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Epithelioid granulomatosis with initial and predominant manifestation in the spleen

Morphological and immunohistochemical analysis of six cases

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Summary. In six patients with systemic symptoms, four of which had lymphoplasmacytic infiltrates in the bone marrow splenectomy was performed because of suspected malignant lymphoma, with resolution of clinical symptoms. The spleen showed epithelioid granulomas, and neither splenic tissue nor clinical follow-up revealed evidence of malignant lymphoma. Immunohistochemical analysis documented an identical phenotype in epithelioid and giant cells as well as in large numbers of CD4+ lymphocytes and S100+ interdigitating reticulum cells within the granulomas. These cases are interpreted to represent epithelioid granulomatosis with primary and/or predominant manifestation in the spleen.

Key words: Epithelioid cells – Granuloma – Immunohistochemistry – Macrophages – Spleen

Introduction

Most granulomatous lesions of the spleen do not possess clinical significance and are discovered incidentially in splenectomy specimens removed for a variety of reasons or at autopsy (Collins and Neiman 1983). However, splenic involvement in systemic granulomatous disorders may cause clinical symptoms by splenic enlargement and/or hypersplenism and may have diagnostic implications. In most of these cases the diagnosis of a granulomatous disease has been established prior to splenectomy. In the present study six cases diagnosed by morphological examination of the spleen removed for suspected malignant lymphoma, were subjected to clinicopathological and immunohistochemical examination.

Material and methods

Clinical records of six patients (four females and two males, aged between 17 and 63 years with a median of 38.6 years) identified by a retrospective survey of the splenectomy specimens on file with the Department of Pathology were reviewed. The six cases were selected on the basis of the presence of granulomatous inflammation in the splenectomy specimen without prior clinical or histological diagnosis of the disease and without known concomitant infectious or neoplastic lesions (e.g. Hodgkin's disease). After splenectomy the patients were followed for a mean follow-up time of 19 months.

Spleen sections stained with haematoxylin-eosin, Giemsa, PAS and Gomori's silver stain, Ziehl-Neelsen and toluidine blue were examined under code. In addition, fresh-frozen (four cases) and paraffin-embedded splenic tissue (all cases) was subjected to an immunohistochemical analysis using a panel of relevant antibodies (Table 1). Visualization of antibody binding was accomplished by an immunoperoxidase and a modified immunoalkaline-phosphatase method. Bone marrow biopsies obtained prior to splenectomy in four patients were also reviewed under code.

Results

All patients suffered from nonspecific constitutional symptoms such as low grade fever, weight loss, night sweats, and malaise. Apart from one case (see below) there was no peripheral lymphadenopathy; peripheral blood counts were normal in all cases, there was no lymphocytosis. The erythrocyte sedimentation rate was raised in three cases.

In 4/6 cases ultrasound and/or CT examination revealed moderate to massive splenomegaly.

In two patients moderate pancytopaenia had been present for up to two years before splenectomy. In one patient a plasma cell granuloma of the lung had been resected 11 months prior to

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Table 1. Antibodies employed in the present study

Antibody	*Specificity*	*Source*
MT 1	T-Cells	Biotest
MB 2	B-Cells	
UCHL-1	T-Lymphocytes	Dako
Leu 4*	T-Cells (CD3)	Becton-
Leu 2*	T-Cells (CD8)	Dickinson
Leu 3*	T-Cells (CD4)	
Ki M-1 – Ki M-8*	Mononuclear Phagocytes	Behring
EBM 11*	Pan-Macrophage	Dako
BerMacDRC	Dendritic reticulum cells	Prof. H. Stein, Berlin
α-1-Antichymotrypsin	Macrophages, epithelioid cells, giant cells	Dako
α-1-Antitrypsin	s.a.	Dako
S-100	Interdigitating reticulum cells, some macrophages	Dako
Vimentin	Cytoskeleton proteins	Dako

* Denotes studies done on frozen sections

splenectomy, and in two additional patients chest x-rays had shown some degree of interstitial fibrosis without hilar lymphadenopathy. One patient had been intermittently treated with corticosteroids without clinical improvement for two years.

