MORPHOLOGY OF THE SPLEEN WHITE PULP AS AN INDICATOR OF THE IMMUNOLOGICAL STATE IN PATIENTS WITH WIDESPREAD MALIGNANT MELANOMA OF THE SKIN

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Summary

The white pulp of the spleens collected from twelve patients who died with widespread malignant melanoma of the skin, and the same number of spleens from an age and sex-matched group of patients who died with myocardial infarction were histologically assessed by using a standardized reporting system with special emphasis placed upon the cell populations involved in immunological reactions.

Histological characteristics suggesting an active function of both the cell-mediated and humoral immune reactions were found to be within normal limits in the control series, whereas in the melanoma series both these elements were profoundly deranged. The significance of the histological observations made was discussed in the light of the previously demonstrated immunological reactions against human malignant melanoma cells, and a conclusion was drawn that an impairment of both the humoral and cell-mediated immune responses must exist in patients dying in widespread malignant melanoma of the skin.

Both the cell-mediated immunity[1,2,3,4] and circulating antibodies[5,6] have been demonstrated against the cells of human cutaneous malignant melanoma. A definite correlation was established between the cell-mediated immunological reactivity and the clinical course of the tumor[4] as determined by the tests for delayed cutaneous hypersensitivity.

It seems to be firmly established that two distinct populations of lymphocytes (T- and B-cells) are encountered in human lymphatic system, including the spleen white pulp[7,8,9,10,11,12]. Both these lymphocyte populations have a homing site of their own in the spleen, i.e. T-cells are localized in the central peripheral lymphoid sheet (C-PALS), and B-cells are found in the peripheral periarterial lymphoid sheet (P-PALS)[7,8,9,10,11,12,13]. Recently, a standardized system of reporting human lymph node morphology in relation to the functions of the T- and B-cells involved in the immunological reactions was introduced[14], and the applicability of this system has been established by assessing the morphology of the lymph nodes draining various human malignancies[15,16,17]. To render the assessment of the morphological alterations in the spleen white pulp unequivocal, a standardized system analogous to that described for the lymph nodes was instituted[11], and tested in experimental animals treated with immunosuppressive agents. Thus far, the system has not been tested in human spleen specimens.

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The aim of the present communication was to evaluate the immunological reactivity of the patients who died with far advanced cutaneous malignant melanoma by assessing the spleen white pulp morphology with the newly introduced standardized system[11].

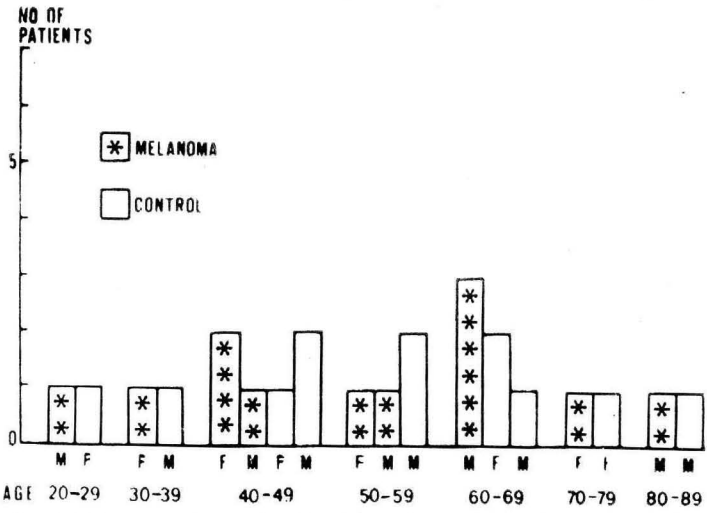

Fig. 1 The age and sex distribution of the patients of the two series

Material and Methods

The present series comprises spleen specimens obtained from 12 patients who died with widespread malignant melanoma of the skin during the years 1971–1979. The material for the study was collected from the files of the Department of Pathology, University Central Hospital of Kuopio, Kuopio, Finland. For the control material, an equally large number[12] of spleen specimens were collected from an age- and sex-matched group of patients who died with myocardial infarction without evidence of any type of malignancy.

