanisms.

The antigenicity of *M. tuberculosis*

M. tuberculosis induces a strong cellular and lesser humoral immune response. The cellular and associated delayed-type hypersensitivity were shown, by Freund *et al.* in 1937, to be enhanced if mycobacteria were administered with paraffin oil.⁷ Subsequent experiments showed that mycobacterial cell walls⁸ or chemically defined fractions of mycobacteria⁹ with certain lipids and emulsifying agents strongly enhanced the immune response or elicited pulmonary granulomas in experimental animals. Why the combination of oils with mycobacteria or mycobacterial fragments produces striking antigenicity has not been explained.

Lipids, antigenicity and bacterial assimilation

The observation that lipids emulsified with Tween 80 together with bacterial fragments promote a powerful antigenic response is known as the

Correspondence: Associate Professor W.E.W. Roediger, M.B., B.Ch., M.Sc., D.Phil.(Oxon.), F.C.S.(S.A.), F.R.A.C.S.

Accepted: 5 February 1991

'adjuvant effect'.¹⁰ Barclay *et al.*¹¹ emphasize that any factor which interferes with the association of oil and mycobacterial fragments reduces or eliminates the immunogenicity. Coating of bacteria to promote phagocytosis or prolonged retention of bacteria in tissue has been suggested for the adjuvant effect. Other concepts also seem possible. All bacterial cell walls lack cholesterol¹² and only mycoplasmas are known to require cholesterol or phospholipid for growth.¹³ Mycoplasmas take up cholesterol/phospholipids from human low density lipoproteins by a simple exchange process,¹³ so altering fluidity of bacterial membranes¹⁴ which in turn may promote uptake of bacteria by epithelial cells or immune cells. Thus the presence of surface receptors for lipids^{15,16} in ileal epithelial cells may promote endocytosis of lipid-associated bacterial fragments. Some mycobacteria require exogenous lipid for chain elongation of intrinsic fatty acids and growth.¹⁷ The presence and absorption of lipid in the distal ileum may enhance mycobacterial growth and hence the frequent manifestation of intestinal tuberculosis in the ileum.

Lamellar-lipid lysosomal bodies in Crohn's disease

Many ileal epithelial cells involved with CD display lysosomes or phagolysosomes containing lipid in lamellar layers showing a whorl-like pattern similar to myelin (Figure 1). The lipid nature of these bodies was inferred from the characteristic lamellation of lipids³⁵ and their affinity on electron microscopy for osmium tetroxide, a characteristic of lipids. These structures were found in 19 out of 19 cases of CD: 17 cases were reported in the

literature¹⁸⁻²⁰ and two further cases of Crohn's disease were personally observed (Table I). None of 6 control cases¹⁸⁻²⁰ and neither of 2 cases of radiation ileitis, now observed, contained these structures. They have not been observed in enterocytes of coeliac disease²¹ or experimental Yersinia infection.²² The lipid-lysosomal bodies appear in the apical, basal and lateral portions of enterocytes, a feature which suggests that these bodies undergo transport across epithelial cells – transcytosis – a well known property of lysosomes.²⁴ These bodies were also found with the electron microscope in macrophages¹⁹ but already 40 years ago lipid bodies were observed by light microscopy in activated macrophages of Crohn's disease.²⁵

Nine electron microscopic studies of colonic epithelial cells (Table II)²⁶⁻³⁴ in ulcerative colitis comprising 159 cases, revealed 4 instances²⁷ where

Table I Electron microscopy of ileal epithelial cells in active Crohn's disease with relevance to phagolysosomes (R bodies)

Crohn's cases	Control* cases	Ileal epithelial cell characteristics in CD	Year
3	1	Small dense vesicles and lysosomes	1979 ¹⁸
8	3	Lysosomal inclusions and myelin-like figures	1981 ¹⁹
6	2 (colon)	Lysosomes with membrane bound vesicles	1984 ²⁰
2	2†	R bodies	1990‡

* No R bodies observed; † radiation ileitis; ‡ current cases.

Figure 1 Ileal enterocyte showing phagolysosomes (R bodies – arrows) from a case of active Crohn's disease. Original magnification × 43,000.

Table II Electron microscopy (EM) of colonic epithelial cells in active and quiescent ulcerative colitis (UC) with relevance to phagolysosomes (R bodies)

No. of cases	Colonic epithelial cells	Year
1	No R bodies	1964 ²⁶
7	R bodies but in shigellosis and Crohn's disease (one case each)	1966 ²⁷
27	No R bodies	1966 ²⁸
9	R bodies in one case of UC*	1967 ²⁹
16	No R bodies	1972 ³⁰
14	No R bodies	1975 ³¹
37	No R bodies	1975 ³²
33	No R bodies	1989 ³³
15	No R bodies	1989 ³⁴

*Other EM features suggest a case of Crohn's colitis.

myelin bodies were found and these appear to be cases of Crohn's colitis and shigellosis rather than ulcerative colitis. Healthy colonic epithelial cells revealed no lipid lysosomal bodies.