In five of the six patients malignant lymphoma was suspected clinically and/or on the basis of a bone marrow biopsy (see below). In one young female patient a lymph node biopsy had been interpreted to show Hodgkin's disease, nodular sclerosis type, although Hodgkin- or Sternberg-Reed cells could not be demonstrated. After a mean follow-up time of 19 months none of the patients had clinical evidence of a malignant lymphoma or tuberculosis. Since the patients showed a marked clinical improvement, repeat bone marrow biopsies were not performed. The pertinent characteristics of the patients are presented in Table 2.

Four trephine biopsies obtained from the posterior iliac crest 14 to two months (mean: 2.5 months) prior to splenectomy were available for study. Smears could not be obtained. In three cases a diffuse and sometimes patchy infiltration by small lymphoid and plasmacytic cells was noted. In two cases lymphoid follicles were evident (Fig. 1). In one patient this infiltrate was present in a markedly hypoplastic marrow. These cases had been diagnosed either as being suggestive of bone marrow infiltration by a low-grade malignant lymphoma (two cases) or as questionable immunocytoma, while one bone marrow biopsy was assumed to show only reactive changes. Upon review, the diagnoses could partly be confirmed: the coded slides were again classified as being suggestive of low grade malignant lymphoma (three cases) or reactive (one case). Granulomatous lesions could not be identified.

Macroscopically, the spleens showed moderate to marked enlargement; splenic weight ranged between 210 and 1600 g with a median of 750 g. The highest weight was reached in a spleen exhibiting the salient features of haemolytic anaemia with red pulp hyperplasia and increased numbers of phagocytosing macrophages. On the cut surface of the spleen a variable number of white nodular lesions with a diameter between 0.3 and 1 cm was visible; some cases, especially those with low splenic weights, showed only a few isolated nodules, while in two instances (splenic weight 630 and 1000 g) the parenchyma was studded with innumerable white nodules simulating the appearance of malignant lymphoma in the spleen.

Microscopically two types of granulomatous lesions could be discerned: the majority (4/6 cases) exhibited large nodular granulomas situated within or at the periphery of the white pulp, sometimes in the immediate vincinity of a blood vessel. These granulomas were composed of typical epithelioid cells with pale nuclei and variable numbers of multinucleated giant cells of the Langhans type (Fig. 2). In two cases, numerous granulomas coalesced into larger nodular lesions; in the vincinity

Table 2. Patient characteristics and splenic pathology

Case	Age	Sex	Lung lesions	Bone marrow	Splenic weight	Splenic granulomas
1	17	f	none	n.d.	210 g	+
2	46	m	yes	n.d.	300 g	++
3	53	f	none	?ML	1000 g	+++
4	59	f	yes	?ML	630 g	+++
5	38	f	none	reactive	1600 g	+++
6	42	f	yes	?ML	n.d.*	++

?ML denotes lymphoplasmacytic infiltrates suggestive of malignant lymphoma in the bone marrow specimen

