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The ruptured spleen. A histological, morphometrical and immunohistochemical study

H. Barnard, E.J. Dreef and J.H.J.M. van Krieken
Department of Pathology, University Hospital Leiden, The Netherlands

Summary. A traumatically ruptured spleen is regarded as a proper control in many histological and immunological studies on the human spleen. This paper compares spleens that ruptured due to trauma and spleens which were removed during surgery in patients without splenic pathology. Based on a histological, morphometrical, and immunohistochemcial description of the control spleens it is shown that the traumatically ruptured spleens contain alterations in the lymphoid tissue. The amount of white pulp is increased due to a larger amount of CD4-positive lymphocytes. Furthermore there are alterations in lymphocyte populations in the different splenic compartments. It is concluded that spleens that rupture may be predisposed due to immunological stimulation.

Key words: Spleen, Rupture, Histology, Immunohistochemistry, Morphometry

Introduction

The spleen may rupture due to trauma or «spontaneously», i.e. without apparent trauma (Mitchell and Morris, 1983). Traumatically ruptured spleens are mostly regarded as normal. Although it is diffucult to define what an adequate trauma is, there are several disorders which may facilitate spontaneous splenic rupture; infectious mononucleosis is one of the most well known examples (Andrews et al., 1980; Rogers and Shah, 1980; Armitage et al., 1981; Berlin et al., 1982; Adouin and Diebold, 1983; Bennett et al., 1984; Caruso and Hall, 1984; Chamberlain et al., 1984; Cochrane, 1984; Doehrmann and Scheele, 1984; Cooperberg et al., 1985; Fallingborg et al., 1985; Mirchadani et al., 1985; Birenbaum et al., 1986; Majewski and Stahlknecht, 1986).

Offprint requests to: J.H.J.M. van Krieken, MD, PhD, Laboratory of Pathology, University of Leiden, PO Box 9603, 2300RC Leiden, The Netherlands

A previous study has shown that there is an increase in the amount of white pulp in traumatically ruptured spleens compared to spleens which were removed incidentally during abdominal surgery without apparent splenic pathology (van Krieken et al., 1983). This leads to the suggestion that in many cases of so-called traumatic rupture of the spleen there might be a predisposing factor, such as, for instance, a viral infection.

In order to investigate the lymphoid tissue in ruptured spleens in more detail, it was compared with the lymphoid tissue of spleens without splenic pathology using morphological, immunohistochemical, and morphometrical methods.

Materials and methods

All spleens removed were processed immediately, which included methylmethacrylate embedding and freezing of tissue blocks as described before (van Krieken et al., 1983, 1986). From ruptured spleen blocks were taken from macroscopical normal tissue at a distance from the rupture. Extensively crushed specimens were excluded from study.

For this study ten spleens with minor rupture due to trauma were available and compared to the ten most recent spleens removed during cancer surgery (mainly partial gastrectomy for early gastric cancer), and to nine spleens removed during surgery for non-malignant disease without splenic pathology. The latter group consists mainly of cases of highly selective vagotomy or implantation of aortic prothesis.

Immunohistochemistry

Cryostat sections were stained using a three-step immunoperoxidase technique with the following antibodies: Leu 4 (CD3), Leu 3 (CD4), Leu 1 (CD5), Leu 2 (CD8), Leu 14 (CD22) all Becton & Dickinson; B1 (CD20), B2 (CD21), both Orthoclone; Tu1 (CD23), Biotest.

Morphometry

All measurements were performed by one of the authors (H.B.) without knowing the reason for removal of the specimens as described before (van Krieken et al., 1983). In short, with a light-bearing cursor areas of a section were encircled using a Leitz Prism-microscope; perimeter and surface area were calculated using a Kontron MOP-amo 3. This procedure gives the percentage of the measured compartment in the spleen. The amount of a compartment was calculated by multiplying this percentage and the weight of the spleen. In methylmethacrylate sections, stained with methenaminesilver/H&E, white pulp and perifollicular zone were measured (Fig. 1); in cryostat sections stained for CD3 the T-cell areas (Fig. 2), and in sections stained for CD22 the B-cell follicles were measured.

In the cryostat sections stained for the different antigens the number of positive cells for each lymphoid compartment (Van Krieken and Te Velde, 1989) (Figs. 1, 3) was semiquantitatively scored in four categories (0 = no positive cell; + + + = more than 90% of the cells positive).

Statistical methods

The morphometrical data were analysed by comparing two groups using Students's t-test; the semiquantitative data were compared using the square-chi test. P-values < 0.05 were considered statistically significant.

Results

Histology

All specimens showed a normal splenic architecture, with the normal variation in the prominence of white pulp (van Krieken and Te Velde, 1988).

