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The cellular composition of granulomas in mesenteric lymph nodes from patients with Crohn's disease

K. Geboes, J. van den Oord, C. De Wolf-Peeters, V. Desmet P. Rutgeerts, J. Janssens, G. Vantrappen, F. Penninckx, and R. Kerremans

Department of Medical Research, Laboratory of Histochemistry and Cytochemistry, Department of Gastroenterological Surgery, University Hospital St. Rafaël, K.U. Leuven, Belgium

Summary. The immunohistochemical findings in granulomatous lymphadenitis in patients with Crohn's disease are presented and compared with conventional light microscopic findings. The cellular composition of the granulomas in mesenteric lymph nodes was examined with a broad panel of monoclonal and polyclonal antibodies directed to B-cells, T-cells, monocytes/macrophages, dendritic reticulum cells, HLA-DR antigens and the transferrin receptor. The centre of the granulomas contains OKIa$^+$, OKM1$^+$, OKT9$^+$, DRC$^-$, To5$^-$ epithelioid cells and giant cells and OKT3$^+$ lymphocytes. In general, the majority of the small lymphocytes within the granulomas expresses the OKT3$^+$, OKT4$^+$ Leu 3a$^+$ phenotype which points toward T-helper cells. Fewer OKT3$^+$ OKT8$^+$ T suppressor/cytotoxic cells are observed. At the periphery of the granulomas a lymphocytic corona composed of BA1$^+$, B1$^+$ B lymphocytes may be present.

From these findings it can be concluded that the granulomas in mesenteric lymph nodes from patients with Crohn's disease are composed of centrally located T-lymphocytes and of epithelioid cells which are of monocyte/macrophage origin and have the characteristics of antigen-presenting cells.

Key words: Crohn's disease – Granulomas – Mesenteric lymph nodes – Immunohistochemistry – Lymphocytes.

Introduction

Although granulomas are not a sine qua non for the diagnosis of Crohn's disease (Rotterdam and Sommers 1981), they are considered to be the most reliable histopathological criterion for differentiating Crohn's disease from

Offprint requests to: K. Geboes at the above address

ulcerative colitis in those countries where other causes of granulomatous inflammation in the gastrointestinal tract are rare (Surawicz and Rubin 1982).

In Crohn's disease granulomas are found in the bowel wall in 50–87% of the cases (Morson 1968; Lockhart-Mummery and Morson 1964) and in regional lymph nodes in 20%–38% of the cases (Williams 1964; Warren and Sommers 1948; Van Patter et al. 1954; Cook 1972). The presence of granulomas in the bowel wall and in the lymph nodes has been related to the prognosis (Glass and Barker 1976; Wolfson et al. 1982; Chambers and Morson 1979) but no consistent conclusions have been reached. The lymph node granulomas of Crohn's disease have been considered to be derived from enlargements of the germinal center histiocytes or from accumulations of sinusoidal histiocytes (Mottet 1971). This difference has been considered important in considerations of the aetiology (Mottet 1971). However, at present the significance of the granulomata in Crohn's disease remains unclear. According to Warren (1976) and Boros (1980) granulomas can be divided into immunological-hypersensitivity or cell-mediated types and nonimmunological-foreign-body types. Immunological and non-immunological granulomas differ in cellular composition. Whereas the latter are composed essentially of macrophages, fibroblasts and multinucleated giant cells, the immunological granulomas contain specific cells such as lymphocytes and plasma cells (Boros 1980). Recent studies using immunohistochemistry have demonstrated that the cellular composition of the immunological granulomas is different in various conditions (van den Oord et al. 1984). T-lymphocytes, mainly of the helper/inducer subset ($OKT4^+$), and plasma cells were found infiltrating various sarcoid lesions obtained from different sites of activity in patients with established sarcoidosis (Semenzato et al. 1983). In Kveim reactive papules from patients with clinically active, untreated sarcoidosis, OKIa1 negative epithelioid cells were found in the center of the lesions. In the surrounding lymphocytic mantle T-lymphocytes, especially of the helper type ($OKT4^+$) were seen (Konttinien et al. 1983). In contrast, OKIa1 positive epithelioid cells, few T helper and many T suppressor cells ($OKT8^+$) were described in the granulomas of sarcoid lymph nodes. These lesions were surrounded by a mantle of B lymphocytes (van den Oord et al. 1984). A predominance of T suppressor cells ($OKT8^+$) was described in the lymphocytic corona of sarcoid skin lesions (Modlin et al. 1983). Variable results were also obtained in other granulomatous conditions such as leprosy (Modlin et al. 1983; Narayanan et al. 1983, 1984), tuberculosis (van den Oord et al. 1984), rheumatoid arthritis (Duke et al. 1984), and suppurtive granulomatous lymphadenitis (van den Oord et al. 1985). In leprosy as well as in suppurative granulomatous lymphadenitis, two types of granulomas, characterized by a shift in the nature of the intragranulomatous T-cell subset have been described (Modlin et al. 1983; van den Oord et al. 1985). In leprosy, the lesions have been correlated with differences in the host's immune response. The present study was performed in order to define the cellular composition of the granulomas in mesenteric lymph nodes from patients with Crohn's disease. The characterisation of

