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Morphological and immunohistochemical studies of Hodgkin's disease in the spleen

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Summary. Hodgkin's disease in the spleen – with the exception of its B cell variant – behaves quite differently from non Hodgkin's lymphomas with respect to both its spread and microenvironment. Each type of HD appears to create its own microenvironment by the secretion of cytokines responsible for the characteristic cellular composition of the infiltrates and thereby alters the normal immunoarchitecture of the spleen profoundly. While some histological findings seem to imply the presence of a host response against HD especially in the nodular sclerosis subtype, morphological and immunohistochemical evidence in the spleen cannot conclusively substantiate this hypothesis.

Key words: Hodgkin's disease – Immunohistochemistry – Microenvironment – Spleen

Introduction

While the spread of Hodgkin's disease (HD) to the spleen is dependent on the histological subtype and the duration of the disease, foci of HD are encountered in up to 42% of spleens removed at exploratory laparotomy (Askergren et al. 1981; Kadin et al. 1971; Kaplan 1980). Splenic HD exhibits a peculiar growth pattern; unlike most of the non-Hodgkin's lymphomas it produces larger nodules by the coalescence of smaller foci (Burke 1981; Falk et al. 1987; Farrer-Brown et al. 1972; Halie et al. 1978; Yam and Li 1976). The present study is intended to examine the relationship between the neoplastic cells of HD, the cellular composition of the microenvironment and the surrounding splenic tissue, and the structural changes in the spleens affected by HD, using histological and immunohistochemical methods.

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Material and methods

The spleens of 27 patients with untreated Hodgkin's disease of all major subtypes and six age and sex-matched controls from otherwise healthy patients with traumatic rupture of the spleen were studied. The diagnosis of HD and its subtype according to the Rye classification (Lukes et al. 1966) had already been established by a lymph node biopsy. Three to five blocks from involved and noninvolved areas of the spleen were fixed in 10% buffered formalin and processed for histological examination with haematoxylin-eosin, Giemsa, PAS, Masson-Goldner, and silver stains as well as for immunohistochemical studies with a panel of monoclonal antibodies (Table 1). Antibody binding was visualized by a modified immunoalkaline phosphatase method (Cordell et al. 1984). PALS, splenic follicles, and MZ could be discerned morphologically and by the

Table 1. Panel of antibodies used in the present study

Reagent	Reactive cells	Source
UCHL-1	T cells	Dako
MT-1	T cells	Biotest
MB-2	B cells	
L26	B cells	Dako
Actin	Smooth muscle cells, myofibroblasts	Enzo
Alpha-1 antichymotrypsin (ACT)	Histiocytes, epithelial cells, endothelial cells	Dako
Lysozyme	Monocytes, neutrophils	
S-100 protein	Interdigitating reticulum cells, Langerhans cells	Camon
BerMacDRC	Dendritic reticulum cells	Prof. H. Stein, Berlin
Vimentin	Mesenchymal cells, endothelial cells	Dako
Factor VIII	endothelial cells	

immunohistochemical identification of the cells populating these regions of the splenic WP. The network of dendritic reticulum cells within the follicles and the MZ was detected by the monoclonal antibody BerMacDRC (a kind gift from Prof. H. Stein Berlin), while interdigitating reticulum cells were visualized by an antibody against S-100 protein.

According to the number of T or B lymphocytes within the affected WP, i.e. within foci of HD, three different cellular patterns ($T \gg B$, $T = B$ and $T \ll B$) could be distinguished. The number of lesions exhibiting each of these characteristics in a given splenic section was determined. The number of alpha-1 antichymotrypsin (ACT)+and/or lysozyme+epithelioid cells (including epithelioid granulomas), S-100+cells, and neutrophils (especially eosinophils), as well as plasma cells within foci of HD, noninvolved WP and the surrounding red pulp were recorded semiquantitatively and compared. Previous studies had shown that these semiquantitative scores were closely correlated with the results obtained by counting the different cells.

The distribution pattern of the Sternberg-Reed cells (SRC) indicating infiltration by HD in three splenic compartments (PALS, MZ and throughout the WP) was assessed in each case. Vascular proliferation with or without hyalinized vessel walls, reticulin fibers, stromal fibrosis and collagen deposition with or without hyalinisation were also studied. Antibodies against actin, vimentin, and factor VIII as well as HE and PAS strains were used for the detection of the vessels.