Immunohistochemistry (Table 1)

There were no differences between spleens removed during cancer surgery and those removed in non-malignant disorders. The results of these groups are combined and given in Table 1. The white pulp consisted of T-cell areas, mainly CD4-positive (Fig. 2), and B-cell follicles. The B-cell follicles may contain a germinal centre. There was no difference between primary follicle and mantle zone lymphocytes: CD19, CD22, CD23, all positive, CD5 weakly positive. The marginal zone consisted of somewhat larger lymphocytes with the following phenotype: CD19- and CD22-positive, CD23- and CD5-negative. Several CD4-positive cells were also present (Fig. 4a). In the red pulp CD19-, CD22- and CD23-positive lymphocytes were intermingled with CD8-positive lymphocytes (Fig. 5a). In the non-filtering areas the B-cells dominated, and there were also some CD4 cells; in the perivascular rim the CD8 cells dominated.

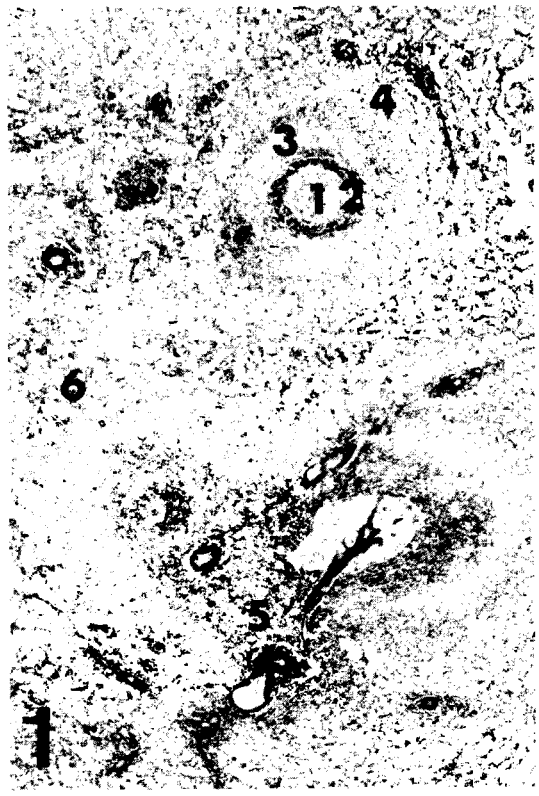

Fig. 1. Traumatically ruptured spleen. Methylmethacrylate embedding. This figure shows at small magnification the compartments of the white pulp. Note the presence of the relatively large perifollicular zone.
1 = germinal centre
2 = mantle zone
3 = marginal zone
4 = perifollicular zone
5 = T-cell area
6 = red pulp

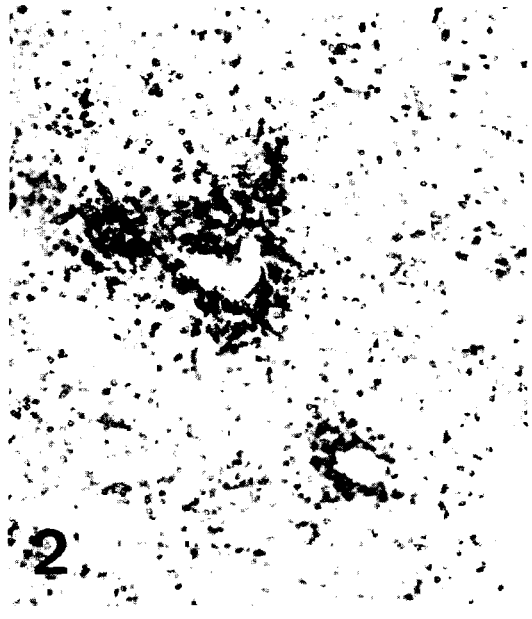

Fig. 2. Spleen removed during highly selective vagotomy procedure. Three-step immunoperoxidase technique using Leu3 as antibody (CD4). Strongly positive T-cell areas and scattered positive cells in the red pulp.

Table 1. Summary of the results of the immunohistochemical analysis. Semiquantitative score of the presence of cells positive for each antibody used in each splenic compartment. 0 = no positive cell; +++ = almost all cells positive.

	B-Cell antigens				T-cell antigens			
	CD20	CD21	CD22	CD23	CD5	CD3	CD4	CD8
Germinal centre	+++	+	+++	++	0	+	+	0
Mantle zone	+++	+	+++	+++	+++*	0	0	0
Marginal zone	+++	+	+++	+	+	+	+	+
T-cell area	0	0	0	0	+++	+++	+++	+
Perifollicular zone	+	+	+	+	+	++	+	++
Red pulp	0	0	0	0	+	+	0	+
Non-filtering area	+	+	+	+	+	+	0	+

