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A morphological assessment of immunoreactivity in colonic Crohn's disease and ulcerative colitis by a study of the lymph nodes

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SYNOPSIS The mesenteric lymph nodes in total colectomy specimens from patients with ulcerative colitis or Crohn's colitis were assessed for immunological reactivity. Despite the suggestion of previous work using different methods that immunological difference between the diseases exist and may be important in pathogenesis, no significant differences were found in this study. Changes were observed in both diseases and when ulceration was present these consisted of both cellular and humoral immune reactivity. There was no evidence of diminished cell-mediated immunity in Crohn's disease and the presence of granulomata did not constitute any special immunological group.

Certain aspects of the immunological reactivity of patients with ulcerative colitis and Crohn's disease have already been investigated. Circulating antibody production to colonic antigen (Broberger and Perlmann, 1959; Harrison, 1965; Broberger and Perlmann, 1962; Marcussen and Nerup, 1973), to milk proteins (Taylor and Truelove, 1961; Wright and Truelove, 1965, 1966), and to bacterial antigens (Lagercrantz, Hammarström, Perlmann, and Gustafsson, 1968; Thayer, Brown, Sangree, Katz, and Hersh, 1969) have been demonstrated. Studies on lymphocyte transformation (Hinz, Perlmann, and Hammarström, 1967; Stefani and Fink, 1967) and lymphocyte cytotoxicity (Shorter, Spencer, Huizenga, and Hallenbock, 1968; Watson, Quigley, and Bolt, 1963) have also been reported and other aspects of delayed hypersensitivity have been investigated such as dermal reactivity to dintrochlorobenzene (DNCB) (Verrier-Jones, Housley, Ashurst, and Hawkins, 1969) and tuberculin (Fletcher and Hinton, 1967).

The variation in response to Kveim antigen has also been investigated particularly in Crohn's disease (Mitchell, Cannon, Dyer, Hinson, and Willoughby, 1970; Siltzbach, Vieira, Topilsky, and Janowitz, 1971).

Although these workers have shown some immunological differences between the two groups these are not sharply defined.

Experimental work in animals using a variety of antigenic stimuli (Nossal, Ada, and Austin, 1964; Nossal and Ada, 1971) has shown that different morphological patterns of lymph node reactivity can be evoked. Other animal work, using thymic and bone marrow ablation techniques (Miller and Osoba, 1967; Perey and Good, 1968; Sutherland, Archer, and Wood, 1964), has characterized the thymic-dependent and bone-marrow-dependent areas of lymph nodes. The thymic-dependent paracortical tissue is increased when there is other evidence of stimulation of cell-mediated activity and reactive follicles in association with increased plasma cells in the medullary cords are noted when circulating antibody levels are raised. The examination of lymph nodes now offers the opportunity of assessing whether cellular or humoral mechanisms are important in any particular instance. Previous examination of the lymph nodes in Crohn's disease and ulcerative colitis has been concerned only with the presence of granulomata and node size (for review see Cook, 1972).

We decided to make an immunological assessment of ulcerative colitis and Crohn's disease of the colon by morphological study of the lymph nodes in colectomy specimens from patients operated upon in the Radcliffe Infirmary from late 1970 onwards.

Material and Methods

Between November 1970 and June 1973 85 colectomy specimens were examined for the purposes of this study. Two cases with no detectable lymph nodes