Results

The 27 spleens from 15 male and 12 female patients (age: 5 to 54 with a mean of 33 years) weighed between 80 and 550 g (Mean: 275 g). Several cases of each subtype were present: lymphocytic predominance, nodular (LP; nodular paragranuloma): three, nodular sclerosis (NS): nine, mixed cellularity (MC); 12, and lymphocytic depletion (LD); three cases.

Almost all cases showed an infiltration of the PALS, the MZ, or a combination pattern (PALS and MZ). Early splenic lesions of HD with few SRC occuring within the PALS and/or the MZ as well as advanced lesions comprised of splenic white pulp containing many SRC in all WP regions and/or expanding nodular lesions transcending the erstwhile borders of the WP were also present. In general, there was no appreciable difference between the different subtypes of HD with respect to the growth pattern, but in five of the NS and MC cases massive nodular lesions with diameters ranging from 2 to 5 cm were observed; these represented numerous coalescing foci separated by strands of fibrosis. In LD cases diffuse invasive infiltrates of HD were present in addition to nodular lesions.

In five cases (three MC and two NS) enlarged germinal centers were surrounded by PALS and MZ infiltrates, but did not contain neoplastic cells. Two MC and LD cases each showed a few scattered SRC within the sinus of the RP outside of HD foci.

The cellular composition of the HD foci and the surrounding RP are shown in Table 2. In early lesions, there were many T cells accompanying the SRC in the PALS and MZ. In more advanced lesions SRC surrounded by T cells were found in central parts of the WP, but still near the marginal zone in almost all cases. Only a few cases exhibited SRC without associated T cells in the B cell areas of the WP. All three cases of nodular paragranuloma showed a diffuse infiltration by B cells with a few admixed epithelioid cells, granulocytes, and plasma cells in advanced lesions.

In contrast, HD foci in almost all cases of MC, NS, and LD contained large numbers of T cells and only focally preserved or enlarged B cell areas ($T \gg B$). In six spleens from MC and in seven NS cases nodular aggegates of small B lymphocytes could be observed within the HD lesions. Two NS cases exhibited medium-sized nodular lesions with a predominance of B cells ($T \ll B$).

In the NS and the MC subtypes, in which T cells occurred chiefly in MZ infiltrates, a balanced cellular composition ($T = B$) was frequently observed. The number of epithelioid cells and of granulocytes within the B cell areas was low.

S-100+cells, almost evenly divided between cells with indented nuclei and slender cytoplasmic projections (interdigitating reticulum cells) and small round cells with central nuclei (macrophages) were scattered mainly at the margins of the lesions. The S-100+cells were intimately associated with T cells and numerous SRC (Fig. 1). In LP, NS, and MC cases they showed a four-fold increase when compared with either nonaffected WP or with WP in spleens of age- and sex-matched controls ($p < 0.05$, t-test), while in LD cases the number of S-100+cells was not appreciably increased ($p = \text{n.s}$).

In early HD foci, the network of BerMacDRC+DRC in the MZ was partially preserved and was disrupted only in the vicinity of neoplastic cells (Fig. 2), while in advanced lesions DRC were obliterated by the neoplastic infiltrates.

ACT+/Lysozyme+mononuclear cells were also quite numerous in the HD foci. Highest numbers were reached in the MC cases, but NS cases also contained many of these cells. The ACT/Lysozyme+cells mainly occurred within the vascular and fibrous proliferations (Fig. 3; vide infra). Some ACT/lysozyme+fibroblasts were found in the fibrosing areas. The number of ACT+histiocytes in the vicinity of HD foci was normal or slightly decreased in comparison with RP areas dis-