cell subtypes may provide a better insight into the significance of the granuloma in Crohn's disease.

Methods

Patients. The resection specimens and draining lymph nodes from 114 patients operated on for Crohn's disease from October 1980 to April 1982 have been re-examined in order to define the incidence of granulomas. There were 56 men and 58 women, aged 17 to 62 years. All these patients agreed to a follow up examination and underwent ileocolonoscopy with biopsy, in order to study potential recurrence. Recurrence was defined as the presence of macroscopic lesions observed during endoscopy which was performed at various intervals after surgery (Rutgeerts et al. 1984). Mesenteric lymph nodes, containing granulomas from 12 operative specimens were used for immunohistochemical studies in order to study the cellular composition of the granulomas. The lymph nodes used for immunohistochemical study were obtained from patients operated because of obstruction (7), internal fistulae (3) or both (2). This subgroup was composed of 9 female and 3 male patients with a mean age of 32 years (range 22–50 years). Three patients had an isolated ileitis. In eight cases an ileocolitis was found and one patient had ileocolitis and intrinsic Crohn's disease of the stomach. Two patients had a relative in whom the diagnosis of Crohn's disease had also been established. In the 12 patients the diagnosis of Crohn's disease had been made 3.8 years (range 1–6 years) before surgery. Only one patient was treated with steroids when he was operated on. None of the other patients had ever received corticosteroids. In all patients the diagnosis of Crohn's disease was based on classical clinical, radiological, endoscopic and histological criteria.

Tissue selection. The mesenteric lymph nodes used for immunohistochemistry were immediately removed from the freshly obtained surgical specimens. Representative portions of each lymph node were quickly frozen in liquid nitrogen-cooled isopentane and stored at −70° C until use. The remainder of the lymph node was fixed in Bouin's solution and routinely processed for diagnostic purposes. The bowel specimens and the lymph nodes from the other patients were routinely processed (Hamilton 1983).

Immunohistochemistry. Five micrometer cryostat sections were cut from the frozen lymph node biopsies and stained with haematoxylin and eosin in order to identify granulomas. When these were present, serial sections were made, dried overnight at room temperature, subsequently fixed in acetone for 10 minutes at room temperature and used for immunohistochemistry.

An indirect immunoperoxidase procedure as described by Mason et al. (1982) was performed. The following panel of monoclonal antibodies was used: BA1, defining mature B-lymphocytes (Abramson et al. 1981) and reacting with the majority of lymphocytes in primary lymphoid follicles and in the lymphocytic corona of secondary lymphoid follicles (Hsu and Jaffe 1984); B1, reacting with all mature B-cells (Nadler et al. 1981); To5, defining human complement C3b-receptors (Gerdes et al. 1982); OKT3, reactive with more than 95 per cent of peripheral T cells and 20 per cent of thymocytes (Kung et al. 1979); OKT4 and Leu 3a, defining the helper/inducer T cell subset (Reinherz et al. 1980b); OKT6, reacting with 70% of thymocytes and not with peripheral T cells (Reinherz et al. 1980a); OKIa1 directed against MHC class II products (HLA DR or Ia-like antigen) (Reinherz et al. 1979b); and reacting with activated T lymphocytes, monocytes and 90% of B lymphocytes; OKM1, reacting with monocytes and polymorphonuclear granulocytes (Breard et al. 1980); OKT9 defining the transferrin receptor; DRC1, reacting with an antigen present on dendritic reticulum cells present in all lymphoid follicles. The OK-series of monoclonal antibodies was purchased from Ortho Pharmaceutical Co., Raritan NJ, USA; Leu 3A, BA1 were obtained from Becton Dickinson, Sunnyvale, CA, USA; B1 was obtained from Coulter Electronic, Hialeah, FL, USA. To5 was a generous gift from Dr. D.Y. Mason, Oxford, U.K. and DRC1 was purchased from Dakopatts, Copenhagen, Denmark.