The paraffin-embedded tissue blocks were sectioned to an average thickness of 4 microns, and processed for light microscopic study according to the routine procedures. The histologic sections were stained with hematoxylin eosin and methyl green-pyronine.

The morphology of the spleen white pulp was assessed by using the standardized reporting system previously introduced by the author[11]. In this system, the T- and B-cell areas, C-PALS and P-PALS, respectively, are separately analyzed with regard to their size and cellular contents as described[11,14]. In determining the final record of the change concerned (− − −, − −, −N+, ++, +++) ten white pulp units (C-PALS, P-PALS, Germinal centers) surrounding the central artery were evaluated randomly selected in the section, and the average degree of change was selected for the final record (Figs. 3–5).

Results

The age and sex distribution of the patients in the two series is shown in Fig. 1. There is a male preponderance of 7/5 in both series, as indicated in the figure.

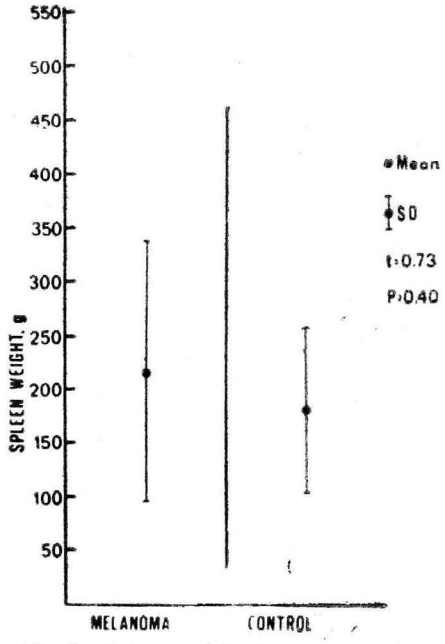

Fig 2 Spleen weights in the two series

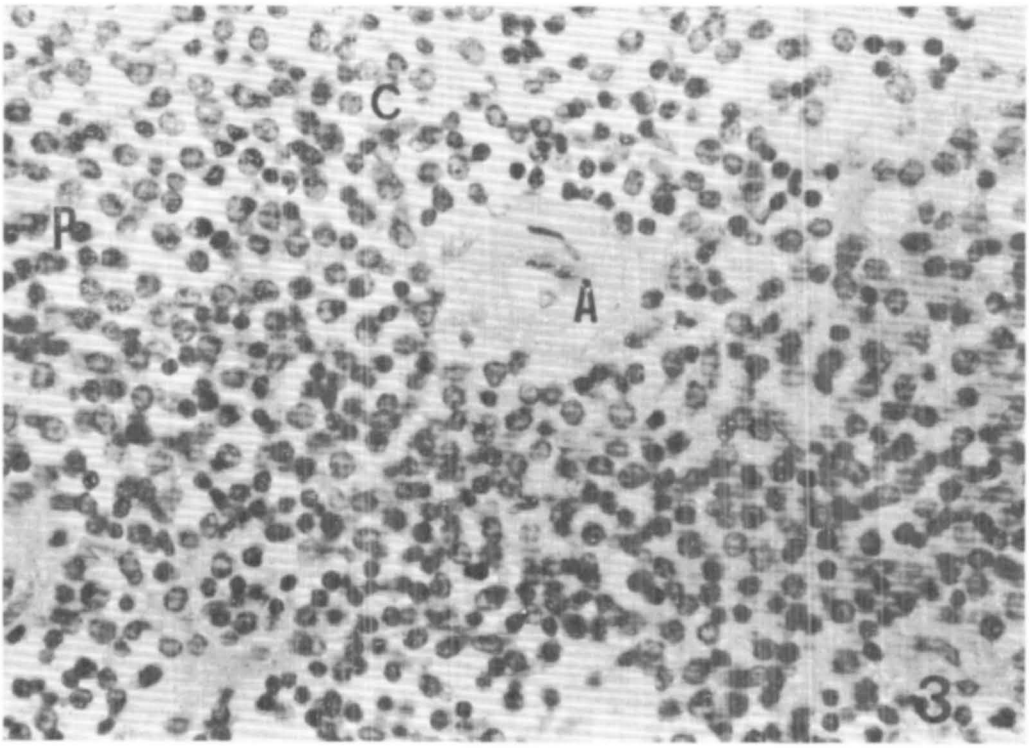

Fig. 3 A medium power view on a spleen white pulp of a patient in CO series. Around the central artery (A), central periarterial lymphoid sheet (C), and peripheral periarterial lymphoid sheet (P) are seen, with their size and cellular constituents within the normal range (— N +). In this field, no germinal center is found. (H and E, original magnification × 250)

The spleen weights of the patients with melanoma (ME) and control (CO) series are given in Fig. 2. The mean weight (214.5 g) of the spleens in ME series did not statistically significantly deviate from that (183.7) of the spleens in CO series.

Table 1 summarizes the histological findings in the spleen white pulp of ME and CO patients. The architecture was considered diffusely altered when the size of both C-PALS and P-PALS was deviated from the normal range (− N +), and focally altered when only either of these areas was changed in size. When these criteria were applied, diffuse alteration (to the direction below the normal range, − − or − − −) was more frequent in ME series. Indeed, diffuse alteration was not encountered in CO series. Similarly, the frequency of the focally altered spleens was greater in ME series than in CO series.