Table 2. Cellular elements in the lesion and the surrounding red pulp

Case No	Lymphocytes			Histiocytes		S100+ cell	DRC	Neutrophils
	T≫B	T=B	T≪B	ACT+	Lyso+			
Lymphocyte Predominance								
1	0	0	6(+)	−(+)	−(+)	++(+)	+F	−(++)
2	0	0	5(+)	nt	+(++)	+(+)	+P	−(+)
3	0	3	2(+)	−(+)	−(++)	+(+)	+F	−(++)
Mixed Cellularity								
4	24	3	1(+)	−(+)	+(+)	+(+)	+F	+(+++)
5	8	1	0(+)	++(++)	nt	+(+)	−	+(++)
6	6	2	0(++)	++(+)	++(+)	+(+)	+F	−(+)
7	10	1	0(+)	+(+)	nt	+(+)	+F	+(++)
8	6	3	1(++)	+(++)	nt	+(+)	−	+(+++)
9	6	0	0(+)	++(+)	++(+)	+(+)	−	+(+++)
10	7	2	0(++)	+(++)	++(++)	+(++)	+P	+(+)
11	7	1	0(++)	++(+)	++(++)	+(+)	−	+(+++)
12	8	2	2(+)	+(+)	+(+)	++(+)	−	−(+)
13	12	2	0(+)	++(+)	++(++)	++(++)	−	−(++)
14	20	0	0(+)	++(+)	++(++)	++(++)	+F	++(++)
15	8	1	1(++)	++(++)	++(++)	+(+)	−	++(+++)
Nodular Sclerosis								
16	17	5	3(+)	+(+)	−(+)	++(+)	+F	+(+++)
17	6	2	0(+)	++(+)	++(+)	++(+)	+P	−(+)
18	5	0	0(+)	++(+)	nt	+(+)	−	++(+++)
19	9	4	1(+)	+(+)	+(++)	+(+)	+F	+(+++)
20	7	3	2(+)	++(+)	++(++)	++(++)	−	+(++)
21	1	4	6(+)	+(+)	+(++)	++(++)	+F	+(+++)
22	7	1	0(+)	++(+)	++(++)	+(+)	−	+(++)
23	2	5	16(+)	nt	+(+)	++(++)	+F	+(+++)
24	7	3	0(++)	+(++)	+(+)	+(+)	−	++(+++)
Lymphocyte Depletion								
25	11	0	0(++)	++(+)	++(++)	+(+)	−	−(+)
26	4	0	0(++)	++(+)	++(+)	+(+)	−	−(+)
27	5	0	0(++)	++(+)	nt	++(+)	−	−(+)

The first three columns denote the number of HD foci in a spleen section showing a predominance of T or B cells or their occurrence in equal numbers. ( ): reaction in the surrounding red pulp, F: focal, P: patchy, nt: not tested

tant from the lesion. Lysozyme+cells were normal or slightly increased in the RP.

One NS, five MC, and all LD cases were found to harbour slightly increased number of lymphocytes (mainly T cells) in the sinuses. Three MC and LD cases each showed small clusters of T cells within the RP that were partly situated in the neighbourhood of sheathed capillaries.

Granulocytes (mainly eosinophils) were located predominantly at the margin in MC and NS cases. In the latter, they were invariably associated with a band-like fibrosis. All cases showed aggregates of granulocytes around the perifollicular areas in the RP; their density here exceeded their numbers in the involved WP. The number of neutrophils and plasma cells in both the lesion and the RP were lower in LP and LD than in NS and MC cases.

The structural changes associated with splenic foci of HD are shown in Table 3. In most cases the most conspicuous feature was a proliferation of capillaries and an associated fibrosis (Fig. 4). In noninvolved areas and in spleens from controls several capillaries were observable in the outer MZ, whose number was slightly increased in the presence of enlarged germinal centers. All WP foci of HD exhibited capillary proliferations in the MZ. LP cases showed a mild increase of capillary density at the outer MZ that resembled the situation around reactive germinal centers. About half of the MC and all of the NS cases contained moderate (MC) to marked (NS) capillary proliferations in the center of advanced lesions as well as at the margins. A moderate increase of capillaries was seen throughout the LD lesions. High endothelial venules could not be detected in any case.