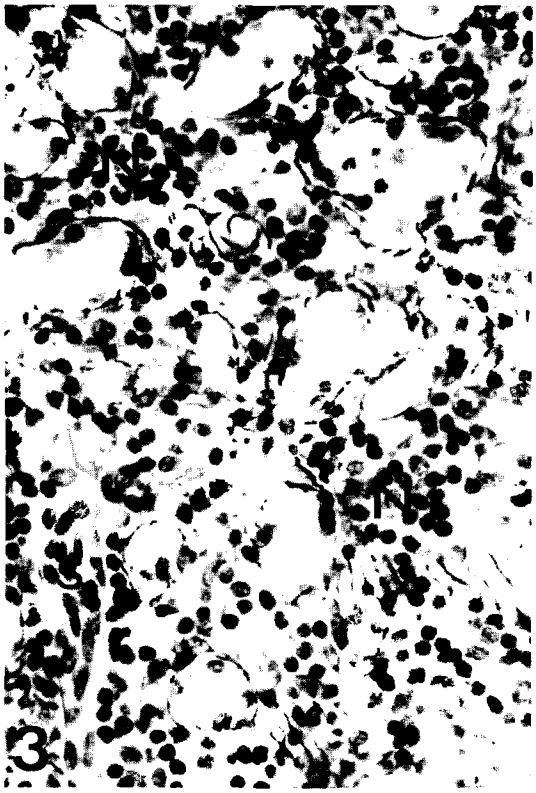

Fig. 3. Same specimens as figure 1. Detail of the red pulp showing sheathed capillary (S) and groups of lymphocytes in non-filtering areas (N).

The main distribution of lymphocyte subsets was the same in the ruptured spleens, but some differences were found. In the marginal zone there was a significant increase of CD23- positive cells, whereas the number of CD4- positive cells decreased (Fig. 4b). CD21 has disappeared from a part of the cells in the follicles. In the red pulp there was a decrease of the number of CD8-positive lymphocytes (Fig. 5b).

Morphometrical results (Figs. 6, 7)

Again no differences were found between spleens removed for malignant vs non-malignant disease. Ruptured spleens showed a significant increase in the percentage of the perifollicular zone compared to both other groups, and the percentage of white pulp slightly increased.

Due to the inclusion of a part of the perifollicular zone, which could not be recognized in cryostat sections, the summation of the percentage of CD23- and CD3-positive areas exceeds the percentage of white pulp as measured in methylmethacrylate-embedded sections. As this was the case in all three groups, it does not influence the comparison. The CD22-positive areas were in all three groups similar, but there was more T-cell area in ruptured spleens compared to both other groups. This becomes especially clear after correction for splenic weight. That means that there is an absolute increase in the amount of perifollicular zone and T-cell area in ruptured spleens compared to the other groups.

Discussion

Known causes of splenic rupture include: perforating trauma, blunt trauma (Sherman, 1980; Seufert, 1983), infectious mononucleosis (Chamberlain et al., 1984; Cochrane, 1984; Majewski and Stahlknecht, 1986), AIDS (Mirchani et al., 1985), malignancies (Andrews et al., 1980; Rogers and Shah, 1980; Armitage et al., 1981; Bennet et al., 1984; Cooperberg et al., 1985), peliosis (Berlin et al., 1982; Audoin and Diebold, 1983) and extra-uterine pregnancy (Caruso and Hall, 1984). Splenomegaly enhances the changes of splenic rupture following blunt trauma (Sherman, 1980; Bisshop and Lansaing, 1982; Cochrane, 1984). In many studies a traumatically ruptured spleen is considered to be normal and the ruptured spleen is, for instance, not discussed in a recent textbook on the pathology of the spleen (Wolf and