* Partial splenectomy

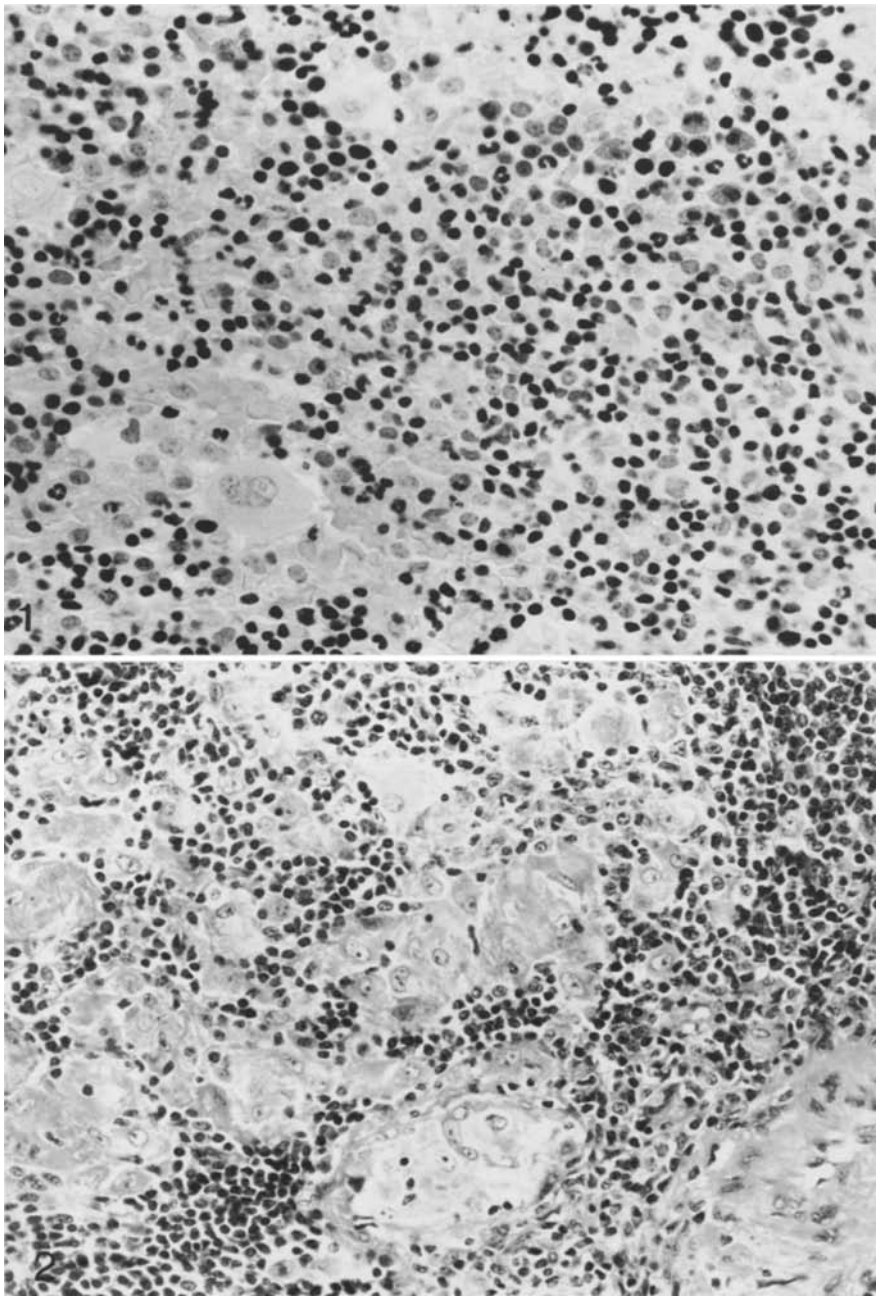

Fig. 1. Bone marrow biopsy showing hyperplastic haematopoiesis and a nodular aggregate of lymphoid cells that probably corresponds to a lymph follicle. Giemsa, × 512

Fig. 2. Granuloma composed of epithelioid and giant cells within a splenic follicle. Giemsa, × 320

of the granulomas concentric areas of sclerosis with some mast cells with toluidine blue-positive granules were present. Typical asteroid (Schaumann) bodies were evident in two instances; focal calcifications within the granulomas could be seen in another instance. Necrosis, suppurative changes or caseation were absent in all cases.

Two spleens showed loose aggregates of epithelioid cells with only a few Langhans cells within the splenic red pulp; Schaumann bodies or calcifications were absent in these cases. These granulomas were distributed randomly throughout the red pulp and were closely associated with numerous plasma cells (Fig. 3). Microorganisms could not be demonstrated by either the PAS reaction or the Ziehl-Neelsen stain. In one case an increased number of macrophages which contained a granular PAS-positive material signifying increased phagocytosis of thrombocytes was evident; this patient also suffered from thrombocytopaenia.