A new hypothesis for the aetiology of Crohn's disease (R bodies)

The hypothesis now proposed is that the lamellar lipid lysosomal bodies, together with bacterial fragments, are the antigenic stimulus and underlying cause of CD (Figure 2). The lysosomally derived bodies are termed R (reactant) bodies to

distinguish them from non-reactant, non-tissue damaging lysosomes or phagolysosomes. The lamellar lipid layering in R bodies may vary in character but the configuration of thin lamellae is suggestive of cholesterol esters³⁵ while the configuration illustrated in Figure 1 is suggestive of phospholipids. The current proposal implies that the combination of a non-antigenic lipid, with bacterial fragments or bacterial cell wall together become strongly antigenic. Such an antigenic stimulus may be of long duration (6–12 months) as shown with dermal injection of mycobacteria in oily suspensions.³⁶

Cholesterol esters are taken up selectively by a variety of cells where both lysosomes and extra lysosomal sites³⁷ participate in their hydrolysis and further metabolism. Macrophages in particular take up cholesterol esters where they are also normally hydrolysed.³⁸ Hydrolysis appears impaired in Crohn's disease judged by the appearance of lipid lamellar bodies in macrophages¹⁹ of active disease but not in health.

The conditions prevalent in the distal ileum for the causation of CD – cholesterol absorption, presence of emulsifying agents (bile) and specific bacteria – are analogous to the conditions required for inducing tuberculosis in experimental animals. These conditions are the presence of a lipid, an emulsifying agent (Tween) and mycobacteria. Such analogous sets of requisites may account for why both tuberculosis and Crohn's disease are so frequently found in the ileo-caecal region.

Figure 2 Proposed events in the causation of Crohn's disease.

Clinical and experimental evidence for the hypothesis

Besides the biochemical and electron microscopic data a number of clinical observations uphold the present contentions.

1. The content of lipids in the intestinal lumen should determine the formation of R bodies and the subsequent disease process. Total parenteral nutrition, which curtails lipid and other luminal nutrients, produces remission in 66–89% of cases of active Crohn's disease.³⁹ More specific evidence that removal of lipid induces remission was obtained in three prospectively controlled trials with elemental diets for active CD.^{40–42} They showed that elemental diets were as effective as prednisolone in inducing remission of disease and even reversed the radiological lesions of Crohn's disease.⁴² All three diets^{40–42} reflected a drastic reduction in lipid intake from the 20–30% of a normal diet to 0.66% of the total nutritive value. Most of the lipids in elemental diets were vegetable oils. These oils when used with mycobacteria do not promote antigenicity as seen with mineral or other oils.⁴³
2. The hypothesis suggests that CD should occur in those regions of the bowel that have a good capacity for absorption of fatty acids and bile and where bacteria are plentiful. The distal ileum and colon fulfil these criteria and correspond to the predilect sites of disease.
3. Crohn's disease in Africa rarely occurs in black population groups compared with those of European descent.⁴⁴ The fat intake of blacks is very significantly less than that observed in whites⁴⁵ and is reflected in differences in manifestation of other bowel diseases in the black populations.
4. Guthy of Germany⁴⁶ put forward the hypothesis that the lipid content of Western diets may cause CD. He then showed that a high lipid diet in pigs produced intestinal inflammation when intestinal transit was slowed by reversed ileal loops.⁴⁷
5. Because R bodies contain no viable bacteria, bacterial growth from tissue with Crohn's disease would not be expected. This is in keeping with the general microbiological observations of the disease.

Experiments with animals provide further support for the hypothesis:

1. Suspensions of bacterial cell fragments (Streptococci group A or D) injected into the ileal wall of rats produced chronic inflammation and granulomas in 46% of animals,⁴⁸ while injection of live or irradiated B C G (Pasteur) into bowel of guinea pigs gave a good model of granulomatous bowel disease,⁴⁹ observations which, to some degree, are supportive of the hypothesis.
2. R bodies of CD closely resemble structures found in macrophages of chronic arthritis produced by injecting fragments of streptococci into joints. No bacteria but morphological arrangements similar to R bodies were found in inflamed joints after 4 months.⁵⁰

Conclusions

Evidence from several disciplines has led to proposals that antigens causing CD are a combination of luminal lipids, emulsifiers (bile) and bacterial fragments which together become powerfully antigenic when normally each individual substance is either non-antigenic or only weakly antigenic. The agents are amalgamated into phagolysosomes, now termed R (reactant) bodies, which, after transcytosis, activate immune cells in the lamina propria. Intestinal tuberculosis is analogous to CD but viable bacteria are taken up where absorption of bile and cholesterol is optimal. The analogy explains the siting of both diseases in the ileo-colonic region. Clinical and dietary evidence suggests that luminal lipids play a role in the causation of CD but the precise lipid and precise bacterial fragments that are involved remain to be defined. A case can be made for fragments of mycoplasma or atypical mycobacteria based on their metabolic needs for exogenous lipids. These bacterial fragments would ideally be detected by DNA hybridization techniques⁵¹ in Crohn's disease tissue. The new hypothesis has strong implications for the treatment of CD. Modification of specific lipid intake and elimination of luminal mycoplasma/atypical mycobacteria with specific therapy may be future strategies for the treatment of Crohn's disease.

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