The cryostat sections were incubated with the monoclonal antibodies for 30 min. A 15 min wash in three changes of phosphate buffered saline (PBS), pH 7.2, was followed by incubation

Table 1. Relation between granulomas, recurrence and duration of Crohn's disease

Duration of the disease	Granulomas in the bowel wall		Granulomas in lymph nodes	
	Recurrence	No recurrence	Recurrence	No recurrence
0–36 months	34 (39%)	–	11 (13%)	2 (7%)
37–72 months	21 (24%)	7 (26%)	24 (27%)	6 (23%)
>72 months	21 (24%)	6 (22%)	3 (3%)	2 (7%)
Total	76 (87%)	13 (48%)	38 (43%)	10 (37%)

for 30 min with peroxidase-conjugated rabbit antimouse IgG (DAKO-immunoglobulins, Denmark).

The sections were washed again in three changes of PBS, pH 7.2, and the reaction product was developed using 0.05% 3-amino-9-ethylcarbazole and 0.02% H_2O_2. The slides were washed in a 0.1 M acetate buffer pH 4.9, for 10 min, counterstained with Mäyer's haemalum and mounted. Helper/inducer T cells were demonstrated by the simultaneous application of OKT4 and Leu 3a, as previously reported (van den Oord et al. 1985).

Cytoplasmic immunoglobulins (IgA, IgM, IgG, IgD) were demonstrated on Bouin's fixed, paraffin embedded sections by the peroxidase-antiperoxidase (PAP) method (Sternberger 1979). The polyclonal antibodies were obtained from Dakopatts, Copenhagen, Denmark.

Results

Granulomas were observed in the bowel wall in 89/114 (78%) of the patients and in the mesenteric lymph nodes in 48/114 (43%) of the resected specimens. The crude recurrence rate of Crohn's disease in the 114 patients amounted to 77% (87). Granulomas were present in the bowel wall in 76/87 (87%) of the patients having recurrence when examined endoscopically and in 13/27 (48%) of the patients without recurrence. In the mesenteric lymph nodes, granulomas were observed in 38/87 (43%) of the patients with endoscopic recurrence and in 10/27 (37%) of the patients without recurrence. The patients showing recurrence had a mean duration of the symptoms of 64 months (0–221). For those without endoscopic postoperative lesions, the mean duration was 84 (0–292) months. When the patients having granulomas in the resection specimen were divided into groups according to the duration of their symptoms, a weak relationship between the duration of the symptoms and the presence of granulomas was found (see Table 1). Various types of granulomas were observed in the bowel wall as well as in mesenteric lymph nodes: a) microgranulomas which are small clusters of three to five epithelioid cells with lymphocytes; b) well formed granulomas composed of epithelioid cells, intermingled with lymphocytes and one or more centrally located giant cells; c) well formed granulomas surrounded by a mantle of small lymphocytes; and d) a diffuse loosely arranged histiocytic infiltrate. The component cells of the well formed granulomas were either tightly packed, or showed a loose cellular arrangement. In the lymph nodes fibrosis was present in some areas, whereas necrosis was never observed.