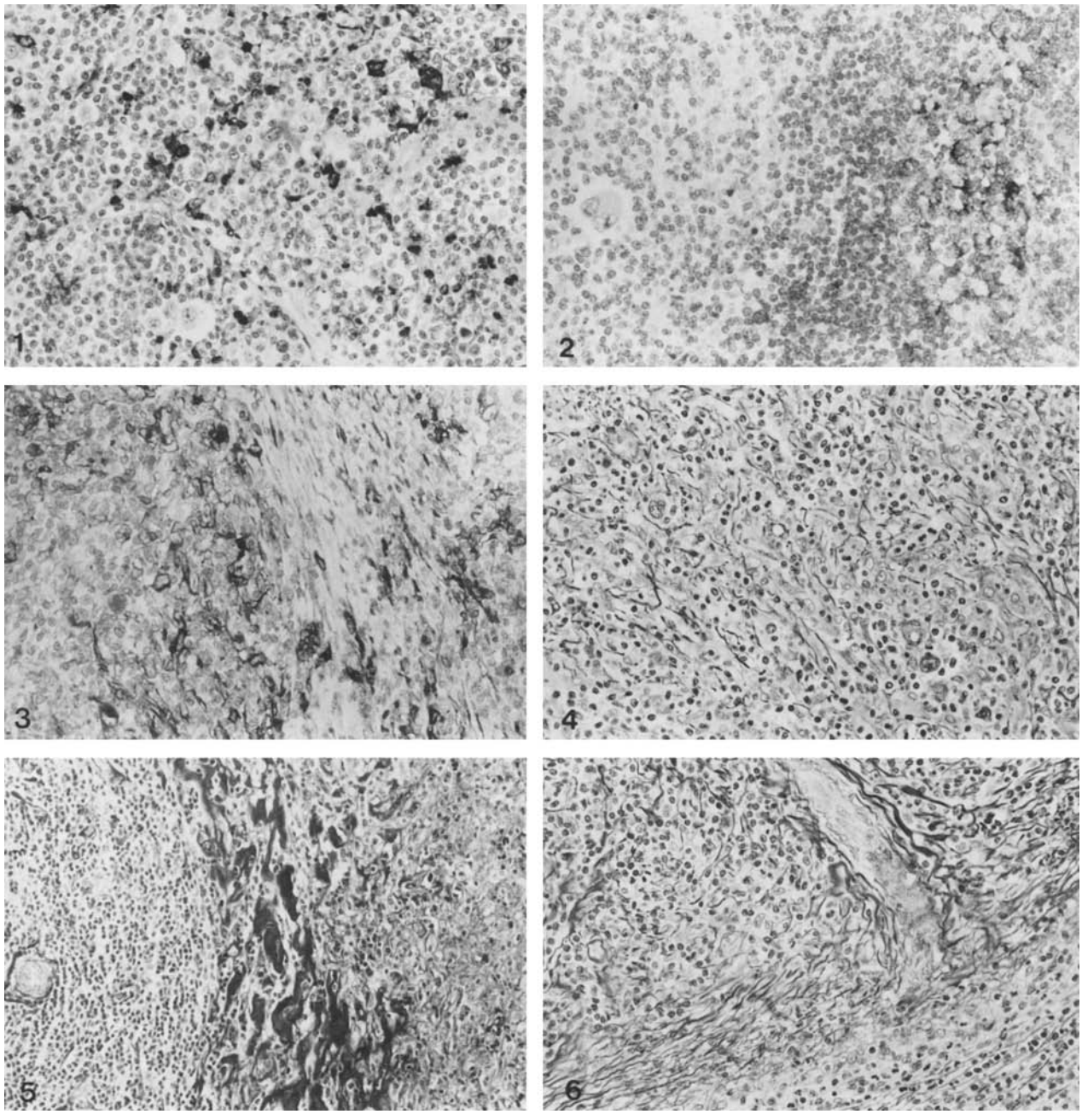

Fig. 1. Marked increase of protein S-100-positive cells in a splenic focus of mixed cellularity Hodgkin's disease. Note close vicinity of Sternberg-Reed cells and interdigitating reticulum cells. APAAP, × 280

Fig. 2. Intact network of dendritic reticulum cells (*right*) in a splenic follicle affected by a marginal zone infiltrate of Hodgkin's disease (*left*). BerMacDRC, APAAP, × 280

Fig. 3. Occurrence of numerous α-1-antichymotrypsin-positive cells within fibrotic areas. Nodular sclerosis, APAAP, × 280

Fig. 4. Mixed cellularity Hodgkin's disease in the spleen with increased reticulin fibers in the center. Note proliferation of small capillaries. Gomori, × 280

Fig. 5. Marked peripheral hyalinisations (*center*) and perifocal sclerosis (*right*) in a splenic focus of Hodgkin's disease, nodular sclerosis subtype. PAS, × 225

Fig. 6. Nodular sclerosis case showing marked periarteriolar fibrosis intimately associated with marginal sclerosis. Gomori, × 280