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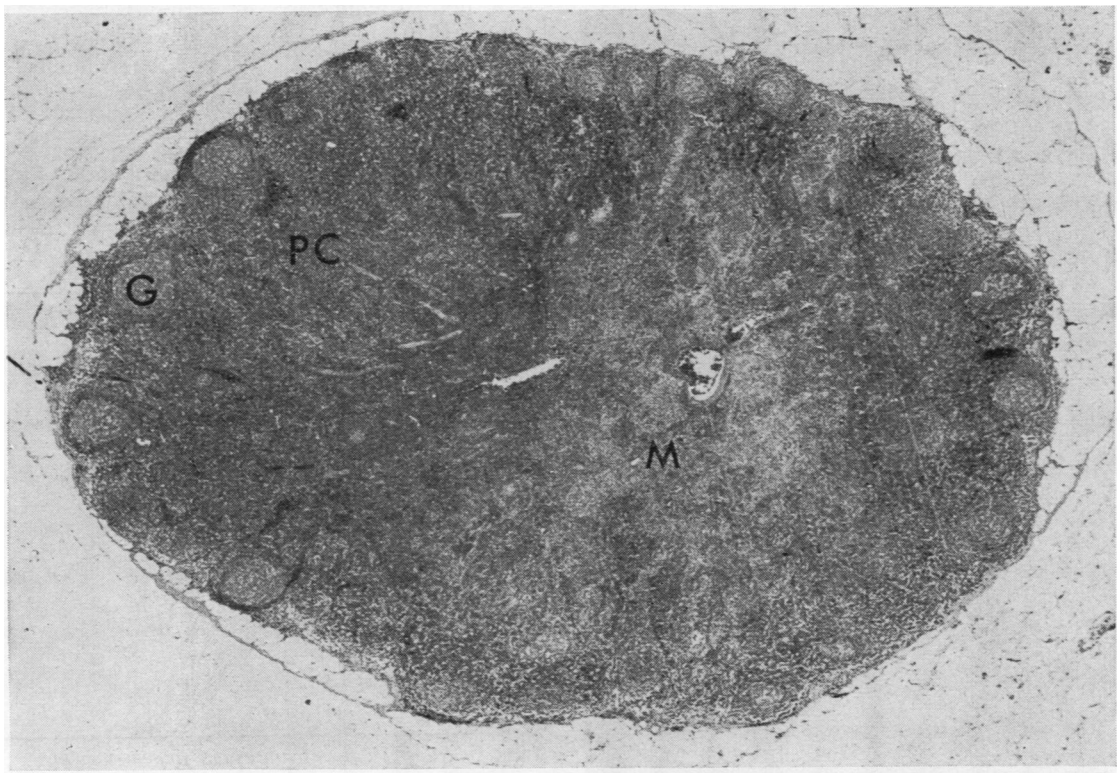

Fig 1 *A low-power (× 35) photomicrograph of a lymph node showing prominent reactive germinal centres* (G), *increased paracortical lymphocytes* (PC), *and in the medullary region* (M) *sinuses and cords packed with plasma cells.*

were excluded and 10 others of acute toxic dilatation and four severely ill cases on the intensive medical regime of Truelove and Lee (1973) were also excluded because of the high steroid dosage given over the preceding days as this may have suppressed some signs of immunoreactivity, especially in the paracortical tissues (Campbell *et al*, personal communication).

A single case on azathiaprine was excluded as this has been shown to suppress B-cell activity (Campbell, Hersey, Harding, Hollingsworth, Skinner, and MacLennan, 1973). Thus 68 cases were available for analysis.

At least 20 blocks of bowel were taken from each case and as many lymph nodes in the mesentery as could be found up to a maximum of 10. The cases were classified as either ulcerative colitis or Crohn's disease on accepted pathological criteria (Lockhart-Mummery and Morson, 1960) leaving an indeterminate group which did not fall readily into either.

The lymph node features on routine haematoxylin-eosin sections were classified as follows and the results put onto punch cards for analysis.

1 Normal nodes with none of the changes in 2-6 below.

2 Reactive paracortical tissue, in which the paracortical lymphocytes were increased in number and showed a high mitotic rate. The postcapillary venules showed a striking hyperplasia of the endothelial cells.

3 Reactive lymphoid follicles have germinal centres which are enlarged and increased in number. They have a pale central area containing large lymphoid cells and 'tingible body' macrophages. Many contain an amorphous pink-staining material and they are surrounded by a tight cuff of small lymphocytes.