Well formed granulomas with and without a lymphocytic corona were

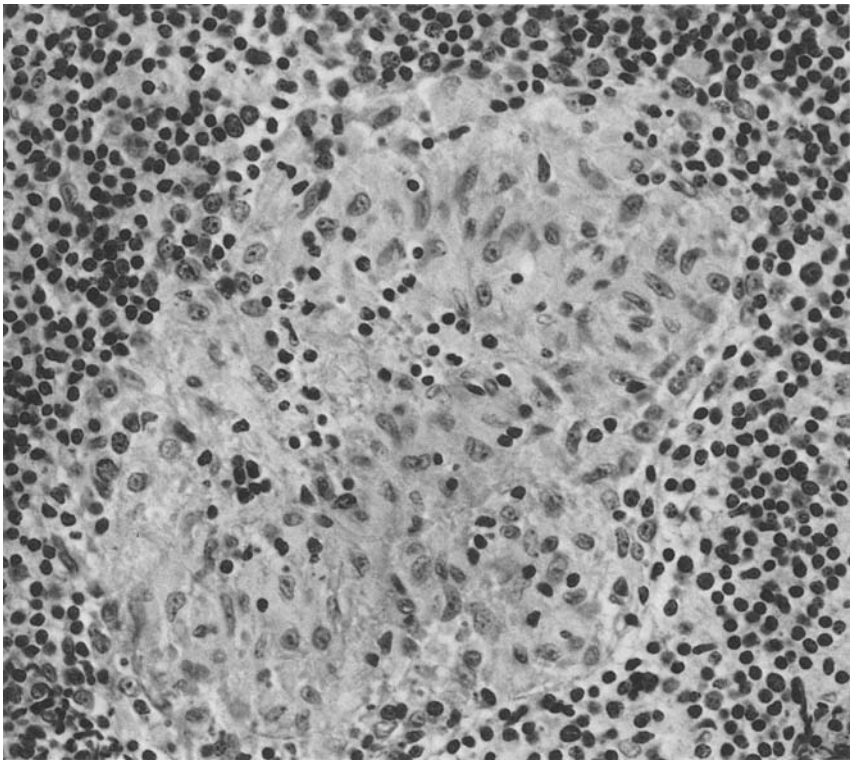


Fig. 1. Well-developed granuloma composed of centrally located epithelioid cells and lymphocytes and surrounded by a mantle of lymphocytes. (Paraffin section, H.E. ×100). A similar mantle is not present in Fig. 4

present together in the bowel wall in 78% of the patients having granulomas ($n=69$) and in the mesenteric lymph nodes in 70% of the patients with granulomas ($n=27$). In the lymph nodes available for immunohistochemical examination, two types of granulomas were observed. In eight patients the granulomas lacked a well developed lymphocytic corona. These granulomas were found in the submarginal sinus area or/and in the paracortical area. The mean duration of the symptoms in these patients was 18 months (12–26). In four patients granulomas with and without a broad mantle of small lymphocytes were found. They were localized in the interfollicular cortical region or in the medulla (in one case). The mean duration of the symptoms for these patients was 48 months (36–70).

The architecture of the lymph nodes was well preserved in all cases. Nonspecific changes including follicular hyperplasia, sinus dilatation and the presence of histiocytes and small lymphocytes within the sinuses were found in all cases. Follicular hyperplasia was however not always present and mostly not prominent whereas sinus dilatation was usually very prominent.

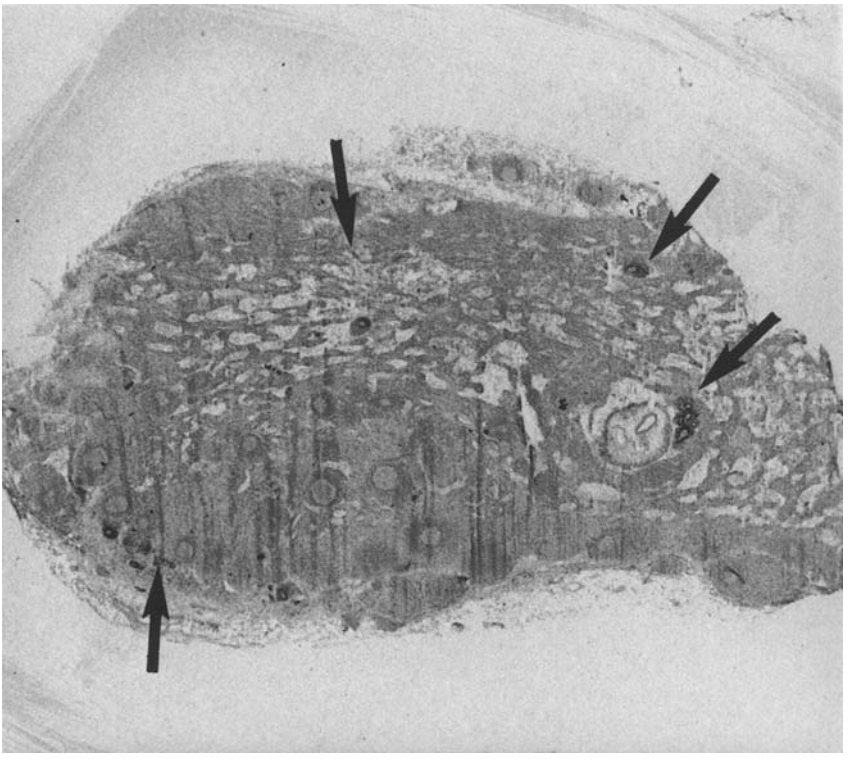


Fig. 2. Microphotograph of a mesenteric lymph node from a patient with Crohn's disease. Several granulomas (*arrows*) are clearly visible as they show a positive staining for the OKM1 monoclonal antibody indicating that they are composed of monocyte derived cells. (Immunoperoxidase ×4)