Table 3. Structural elements in splenic HD lesions and their vicinity

Case No	Cap C	Pro M	Hyalinisation	Ret F		Fibrosis		PCAF		Collagen changes
				C	M	C	M	C	M	
Lymphocyte Predominance										
1	+	+	C (−)	−	+	−	−	−	−	−
2	+	+	C (−)	−	+	−	−	−	−	−
3	−	+	− (+)	−	+	−	−	−	−	−
Mixed Cellularity										
4	+	+	CM (−)	−	+	−	+	−	−	+
5	+	+	− (−)	+	+	−	−	−	−	−
6	−	+	− (−)	−	+	−	−	−	−	−
7	++	+	C (+)	++	++	−	+	−	−	−
8	+	+	C (−)	++	++	−	−	−	−	−
9	++	++	− (−)	++	++	+F	−	−	−	−
10	++	+	CM (+)	++	++	++P	+F	+	−	+
11	++	++	− (−)	++	++	+	+F	+	−	+
12	++	+	CM . (−)	++	+	++P	+F	+	−	−
13	++	+	CM . (−)	++	+	++P	+	++	−	+
14	++	++	C (−)	++	++	+F	+	+	+	+
15	++	++	− (+)	++	++	++P	++P	−	+	+
Nodular Sclerosis										
16	+	++	CM . (−)	+	++	−	++	−	+	+
17	++	++	CM . (−)	++	++	+	++	++	++	++
18	+	++	− (+)	++P	++	+F	++	+	++	++
19	+	++	CM . (+)	+	++	−	++	+	++	+
20	+	++	CM (−)	+	++	+F	++	+	++	++
21	+	++	CM . (+)	+	+++	+F	+++	+	++	++
22	+	++	− (−)	+	+++	−	+++	−	++	++
23	+	++	CM . (+)	+F	+++	+F	+++	+	++	++
24	++	++	CM . (−)	+F	+++	+	+++	+	++	++
Lymphocyte Depletion										
25	++	++	− (+)	++	++	+	++	−	++	+
26	++	++	− (−)	++	++	++	++	++	−	+
27	++	++	C (+)	++	++	+	+	−	−	+

Cap Pro: capillary proliferation, Ret F: reticulin fibers, PCAF: pericentral arterial fibrosis, C: central, M: marginal, F: focal, P: patchy, . : Hyalinized vessels are closely connected with marginal fibrosis

In addition to the pronounced vascularisation, 17 cases (LP: two, NS: seven, MC: seven, LD: one) contained hyalinized branching capillaries in the lesion, which in the LP and MC cases were chiefly located in the center of the lesion. In the vicinity of the hyalinized capillaries an increase in reticulum fibers, fibrosis and a mixed cellular infiltrate were observed in all cases except for the LP subtype. In eight cases (six NS and two MC cases) proliferation and hyalinisation of capillaries was intimately associated with marginal fibrosis (Fig. 5).

An increase of reticulin fibers accompanied by capillary proliferation was found in the outer MZ in the early lesions of each subtype. While the LP cases caused a loose network of reticulin fibers to appear in the perifollicular area, 60% of the MC showed a moderate increase of reticulin fibers in the center of the lesion as well as at its margin. In the MC cases the amount of reticulin fibers depended on the extent of the lesion and the degree of capillary proliferation. All cases of NS showed moderate to marked band-like fibrosis at the lesions' margins, six cases also contained focal fibrosis in the center and/or at the margin. In addition, typical pericentral arterial fibrosis associated with patchy hyalinization was observed. There was no correlation with the number of SRC. In all NS cases the pericentral arterial fibrosis extended to the outer margins of the lesions (Fig. 6) and sometimes to their center, while only two cases showed peritrabecular fibrosis within the lesion. In five cases nodular lesions without accompanying marginal fibrosis were noted. With the exception of LP cases, fibrotic areas always contained large numbers of eosinophils.

Discussion

With the exception of nodular paragranuloma which behaves differently from other subtypes because of its B cell nature (Hansmann et al. 1986; Pinkus and Said 1988), SRC in HD foci in the spleen are accompanied by T lymphocytes (Poppema et al. 1982). Their presence in infiltrated MZ changes the ratio of T and B lymphocytes in this particular splenic compartment, while in involved PALS the number of T cells is substantially increased. This expansion of the T cell compartment accounts for the increased number of T cells found in involved spleens from patients with HD (Baroni et al. 1982; Pinkus et al. 1978; Poppema et al. 1982; Posner et al. 1981).