The immunohistochemical studies demonstrated that the epithelioid cells in all cases exhibited a similar immunophenotype: they were reactive with the macrophage markers EBM 11 as well

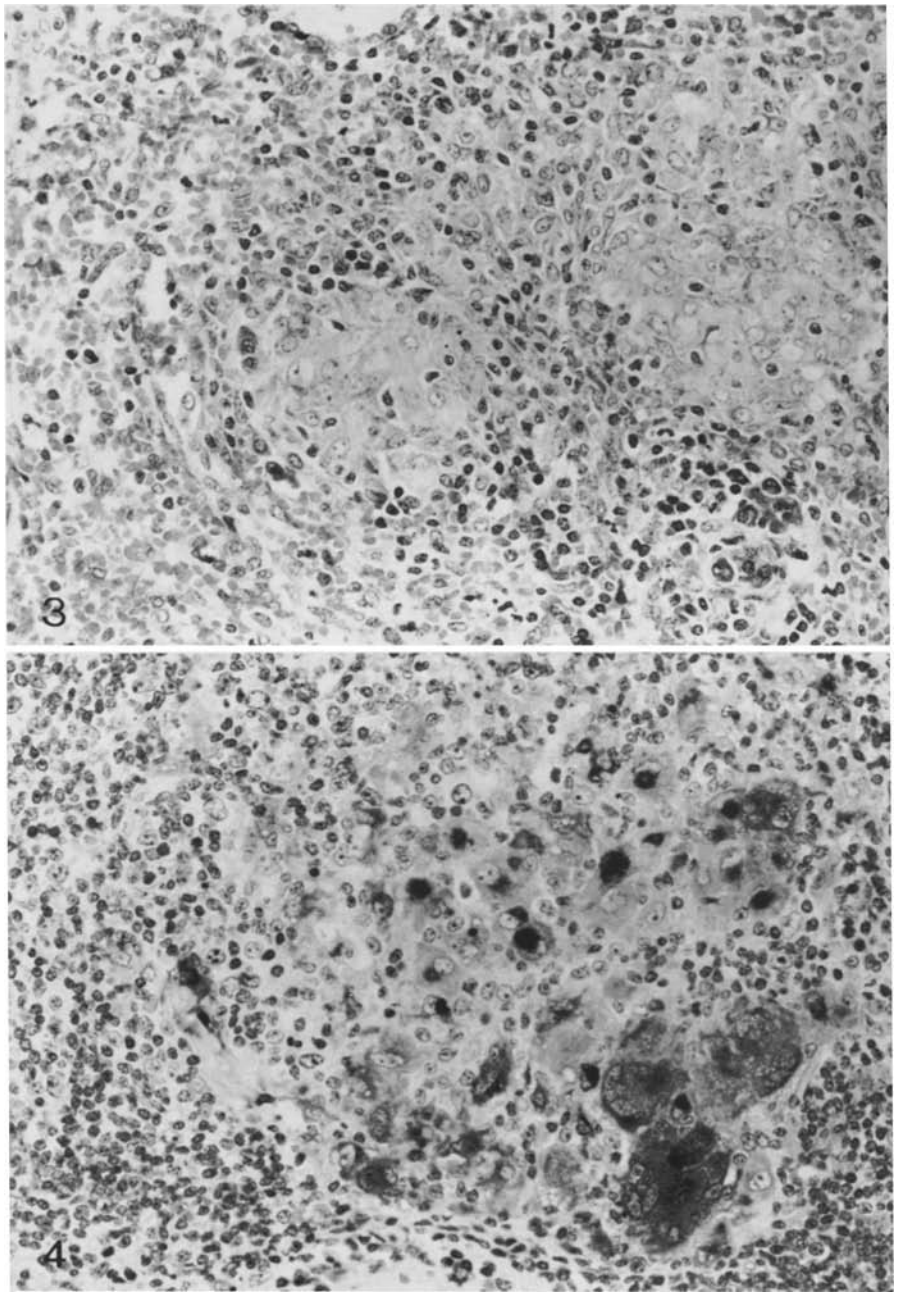

Fig. 3. Epithelioid granulomas within the splenic red pulp. Note the compression of sinus and the association with numerous plasma cells (*lower right*). Giemsa, ×320