In all cases both the epithelioid cells and the multinucleated giant cells showed strong membranous reactivity with monoclonal antibodies OKIa1, OKM1, and OKT9 (Fig. 2). No reactivity was found on these cells with monoclonal antibodies DRC and To5 (Fig. 3), $OKT3^+$ cells were present within and outside the granulomas. In general, the majority of the small lymphocytes, within the granulomas expressed the phenotype $OKT3^+$, $OKIa^+$, $OKT4^+$, Leu 3^+, whereas fewer $OKT3^+$, $OKIa^+$, $OKT8^+$ cells were observed. In some cases the number of $OKT3^+$ $OKT4^+$ Leu $3a^+$ cells in the granulomas was equal to the number of $OKT3^+OKT8^+$ cells (Fig. 4). The granulomas contained no $B1^+$, $BA1^+$ cells. When a lymphocytic mantle was present ($n=4$) it was mainly composed of $B1^+$, $BA1^+$ cells. Within the granulomas no plasma cells were found, but in the periphery of the lesions a few of these cells, carrying either IgA or IgG in their cytoplasm were always detected. IgM or IgD containing cells were rarely present. No OKT6 immunoreactivity was found in any of the lymph nodes.

The immunohistochemical findings in the remainder of the lymph nodes

Fig. 3. The granuloma (*arrows*) is localized in the interfollicular cortical region and stains negative for the monoclonal antibody DRC. This antibody is expressed on the cells of the follicle lying in the immediate vicinity. This indicates that granulomas are not formed from dendritic reticulum cells (Immunoperoxidase × 50)

was similar to previously reported findings (Hsu and Jaffe 1984; Hsu et al. 1983).

Discussion

The significance of the granulomata in Crohn's disease is unclear. Their presence is important for the microscopic diagnosis, but not indispensable and granulomas may be found in other conditions (Surawicz and Belic, 1984). Various studies have examined the relationship between the granulomas and recurrences of Crohn's disease and have yielded conflicting results. Glass and Baker (1976) found that the absence of granulomas was associated with a crude recrudescence rate of 60%. This is broadly in agreement with the results of Chambers and Morson (1979) who showed that granulomas in the larger bowel and anus were associated with a good prognosis, but were of no prognostic significance in the small bowel. These authors concluded also that a small number of granulomas had no real relation with