Concomitantly, the network of DRCs within the MZ is destroyed, and in more advanced lesions SRC and associated T cells populate splenic follicles that contain no residue or only frayed remnants of the DRC. In several cases small nodular aggregates of B cells without associated DRC are visible (Forni et al. 1985). At the same time the number of S-100+IDRC and macrophages that normally populate T cell-associated regions (Müller-Hermelink et al. 1974) increases markedly and in more advanced lesions the normal B cell micro-environment of the splenic follicle and the MZ is replaced by a cellular infiltrate typical of T cell areas. This fact is underscored by the close spatial relationship between the IDRCs' cytoplasmic projections, T cells, and SRC (Sangster et al. 1985; Van Parys et al. 1985) which is reminiscent of the antigen-presenting function of IDRC in normal lymphoid tissue. These changes resemble the findings in lymph nodes with HD that also show increased numbers of S-100+cells (Carbone et al. 1987). However, we could not confirm that S100+ cells are most numerous in NS cases (Sangster et al. 1985).

Macrophage-histiocytes (M-H) that are reactive with antibodies against ACT, and/or lysozyme also are a constant finding in splenic foci of HD. In contrast to other investigations employing lectin-labelling (Ree and Kadin 1985), in our study the highest numbers of M-H are present in MC cases and are intimately associated with vascular proliferation and fibrosis. This especially applies to the NS cases, but also is a constant finding in other HD subtypes, since fibrosis in HD is probably preceeded by increased vascularisation (Crocker et al. 1988; Möller and Lennert 1984). In all our cases a moderate to marked angioproliferation is present, and its extent is dependent on the HD subtype: NS cases show the highest vascular densitiy. In HD foci a direct spatial relationship between central or marginal fibrosis and vascularisation may often be demonstrated. The frequent presence of a fibrinous exsudate in foci of HD that occurs in the vicinity of blood vessels and is associated with fibrosis (Harris et al. 1982) may constitute evidence of the role of vessel proliferation and plasma exsudation in sclerosis.

The findings in our study may be translated into some functional implications for the spread and the biological behaviour of HD in the spleen. First of all, upon spread to the spleen HD unlike most of the non Hodgkin's lymphomas does not show a predilection for either B or T cell areas of the organ. Rather, the cells admixed to the neoplastic elements, i.e. T cells, M-H and IDRC, tend to replace/augment the original microenvironment of the B/T areas, effectively eliminating organized B cell regions. Thus, the immunoarchitecture of the splenic WP is profoundly altered by HD infiltrates.

The accumulation of M-H associated with angioproliferation and fibrosis bears morphological resemblance to granulation tissue. Since HD with the exception of LD cases does not tend to leave the WP and openly invade the RP as most non Hodgkin's lymphoma do, and since in lymph node biopsies of NS cases partially intact so-called composite nodules (Van den Oord et al. 1986) were detected, this tissue reaction has been interpreted as a host response against the tumour (Seemayer et al. 1980; Van Parys et al. 1987). This phenomenon could be held responsible for the containment of HD within the WP and the lack of frank RP infiltration. In addition, it has been implied that the presence and degranulation of eosinophils may also reflect an immunological response against neoplastic infiltrates (Samoszuk et al. 1986).

However, several facts are at variance with this hypothesis. First of all, only very few MC cases show obvious RP invasion despite the absence of a band-like fibrosis. Secondly, the prognostic significance of the Rye classification, especially with respect to the NS subtype, has come under close scrutiny since variants of this type with a very unfavourable prognosis have been identified (Bennett et al. 1985). Moreover, increased numbers of M-H in HD are related to progressive disease (Colby et al. 1981; Coppleson et al. 1973) and to the presence of B symptoms (Ree and Crowley 1983; Ree and Kadin 1985; Ree 1987). Eosinophils are abundant in most MC cases whose prognosis is usually worse than in the NS subtype (Kaplan 1980). Since angioproliferation and fibrosis may be identified in splenic foci of all subtypes and since limited