The size of the C-PALS was reduced significantly more often in ME series, as was the cellular content of this area (small, medium sized and large lymphocytes of the T-cell series). Similar, if not even more accentuated differences were observed between the two series regarding the histology of the P-PALS. A number of spleens with alterations into hyperplastic direction (+ +) was encountered in CO series, but not in ME series.

Germinal centers (GC) which could be classified active (+ +) or even as resting stage (− N +) were exceptional in ME series. The most frequent finding

ME series was a spleen with 0-2 germinal centers per 10 white pulp units evaluated (GC number– – –). The number was graded– –when 3-5 GC:s were found, –N+ when 6-8, and ++ when 9 or 10 GC:s were found. In majority of cases, GC:s were small in size, and contained scanty cells involved in antibody response, i.e. the cells of the B-lymphocyte series.

Discussion

In the present study, morphology of the spleen white pulp in patients who died due to far advanced malignant melanoma of the skin was recorded with special reference to the cell populations involved in immunological functions. Immunological reactivity of both humoral and cell-mediated type has been detected against the malignant melanoma cells in vivo and in vitro ([1],[2], [3],[4],[5],[6]). The recording system used in the present work was analogous to that previously introduced in the evaluation of lymph nodes (14), and identical to that applied in experimental animals (11) to assess their spleen white pulp following the treatment with T-cell suppressive regimen.

The age and sex distribution of the present material was comparable to that of more extensive series ([18]). As a control material, an age- and sex-matched group of patients without any signs of malignancy was used to exclude the possible influence of the patient's age and sex on the size and histologic appearance of the spleen, as recently