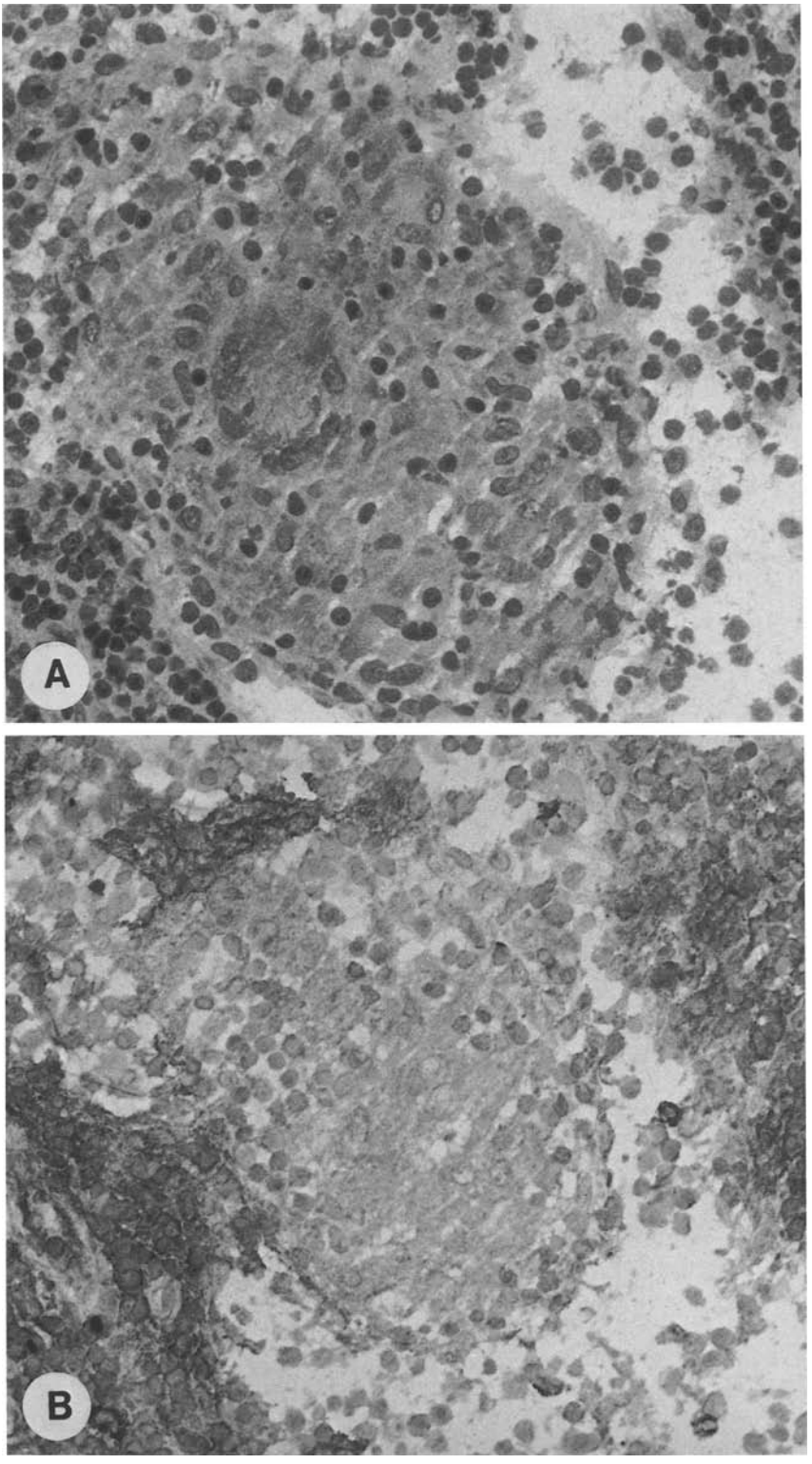
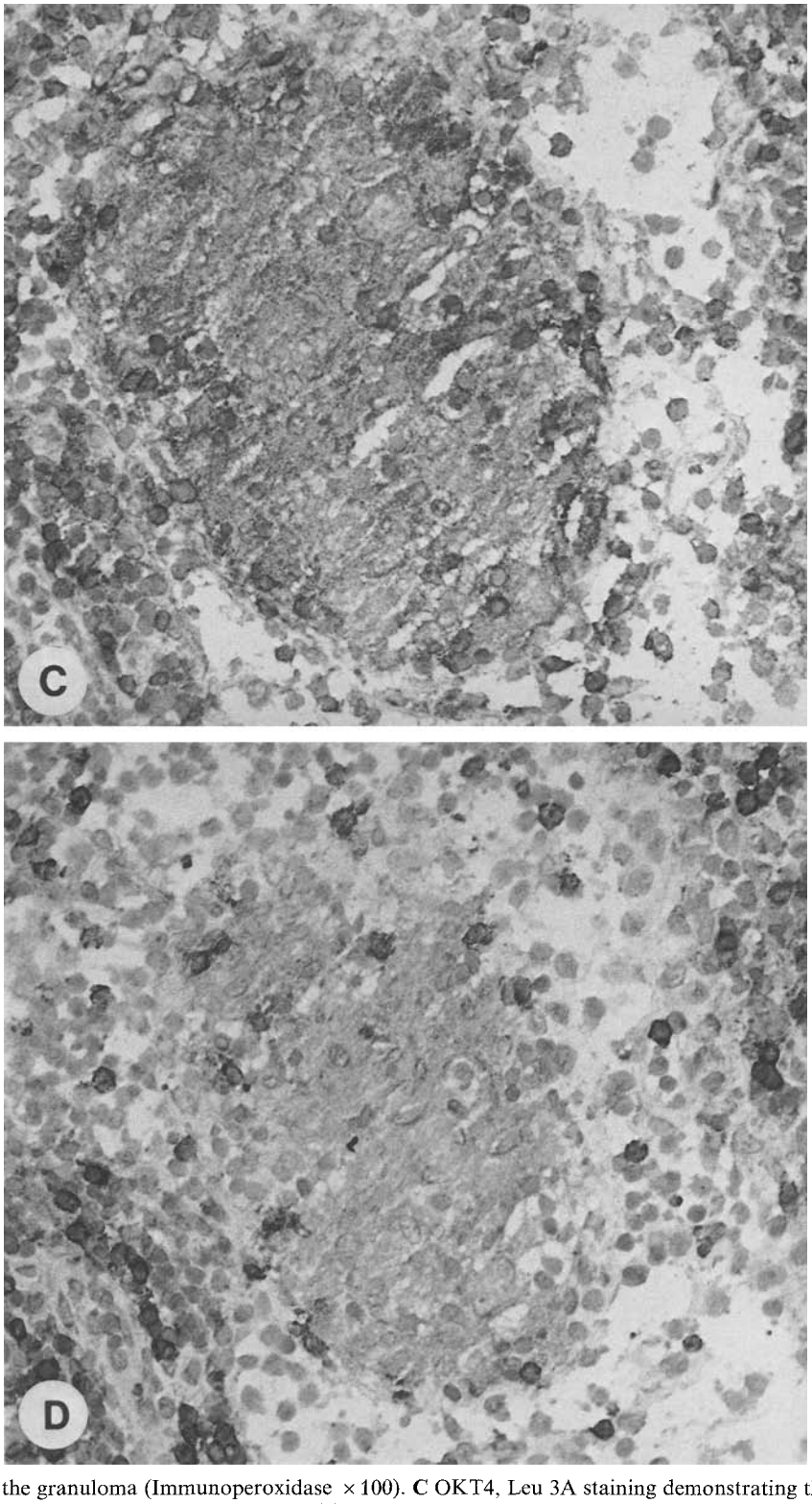