RP invasion could also be detected in NS cases, it appears questionable whether the peculiar fibrotic bands in NS indeed represent a host response against the tumour. In contrast to T cell infiltrates in follicular non Hodgkin's lymphoma (Strickler et al. 1988), T cells and IDRC in HD are not zonally arranged; rather they are intimately associated with neoplastic SRC and are most numerous in the MC subtype. Thus they obviously do not possess a prognostic significance. Rather, the spatial relationship of non-neoplastic M-H, granulocytes, and lymphocytes with SRC appears to support the hypothesis that the powerful cytokines secreted by the neoplastic cells in HD are responsible for the characteristic cellular background of the lesions (Schell-Frederick et al. 1988; Stein et al. 1988). This would also be in keeping with the distinct behaviour of the nodular LP subtype of HD as well as with the cellular composition, of some peripheral T cell lymphomas, including SRC-like elements, which show remarkable similarities in HD. Angiogenesis and fibrosis could also be induced by similar factors secreted by RSC (Newcom and O'Rourke 1982) or by M-H (Nathan 1987). Thus, the absence of spread to the splenic red pulp may not be due to a host reaction but rather to the lack of the peculiar T-cell associated microenvironment of Hodgkin's disease. This appears to be induced by cytokines that presumably are most effective in the preformed T cell areas of the spleen and their immediate vicinity.

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References

Askergren J, Björkholm M, Holm G, Johansson B, Sundblad R (1981) On the size and tumor involvement of the spleen in Hodgkin's disease. Acta Med Scand 209:217–220

Baroni CD, Ruco L, Uccini S, Foschi A, Occhionero M, Marcorelli E (1982) Tissue T lymphocytes in untreated Hodgkin's disease. Morphologic and functional correlations in spleens and lymph nodes. Cancer 50:259–268

Bennett MH, MacLennan RA, Easterling MJ, Vaughan Hudson B, Vaughan Hudson G, Jeliffe AM (1985) Analysis of histological subtypes in Hodgkin's disease in relation to prognosis and survival. In: Quaglino D, Hayhoe FGJ (eds) The cytology of leukaemias and lymphomas. Raven Press, New York, pp 15–32

Burke JS (1981) Surgical pathology of the spleen: An approach to the differential diagnosis of splenic lymphomas and leukemias Part I. Disease of the white pulp. Am J Surg Pathol 5:551–563

Carbone A, Manconi R, Poletti A, Sulfaro S, Menin A, Tirelli U, Betta PG, Volpe R (1987) Reed-Sternberg cells and their cell microenvironment in Hodgkin's disease with reference to macrophage-histiocytes and interdigitating reticulum cells. Cancer 60:2662–2668

Colby TV, Hoppe RT, Warnke RA (1981) Hodgkin's disease. A clinicopathologic study of 659 cases. Cancer 49:1848–1858

Coppleson LW, Rappaport H, Strum SB, Rose J (1973) Analysis of the Rye classification of Hodgkin's disease: The prognostic significance of cellular composition. J Natl Cancer Inst 51:379–390

Cordell JL, Falini B, Erber WN, Gosh AK, Abdulaziz Z, MacDonald S, Pulford KAF, Stein H, Mason DY (1984) Immunoenzymatic labelling of monoclonal antibodies using immune complexes of alkaline phosphatase and monoclonal anti-alkaline phosphatase (APAAP complexes). J Histochem Cytochem 32:219–229

Crocker J, Overton SP, Smith PJ (1988) Type IV collagen in Hodgkin's disease. Am J Clin Pathol 89:57–62

Falk S, Müller H, Stutte HJ (1987) Hodgkin's disease in the spleen. A morphological study of 140 biopsy cases. Virchows Arch [A] 411:359–364

Farrer-Brown G, Bennett MH, Harrison CV, Millet Y, Jeliffe AM (1972) The diagnosis of Hodgkin's disease in surgically excised spleens. J Clin Pathol 25:294–300

Forni M, Hofman FM, Parker JW, Lukes RJ, Taylor CR (1985) B- and T-lymphocytes in Hodgkin's disease: an immunohistochemical study utilizing heterologous and monoclonal antibodies. Cancer 55:728–737

Halie MR, Thiadens J, Eibergen R, Broek AA van den (1978) Hodgkin's disease in the spleen. Virchows Arch [B] 27:39–48

Hansmann ML, Wacker HH, Radzun HJ (1986) Paragranuloma is a variant of Hodgkin's disease with predominance of B cells. Virchows Arch [A] 409:171–181

Harris NL, Dvorak AM, Smith J, Dvorak HF (1982) Fibrin depositis in Hodgkin's disease. Am J Pathol 108:119–129