Fig. 4. Epithelioid and giant cells strongly express lysozyme. Some cells exhibit focally increased reactivity. Immunoperoxidase, ×320

as Ki M-6 and Ki M-7. They also stained positive for Ki M-1 and showed reactivity with antibodies directed against lysozyme, α-1-antitrypsin and α-1-antichymotrypsin. While the monoclonal antibodies identifying mononuclear phagocyte antigens showed a uniform and diffuse staining pattern, lysozyme and proteinase inhibitors were distributed more unevenly; while most epithelioid and giant cells exhibited moderate diffuse positivity for these antigens, some of these cells showed strong staining with globular perinuclear accentuation (Fig. 4). Antiobodies reacting with T lymphocytes (UCHL-1 and MT1) also showed diffuse positivity of moderate intensity. In addition, epithelioid and Langhans cells were also reactive for vimentin; globular and most intense staining corresponded to Schaumann bodies. Numerous S-100 positive cells with indented nuclei and cytoplasmic projections were found scattered within the granulomas and in their immediate vincinity, regardless of their location (Fig. 5). Some round cells with scanty cytoplasm also positive for S-100 were present as

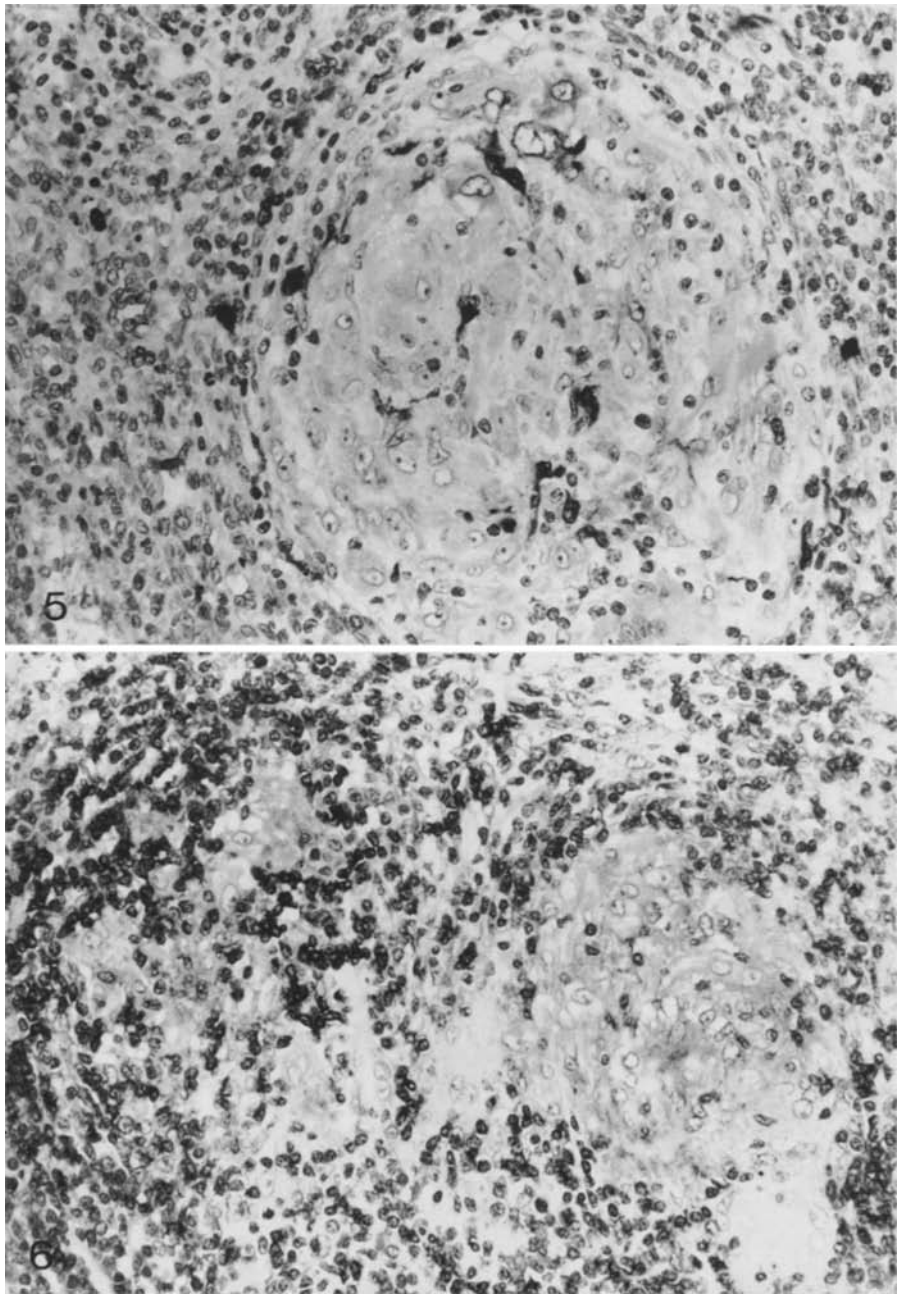

Fig. 5. In all cases the splenic granulomas contain S-100+ interdigitating reticulum cells. Immunoperoxidase, × 320

Fig. 6. Splenic epithelioid granulomas are associated with large numbers of T lymphocytes that occur both around and within the granulomas. MT1, immunoalkaline phosphatase, × 320

well. Among the epithelioid and giant cells numerous CD3+ T-lymphocytes were present; large numbers of these cells were found surrounding the granulomas. Helper/inducer T cells made up the vast majority of the T lymphocytes that were also identifiable by staining with MT1 in paraffin sections (Fig. 6). These T lymphocytes were present not only in granulomas located in the splenic white pulp, but were also associated with epithelioid cell aggregates in the red pulp. Suppressor/cytotoxic T cells were detected only in very small numbers.

Staining for dendritic reticulum cells with Ki M-4 and BerMacDRC as well as for mature B lymphocytes with MB2 revealed that granulomas in the splenic white pulp were preferentially localized within the B-cell areas, displacing the network of dendritic reticulum cells (Fig. 7). In addition to the T-cells the granulomas also possessed a rim of surrounding MB2+ B-cells; however, they did not contain dendritic reticulum cells.

In the two cases with epithelioid granulomas located in the red pulp, these granulomas were as-