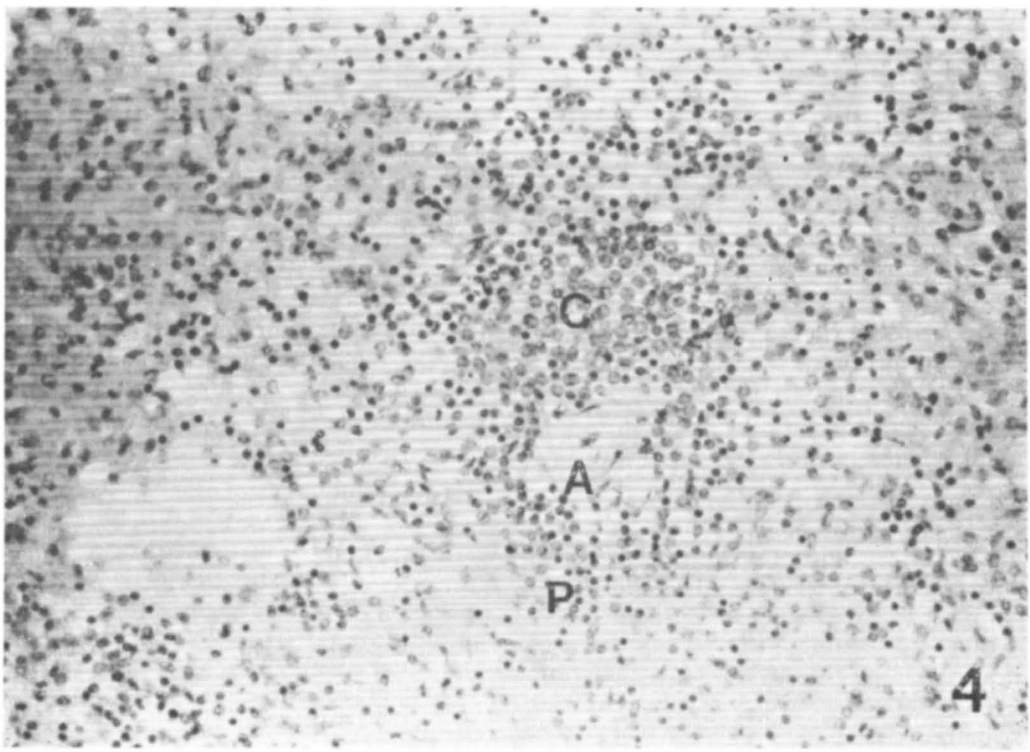

Fig. 4 A low power photomicrograph from the spleen of a patient in ME series. Central periarterial lymphoid sheet (C) adjacent to the central artery (A) seems to be populated mainly by the large lymphocytes, the small lymphocytes being profoundly depleted (– – –). The peripheral periarteial lymphoid sheet (P) is heavily depleted with its cellular populations, and no GC:s are found. (H and E, original magnification × 100)