Kadin ME, Glatstein E, Dorfman RF (1971) Clinicopathological studies of 117 untreated patients subjected to laparotomy for the staging of Hodgkin's disease. Cancer 27:1277–1294

Kaplan H (1980) Hodgkin's disease. Cambridge, Harvard University Press

Lukes RJ, Craver L, Hall T (1966) Report on nomenclature committee. Cancer Res 26:1311

Möller P, Lennert K (1984) On the angiostructure of lymph nodes in Hodgkin's disease. An immunohistochemical study using the lectin I of Ulex europaeus as endothelial marker. Virchows Arch [A] 403:257–270

Müller-Hermelink HK, Heusermann U, Stutte HJ (1974) Enzyme histochemical observations on the localization and structure of T cell and B cell regions in the human spleen. Cell Tissue Res 154:167–179

Nathan CF (1987) Secretory products of macrophages. J Clin Invest 79:319–326

Newcom SR, O'Rourke L (1982) Potentiation of fibroblast growth by nodular sclerosing Hodgkin's disease cells cultures. Blood 60:228–237

Pinkus GS, Said JW (1988) Hodgkin's disease, lymphocytic predominance type nodular – further evidence for a B cell derivation. Am J Pathol 133:211–217

Poppema S, Bhan AK, Reinherz EL, Posner MR, Schlossman SF (1982) In situ immunologic characterization of cellular constituents in lymph nodes and spleens involved by Hodgkin's disease. Blood 59:226–232

Posner MR, Reinherz EL, Breard J, Nadler LM, Rosenthal DS, Schlossman SF (1981) Lymphoid subpopulations of peripheral blood and spleen in untreated Hodgkin's disease. Cancer 48:1170–1176

Ree HJ, Crowley JP (1983) Concanavalin A binding histiocytes in Hodgkin's disease and their relation to clinicopathologic features of the disease. Cancer 52:252–257

Ree HJ, Kadin ME (1985) Macrophage-histiocytes in Hodgkin's disease. The relation of peanut agglutinin binding macrophage histiocytes to clinicopathologic presentation and course of disease. Cancer 56:333–338

Ree HJ (1987) Stromal macrophage-histiocytes in Hodgkin's disease. Their relation to fever. Cancer 60:1479–1484

Samoszuk MK, Nathwani BK, Lukes RJ (1986) Extensive deposition of eosinophil peroxidase in Hodgkin's and non Hodgkin's lymphomas. Am J Pathol 125:426–429

Sangster G, Crocker J, Jenkins R, Leyland MJ (1985) Cells which contain S-100 protein in Hodgkin's disease. J Clin Pathol 38:1021–1024

Schell-Frederick E, Radtke H, Sommer H, Helbing I, Burrichter H, Schaad M, Diehl V (1988) Inhibition of human neutrophil migration by supernatants from Hodgkin's disease-derived cell lines. Eur J Clin Invest 18:290–296

Seemayer TA, Lagace R, Schürch W (1980) On the pathogenesis of sclerosis and nodularity in nodular sclerosing Hodgkin's disease. Virchows Arch [A] 385:283–291

Stein H, Dallenbach F, Dienemann D (1988) Differenzierungslinien physiologischer und maligner Zellen des lymphatischen Systems. Verh Dtsch Ges Pathol 72:57–85

Strickler JG, Copenhaver CM, Rojas VA, Horning SJ, Warnke RA (1988) Comparison of "host cell infiltrates" in patients with follicular lymphoma with and without spontaneous regression. Am J Clin Pathol 90:257–261

Van Parys G, Van den Ooord JJ, De Wolf-Peeters C, De Vos R, Desmet VJ (1985) Reed-Sternberg cells, lymphocytes and interdigitating reticulum cell rosettes in Hodgkin's disease. J Clin Pathol 38:1316

Van Parys G, De Wolf-Peeters C, Van den Ooord JJ, Desmet VJ (1987) Lymph node architecture in Hodgkin's disease: evidence for the role of the composite nodule in nodular sclerosing Hodgkin's disease. Hematol Oncol 5:147–154

Van den Ooord JJ, De Wolf-Peeters C, Desmet VJ (1986) The composite nodule. A structural and functional unit of the reactive human lymph node. Am J Pathol 122:83–91

Yam LT, Li CY (1976) Histogenesis of splenic lesions in Hodgkin's disease. Am J Clin Pathol 66:976–985

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