4 Increased large lymphoid cells and plasma cells in the medullary cords of the nodes.

5 Dilatation of sinuses with hyperplasia of the littoral cells which thus appear plump and active.

6 The presence of typical sarcoid-like epithelioid granulomata was noted separately.

In the latter part of the study one half of each of two lymph nodes was processed by the cold alcohol method of Sainte-Marie (1962) and 'stained' with

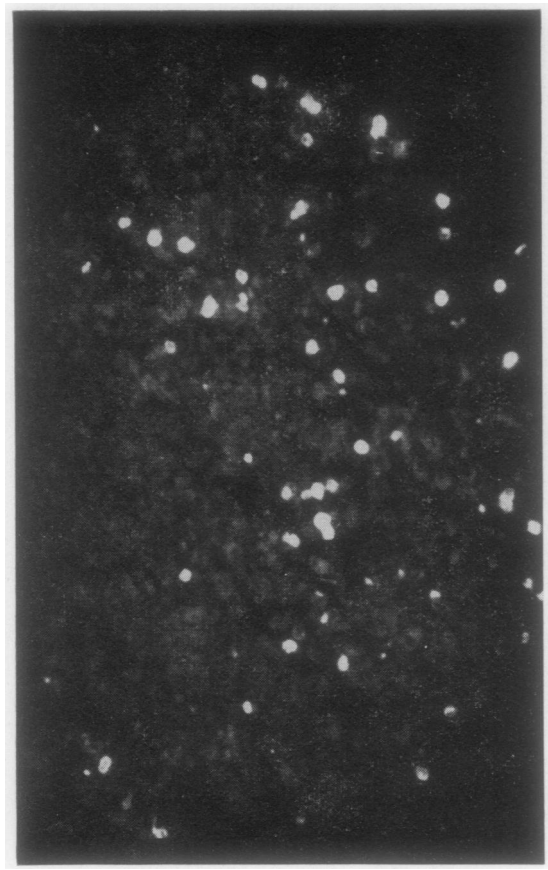

Fig 2. *A fluorescence micrograph (× 150) of the medullary region of a node showing IgA-producing plasma cells identified by a fluorescein conjugated anti α chain antibody. The cell density shown is equivalent to the ++ level in the grading system.*

antibodies to human immunoglobulin heavy chains raised in swine conjugated with fluorescein isothiocyanate (from Nordic Pharmaceuticals). The usual controls were included (Nairn, 1969). Two lymph nodes unassociated with any intraabdominal inflammation or malignancy were used for comparison (figs 1 and 2).

The nodes were examined on a Leitz Ortholux microscope fitted for fluorescence work with a quartz iodine light source and 525μ interference filter (Tomlinson, 1970). The assessment of plasma cell numbers was made at a total magnification of 250 ×.

The number of immunoglobulin-containing cells of classes IgG, A, and M, were graded using a simple + to + + + + system:

+ = normal numbers, + + = approximately 2-4 × normal numbers, + + + = approximately 5-10 × normal numbers, and + + + + = larger numbers.

Results

Table I indicates the number and distribution of the histological features in the lymph nodes of the cases examined and these features are also portrayed in terms of function. Statistical evaluation using a χ^2 contingency test shows that there are no differences in the distribution of the features and hence function between the groups. This also applies whether the indeterminate cases are put into the ulcerative colitis or Crohn's groups. In table II the findings in the cases with and without ulceration are shown. There is no statistical difference between the various features in the ulcerated cases. Statistical comparison between the non-ulcerated and the ulcerated groups is difficult because of the small number of non-ulcerated cases but there seems to be less immunological reactivity in the absence of ulceration although the node features are otherwise normal.

	Ulcerative Colitis	*Indeterminate*	*Crohn's*
Morphological Features			
Totals	35	6	28
Normal nodes	7	1	4
Increased paracortical tissue	11	2	13
Increased reactive follicles	11	4	11
Large lymphoid and plasma cells	13	4	17
Sinus dilatation	25	3	22
Granulomas	1	0	6
Immunology			
Cellular only	4	0	1
Humoral only	8	2	6
Both cellular and humoral	7	2	12
Sinus dilatation only	9	1	5
Total with immunological activity	19	4	19

Table I *Morphological features and immunological interpretation in cases of ulcerative colitis, indeterminate, and Crohn's disease*

Table III shows results of the immunohistochemical findings in the two groups examined. There is no significant difference in the level IgA-, IgG-, and IgM-producing plasma cells in the nodes.