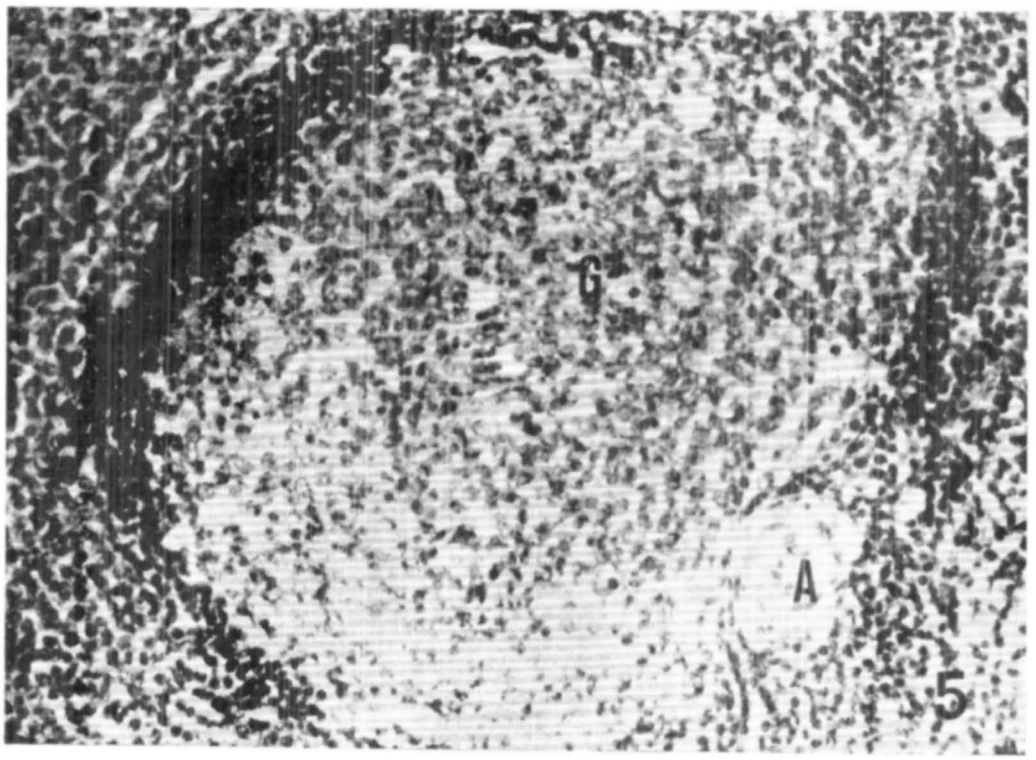

Fig. 5 Adjacent to a central artery (A) a conspicuous germinal center (G) filling almost totally the area of P-PALS is encountered. C-PALS seems to be composed of a narrow rim of small lymphocytes adjacent to the germinal center. Germinal centers as large as the one in this figure were exceptional also in CO series. The cellular constituents of this GC were graded within the normal limits (— N +). (H and E, Original magnification × 100)

discussed ([19],[20]). On the same basis, all the patients with signs of liver cirrhosis were excluded from the control series, because of the observed increase in the spleen size in these patients ([19]).

The mean weight of the spleens in the two series did not deviate significantly from each other (Fig. 2). The slightly higher mean weight in ME series was due to a couple of large spleens (481 and 438 g) both containing melanoma metastases. The other ten spleens, however, were fully comparable in size with those of CO series, and it can be stated that widespread melanoma is not in general associated with either smaller or larger spleen than that in patients with non-malignant diseases. This finding also excludes the possibility that the morphological changes suggesting impairment of the immunologically functioning cells discussed below, could be due to the decreased spleen size in ME series.

The architecture of the spleen white pulp was considered diffusely altered into hypoplastic (——) direction only in ME series. This is in accordance with the findings made in the lymph nodes draining bronchial carcinomas[16], and other human malignancies too[15],[17]. This indicates that the recording systems applied in the evaluation of the morphology of these two lymphoid organs are mutually comparable, and also that various human malignancies seem to be associated with structural derangements in areas homed by the immunologically reacting T- and B-cells.

Table 1

Histology of the Spleen White Pulp in the Melanoma (ME) and Control (CO) Series

	———		——		—N+		++		+++	
	ME	CO	ME	CO	ME	CO	ME	CO	ME	CO
Architecture										
Altered diffusely			7 †	0	1 ‡	10				
Altered focally			4	2						
Central Periarterial Lymphoid Sheet										
Size	1	0	9 ‡	1	2 ‡	11				
Content of small lymphocytes	5 *	0	5	3	2 †	9				
Content of medium-sized lymphocytes	3	0	6 *	1	3 ‡	11				
Content of large lymphocytes	5 *	0	5	2	2 †	9	0	1		
Content of histiocytes	6 †	0	4	1	2 ‡	11				
Mitotic activity	8 ‡	0	4	3	0 ‡	9				
Peripheral Periarterial Lymphoid Sheet										
Size	1	0	7 †	1	4 †	11				
Content of small lymphocytes	4 *	0	7 *	2	1 ‡	10				
Content of large lymphocytes	2	0	8 †	1	2 ‡	11				
Content of histiocytes	3	0	9 ‡	0	0 ‡	12				
Content of plasma cells	5 *	0	7 †	1	0 ‡	9	0	2		
Mitotic activity	5 *	0	7 †	1	0 ‡	11				
Germinal Centers										
Number	11 ‡	3	0	2	1	3	0 *	4		
Size	11 ‡	2	1	5	0 *	5				
Content of large lymphocytes	11 ‡	3	1 *	6	0	3				
Mitotic activity	11 ‡	3	1 *	6	0	3				
Number of macrophages	11 ‡	3	1 *	6	0	3				

Explanation of the symbols: N, normal; one, two or three plus or minus signs indicate slight, moderate or marked deviation above or below the normal range; p, statistical significance calculated with the chi-square test *=P 0.05; †=P 0.01; ‡=P 0.001