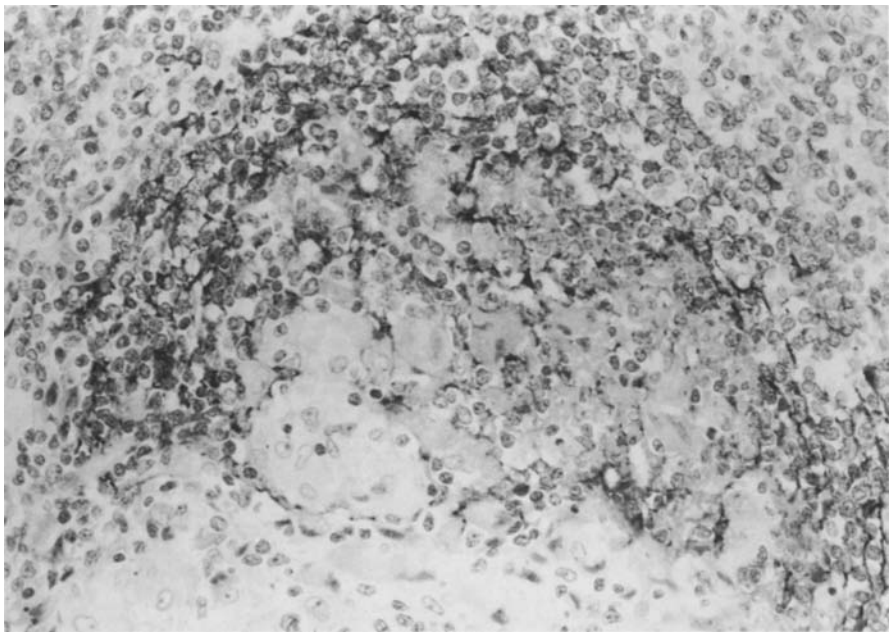

Fig. 7. Dendritic reticulum cells reactive with BerMacDRC antibody are usually found at the periphery (*top*) but not within the granulomas (*bottom*). Immunoalkaline phosphatase, ×320

sociated with numerous plasma cells (Fig. 3) containing polytypic immunoglobulins.

Discussion

Granulomatous lesions of the spleen may appear in a wide range of disorders that encompass infections, storage diseases, neoplasms, rheumatological and autoimmune disorders as well as a variety of ill-defined conditions (Collins and Neiman 1983; Stutte 1984). In the present study six cases with non-caseating epithelioid granulomas of the spleen were analysed in which splenectomy was performed because of suspected malignant lymphoma (five cases) or hypersplenism (one case). The majority of patients exhibited systeme nonspecific symptoms raising the clinical suspicion of malignant lymphoma. This was nurtured by the presence of lymphoplasmacytic infiltrates in the bone marrow, suggestive if not diagnostic of low-grade malignant lymphoma in four cases. Blind review of the bone marrow biopsies substantiated the initial diagnoses. However, histopathological examination of the spleens and clinical follow-up failed to demonstrate a lymphoproliferative disorder, although the macroscopic appearance of the splenectomy specimens showing variable numbers of white nodular lesions was also suggestive of malignant lymphoma. Neoplastic infiltrates obscured by a diffuse granulomatous reaction (Braylan et al. 1977) were not detected. Instead, a variable number of well-formed, sarcoid-like granulomas were demonstrated composed of epithelioid cells and multinucleated giant cells of the Langhans type without evidence of microorganisms.

While the frequent occurrence of epithelioid granulomas of the spleen in the setting of non-Hodgkin's lymphomas (Braylan et al. 1977; Collins and Neiman 1983; Neiman 1977) or Hodgkin's disease (Sacks et al. 1978; Schneider et al. 1977) has been well documented, the six cases analysed in the present study demonstrate that granulomatous disorders with predominant and/or primary (initial) splenic manifestation may also imitate malignant lymphomas. Clinically, the presenting systemic symptoms combined with splenomegaly, which may also be caused by a granulomatous process with splenic involvement, are easily compatible with this diagnosis. Morphologically, however, the presence of infiltrates composed of lymphoid and plasmacytic cells in bone marrow biopsies of four patients which was confirmed upon review is not readily explained. Although they appeared neoplastic and suggestive of malignant lymphoma rather than reactive, clinical follow-up failed to detect evidence of a malignant lymphoma, and splenectomy was followed by rapid clinical improvement in all patients. Thus it appears that while these lesions were not associated with bone marrow granulomas they seem to be related to this granulomatous disorder, chiefly manifest in the spleen.

In order to classify further the granulomatous lesions, immunohistochemical studies were performed. They revealed that the epithelioid and giant cells share a common immunophenotype de-

spite the somewhat variable morphological appearance of the granulomas: they stain for antigens expressed by mononuclear phagocytes (Ki M-antibodies, EBM 11) and contain lysozyme as well as protease inhibitors. In addition they exhibit (nonspecific?) reactivity with antibodies identifying T lymphocytes in paraffin sections.