Discussion

The differential diagnosis between ulcerative colitis and Crohn's colitis poses great problems to clinicians

	Ulcerative Colitis	*Indeterminate*	*Crohn's*
Cases without Ulcers	7	1	1
Normal nodes	3	0	1
Abnormal nodes	4	1	0
Increased paracortical tissue	2	0	0
Increased reactive follicles	1	1	0
Lymphoid and plasma cells	1	1	0
Sinus dilatation	4	0	0
Granulomas	0	0	0
Cases with Ulcers	28	5	27
Normal nodes	4	1	4
Increased paracortical tissue	9	2	13
Increased reactive follicles	10	3	11
Lymphoid cells and plasma cells	12	3	17
Sinus dilatation	21	3	22
Granulomas	1	0	6

Table II *Morphological features in cases with and without ulceration in ulcerative colitis, of indeterminate aetiology, and in Crohn's disease*

Case No.	*Immunoglobulin-containing Cells*		
	IgA	*IgG*	*IgM*
Ulcerative Colitis			
68	+++	++	++
71	++++	+++	++
72	++++	+	+++
82	++++	+++	+++
85	++	+	+
Mean	++++	++	++
Crohn's Disease			
69	++++	+++	+
70	++	++	+
75	+++	+	+
76	+++	++	+
86	+++	++	++
87	++++	+++	++
Mean	+++	++	+

Table III *Analysis of immunoglobulin-containing cells in ulcerative colitis and Crohn's disease*

and pathologists alike. When two diseases of unknown aetiology share so many common clinicopathological features this is to be expected. It is clear why so much effort has been expended in order to highlight the difference between the two diseases and this is especially true in the immunological field. Despite this, although there have been differences demonstrated, the overlap of the different immunological phenomena is considerable. The results of this investigation show that a morphological assessment of immunological reactivity does not allow any distinction to be made between the lymph node appearances in the two diseases. Such differences as occur are related more closely to the presence of ulceration and are largely independent of the disease process involved. In non-ulcerated cases of chronic ulcerative colitis in a quiescent phase when colectomy was performed because of the cancer risk the nodes were thus understandably either normal or showed the minor change of sinus dilatation.

Previous work (Taylor, 1965; Bendixen, 1967, 1969) has suggested that in Crohn's disease there is diminished cell-mediated immunological reactivity. Our results do not support this and in both Crohn's disease and ulcerative colitis a combination of morphological features indicating that there is competence of both cellular and humoral mechanisms and that both are stimulated is shown.

Not only is there architectural evidence of this in the lymph nodes but the humoral response using immunohistochemical methods is also verified by the plasma cell component of the nodes. The preponderance of IgA cells is to be expected in ulcerative inflammation of the gut.

There can be no doubt that this type of analysis is a reliable one for the assessment of immunological function. Recent work by Cottier, Turk, and Sobin (1973) substantiates this and they outline an even more detailed technique of relating lymph node morphology to function, although their basic principles for assessing cell-mediated and humoral immunity are basically the same as the method we adopted. These results only imply that the lymphoid tissue of the mesenteric nodes is behaving similarly in the two diseases and does not preclude differences in the lymphoid reactivity in the gut wall which in fact may well be the most important area for the two diseases pathogenically.

There is evidence that the tissue response which produces granulomata is sometimes dependent on the presence of antigen/antibody complexes in antibody excess (Spector and Heesom, 1969). The six cases of Crohn's disease in this series with lymph node granulomata (an incidence of 21·4%) did not show any differences in other lymph node features when compared with the other Crohn's disease cases and likewise there was no difference in lymph node appearance whether or not granulomata were present in the bowel wall.

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