C-PALS is known to be the homing site for T-lymphocytes responsible for cell-mediated immunity[7,8,9,10,11,12,13]. Treatment of experimental animals with anti-T-cell serum or thoracic duct drainage, produces a profound lymphopenia in C-PALS[11,21], as well as in the lymph node paracortex[22]. Activity of the lymphoid cell population (T-cells) in the lymph node paracortex has been shown to be correlated with the survival of the patients with breast, gastric[23,24] and bronchial carcinomas[25]. These findings are consonant with the recently described depletion of the paracortical lymphoid cells in the nodes draining bronchial carcinoma[16]. Cell-mediated immunological reactivity exerted by the small T-lymphocytes has been shown in patients with malignant melanoma[1,2,3]. Failure to develop delayed cutaneous hypersensitivity (a T-cell activity) in association with malignant melanoma was shown to be typical to the patients with poor prognosis[4]. These observations are substantiated by the results of the present work where C-PALS was reduced in size and contained a reduced number of T-lymphocytes and their precursors. The mitotic activity in this area, too, was very low. Not a single spleen was found in ME

series where these parametres would have been altered into hyperplastic direction. On the other hand, one spleen was encountered in CO series where C-PALS contained an increased number of large lymphocytes. In this case, the histologic picture was much like the one seen in lymph node paracortex following the development of contact sensitivity[26], or during the recovery of the spleen white pulp following the anti-T-cell serum treatment[11] or thoracic duct drainage[21]. The results of the present study indicate that an impairment of the cell-mediated immune reactions evaluable on the morphological grounds exists in majority of patients dying with widespread malignant melanoma of the skin. When compared with the spleen white pulp morphology in patients free from malignancy dying with myocardial infarction, the difference was highly significant in this respect. Similarly, morphological alterations suggesting an impairment of the immunologically reactive cells have been demonstrated in the spleens of the patients suffering from gastric carcinoma[27].

P-PALS, considered to be the homing site for B-lymphocytes[7,8,9,10,11,12] was observed to be reduced in size and depleted in its cellular contents more often in ME series than in CO series. B-lymphocytes, as precursors of the plasma cells are involved in antibody response. The morphologic details of the antibody response in the spleen are extensively studied[28,29,30,31]. Under optimal conditions, the spleen has been claimed to be the major site of antibody synthesis in human lymphatic system[32], and splenectomy has been shown to impair this antibody-forming capacity considerably[33]. In the present work, active germinal centers which could have been graded + + or + + + regarding their size and cellular constituents, were not found in either of the two series. A vast majority of spleen specimens in ME series was totally devoid of GC : s (number - - -), which, however, could be found within normal limits in CO series. The findings indicate that the cells responsible for antibody response are inconspicuously represented even in the spleens of the control, non-cancer patients, and almost absent in the spleens of the melanoma patients. There are, however, reports on the demonstration of circulating antibody against the human malignant melanoma cells[5,6]. These reports are not necessarily contradictory to the present observations, because the material used in these studies was obtained from the patients surgically treated for their disease, which was not yet generalized like that of the patients studied in the present work. No reports are available, thus far, on the morphology of the spleen white pulp in patients with only a localized malignant melanoma. Thus, it seems feasible to explain the observed lack of antibody-synthetizing elements in the spleen white pulp of the present ME series on the basis of the fact that the disease was a generalized one causing an immunological paralysis of the host. This was true for both the cell-mediated and humoral immunity, as discussed above. The present observations do not rule out the possibility that the patients of the present series could have excerted both the cell-mediated and humoral immunity against their tumors at some earlier stage of the disease, but this immunological response was not strong enough to eradicate the tumor, and a generalized, ultimately fatal disease was able to develop.

The standardized reporting system used[11] in the present work proved to be a suitable one to be applied in the assessment of the morphology of human spleen white pulp, too. By recording the changes in the tabular form, one easily gets an insight on the evident differences existing between the two materials studied. By assessing the

morphology of the spleen white pulp, it seems to be possible to gain valuable information about the cell populations responsible for the immunological reactivity of the patient in general, and this approach should be extended into the field of clinical cancer amenable to operative treatment.

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TRUE or FALSE?

Linoleic, linolenic and arachidonic acids are three fundamental and naturally occuring essential fatty acids which can also be synthesised in the body by higher animals from small molecular weight metabolities of CHO and proteins.

(Answer page No. 44)