Fig. 4A–D. This figure shows serial cryostat sections of the same granuloma: **A** H.E. staining showing a centrally located giant cell with epithelioid cells and lymphocytes but without a mantle of lymphocytes ($\times 100$). **B** BA1 staining showing the absence of B lymphocytes from the centre of the granuloma. Some $BA1^+$ lymphocytes form a cluster in close contact with



the granuloma (Immunoperoxidase ×100). **C** OKT4, Leu 3A staining demonstrating the presence of T helper lymphocytes within the granuloma (Immunoperoxidase ×100). **D** OKT8 staining showing the presence of T suppressor lymphocytes within the granuloma (Immunoperoxidase ×100)

the prognosis. In contrast to these findings no relation between the presence of granulomas in the bowel wall and/or lymph nodes and recrudescence of Crohn's disease was found in the studies of Van Patter et al. (1954), Antonius et al. (1960), Wolfson et al. (1982) and Cook (1972). We have found a somewhat higher incidence of recurrent endoscopic lesions in patients with granulomas in the bowel wall but not relation between the presence or absence of granulomas in mesenteric lymph nodes and postoperative endoscopic recurrence. A similar relationship between recrudescence and granulomas was observed by Lindhagen et al. (1983). All these different results can be explained by the wide variation of the definition of recurrence used in the literature, by the variable indications for surgery but probably more by the nature and function of the granuloma. We have used a definition of recurrence based upon endoscopic and histopathological evidence of Crohn's disease. Such a definition provides sensitivity but includes subclinical recurrences which may require no therapy. Yet we have chosen this definition as it probably reflects the natural history of Crohn's disease more adequately.