In all cases numerous S-100+ interdigitating reticulum cells were detectable both within and at the periphery of the granulomas regardless of their localisation. These cells possess accessory cell functions by presenting antigens to T cells. Although they have been demonstrated in a variety of granulomatous inflammations (Schmauz et al. 1987), their role in granuloma formation is not fully understood. Possibly they provide a suitable microenvironment for the T-lymphocytes associated with the granulomas.

In all cases a large number of CD4+ UCHL-1+MT1+ T-lymphocytes was present both within and in the immediate vincinity of the granulomas regardless of their preferential location within the B-cell areas of the spleen. These findings are compatible with numerous studies of granulomas associated with sarcoidosis that also demonstrated CD4+ T-lymphocytes within the granuloma (Kataria and Park 1986; Semenzato et al. 1986) They are assumed to initiate and sustain granuloma formation (i.e. transformation of macrophages into epithelioid and giant cells) by the secretion of certain lymphokines (Hunninghake and Crystal 1981). In the present study, however, CD8+ T-cells that have been reported to occur in sarcoid granulomas (Van den Oord et al. 1984) could not be demonstrated in appreciable numbers. This phenomenon may be associated with a switch in the immunophenotype of the granuloma-associated T-cells during the granulomas' evolution, i.e. a modulation of the immune response at the granuloma site (Van den Oord et al. 1985).

Other findings supporting the diagnosis of sarcoid granulomas are the presence of perigranulomatous fibrosis, of focal calcifications, of vimentin-positive Schaumann bodies (Cain and Kraus 1983) and of lung lesions that had been demonstrated radiographically in two patients. However, these findings are not invariably present in all patients studied. Moreover, in two cases granulomas involving the splenic red pulp predominantly were associated with large numbers of plasma cells. Such rings of plasma cells have been described infrequently in granulomatous disorders (Van den Oord et al. 1985). Thus their significance with regard to the biology and to the classification of the granulomas as well as the possible contribution of the microenvironment of the splenic red pulp to the plasmacytosis remain to be determined.

Apart from these variations, the morphological and immunohistochemical investigations of the splenic granulomas yield identical results in almost all cases; a common pathogenesis may therefore be assumed. Since microorganism could not be demonstrated and there was no clinical evidence of a malignant or infectious process, the epithelioid granulomas in the spleen very probably represent the morphological equivalent of hypersensitivity reactions (so-called hypersensitivity granulomas).

Although the results of immunohistochemical investigations are nonspecific with regard to differentiating hypersensitivity granulomas and do not suffice to support an unequivocal diagnosis of sarcoidosis of the spleen, the high incidence (20–30% of the cases) of splenic involvement in this ill-defined disease (Scadding 1967) as well as the morphological and clinical similiarities to sarcoidosis-associated granulomas support the notion that the epithelioid granulomas in the spleen of the patients studied are related to or even correspond to sarcoidosis of the spleen. It must be noted, however, that even the application of special stains for microorganisms and the negative clinical evaluation of the patients do not wholly rule out splenic involvement in infectious diseases such as tuberculosis, syphilis or toxoplasmosis. A comparison between the morphological appearance of the lesions observed in the present study and splenic granulomas associated with both sarcoidosis and tuberculosis (three cases each) did not demonstrate appreciable differences. Since a definite aetiological classification of the granulomas obviously is impossible, the splenic lesions of the patients analysed in the present sudy should preferably be designated epithelioid granulomas (hypersensitivity type) of unknown aetiology.

In summary, the clinical and morphological analysis of the six cases reveals epithelioid (hypersensitivity) granulomatosis of unknown aetiology presenting primarily and/or predominantly in the spleen. In addition to the known sequelae of granulomatous inflammation of the spleen, such as hypersplenism with leuko- and thrombocytopaenia (Stutte 1984; Zieger and Scheurlen 1980), these cases may apparently be associated with lymphoplasmacytic infiltrates of the bone marrow simulating malignant lymphoma.

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