It means however, that our results cannot simply be compared with those obtained from other series. The high incidence of recurrent lesions in our series and, in fact, in other long term follow-up studies (Hellers 1979) can best be explained as the result of the diffuse abnormality of the entire mucosa of the gastrointestinal tract as evidenced by a variety of studies using different techniques (Hamilton 1983) and must not necessarily be related to the presence of granulomata. We think that the granuloma is the normal response of immunologically competent tissue to the aetiology or aetiologic agents of Crohn's disease. This assumption is based upon the facts that no consistent alterations of the immunological system have been found in Crohn's disease despite numerous investigations (Kirsner, 1984) and that similar granulomatous reactions occur in various diseases and various organ systems (Konttinien et al. 1983). Our studies demonstrate the similarity between Crohn's granuloma's and those occurring in other diseases. Because of its composition, the granuloma can have an influence upon the natural history of the disease. As shown in our study, the well formed granulomas in lymph nodes from patients with Crohn's disease are composed of centrally located epithelioid cells and of T lymphocytes. The epithelioid cells are $OKIa^+$, $OKM1^+$, $OKT6^-$, DRC^-, $To5^-$ cells. This means that these cells are no follicular dendritic cells (which are normally found within the germinal centers of lymphoid follicles and which play a role in the trapping and subsequent presentation of antigens to B-cells) as suggested by Mottet (1971), but rather interdigitating reticulum cells (Hsu et al. 1985). These cells are known to be present in the small bowel (Poulter and Janossy 1985) and in the paracortical area of the lymph nodes, where according to our findings the granulomas are usually found. They express class II MHC antigens (HLA-DR or Ia) on their cell surface and, thus, may interact with and stimulate T lymphocytes.

It is therefore normal that the lymphocytes present within the granulomas from patients with Crohn's disease, are T-lymphocytes, as demonstrated

by our findings, and these are considered to be important in the pathogenesis of Crohn's disease (Ebert et al. 1984; Ferguson 1983). The interdigitating reticulum cells are derived from $OKT6^+$ $OKIa^+$ $ATPase^+$ veiled cells, and increased numbers of veiled cells have been observed in the gut wall of patients with chronic inflammatory bowel disease (Selby et al. 1983) (Wilders et al. 1984). In view of the morphological, enzyme- and immunohistochemical. similarities between these cell types, it is reasonable to assume that the epithelioid cells originate from veiled cells, arrested in their locomotion and phagocytic capacities by soluble products of activated T cells (Veerman 1974). If, as we assume, the granuloma formation is the expression of a normal reaction in immunologically normal tissue, it is expected to occur more frequently although not exclusively in cases with a better prognosis than in patients with a worse outcome. This is in agreement with the findings of Chambers and Morson (1974) who found rather a relation between a high granuloma content and a better prognosis, than between the presence or absence of granulomas and the prognosis. It can also explain why a relapse of symptoms and a resistance to steroid therapy was found in a patient without veiled cells in the intestinal mucosa (Wilders et al. 1984) and thus without granuloma formation. The lack of granuloma formation in some cases would prevent the elimination of the aetiologic agents. Granuloma formation would also be expected to occur more frequently early in the disease and such a relation with the duration of symptoms was found in the studies of Chambers and Morson (1979) and in our findings.

Resection specimens from patients with a long history of Crohn's disease have a low granuloma content (Chambers 1981). From this finding it has been suggested that many of the clinical symptoms of Crohn's disease may be caused by tissue scarring. This phenomenon can also be explained by the nature of the granulomas. The phenotype of the epithelioid cells composing the granulomas corresponds to that of activated macrophages (Poulter et al. 1982) which may stimulate the proliferation of fibroblasts and induce the fibrosis (Bitterman et al. 1982) remaining after the disappearance of the granulomas. The cellular composition of the granulomas, as we describe it, thus explains certain characteristics of the natural history and pathology of Crohn's disease such as the early occurrence of granulomas, the relation between a high content of granulomas and a good prognosis and finally the scarring and fibrosis. It can also explain the lymphoid hyperplasia frequently observed in the gut. We found no $BA1^+$, $B1^+$, B lymphocytes within the granulomas. They were however present as a corona around the epithelioid cells although not always. As the various morphological types of granulomas were frequently present together we assume that these types are the result of a dynamic process and represent stages in the development of the granuloma. Such a relation has already been shown for the size of the granulomata (Schmitz-Moormann and Becker 1981). The lymphocytic corona around the granuloma is the expression of an activation of B-cells, probably recruited by the $OKT4^+$ helper T lymphocytes within the granulomas secondary to the activation of these cells by the real aetio-

logic agent or agents of Crohn's disease. The nature of this aetiologic agent or agents remains obscure. Antigenic elements of it must however be located within the granuloma and must be expressed by the epithelioid cells. At present, no identifiable material has been consistently found within the granulomas. Still they are the most likely place to hide constituents of the aetiologic agents (probably molecular) and as they are mainly found early in the disease, patients and material studied at an early stage will offer the best chances to discover the aetiology or aetiologies of Crohn's disease.

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