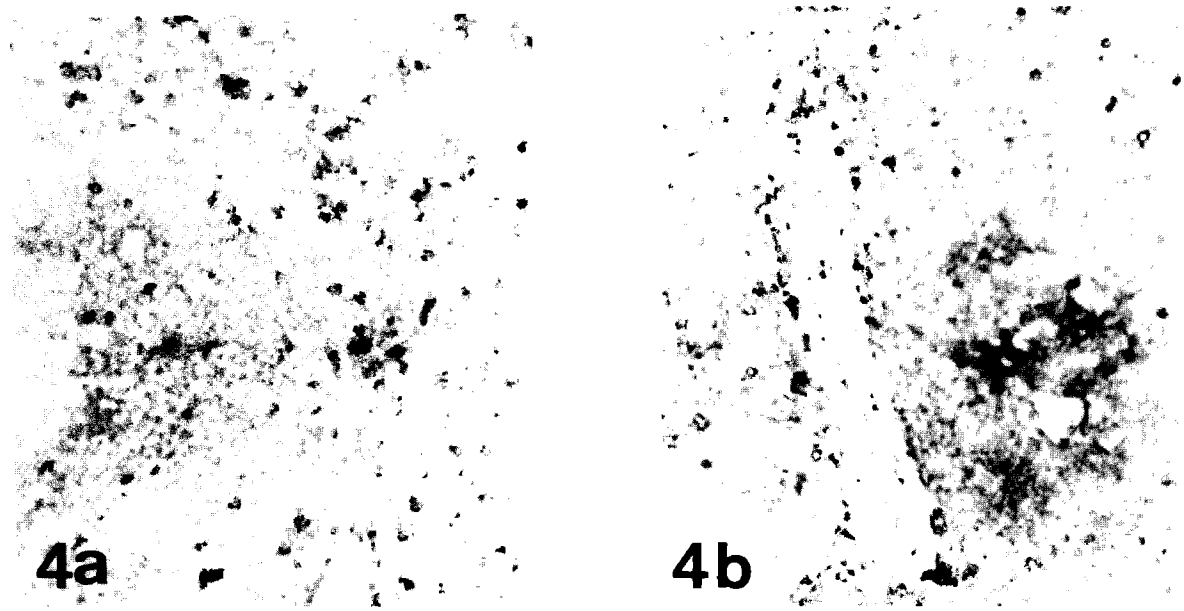

Fig. 4. Comparison of Leu 2 (CD8) staining of marginal zone between ruptured (a) and non-ruptured (b) spleen.

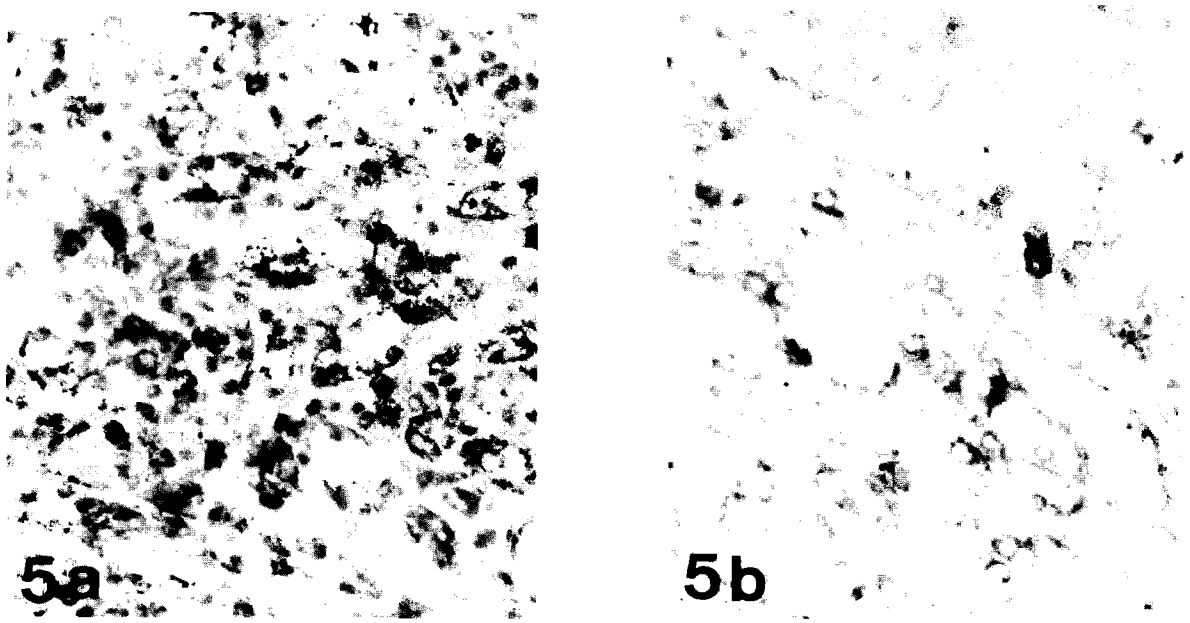

Fig. 5. Comparison of Leu (CD8) staining of red pulp between ruptured (a) and non-ruptured (b) spleen.

Neiman, 1989). A previous study has shown that ruptured spleens contain more lymphoid tissue than spleens which can be considered as normal (van Krieken, 1983). Similar results were found in this study which comprises different, more recently obtained specimens, of which frozen tissue was available. There are some differences between the present group of ruptured spleens and the previous series. Namely, less white pulp and more perifollicular zone. Although these differences are not statistically significant ($p < 0.05$) one possible explanation needs mentioning. In the period when we obtained our first series (1975-1980) spleens with small superficial ruptures were always removed, and splenic repair was not attempted. This is contrasts with the present less agressive approach, and this may have influenced the composition of the present series of specimens. However, the differences compared to our control group, for which the same difference in approach may also have had some influence, is not altered.

Although the number of specimens is inevitably low (spleens with minor rupture nowadays rarely reach our department of pathology), this immunohistochemical study made it possible to show that the increase in white pulp in ruptured spleens is solely due to an increased amount of CD4 (helper/inducer)-lymphocytes. Although

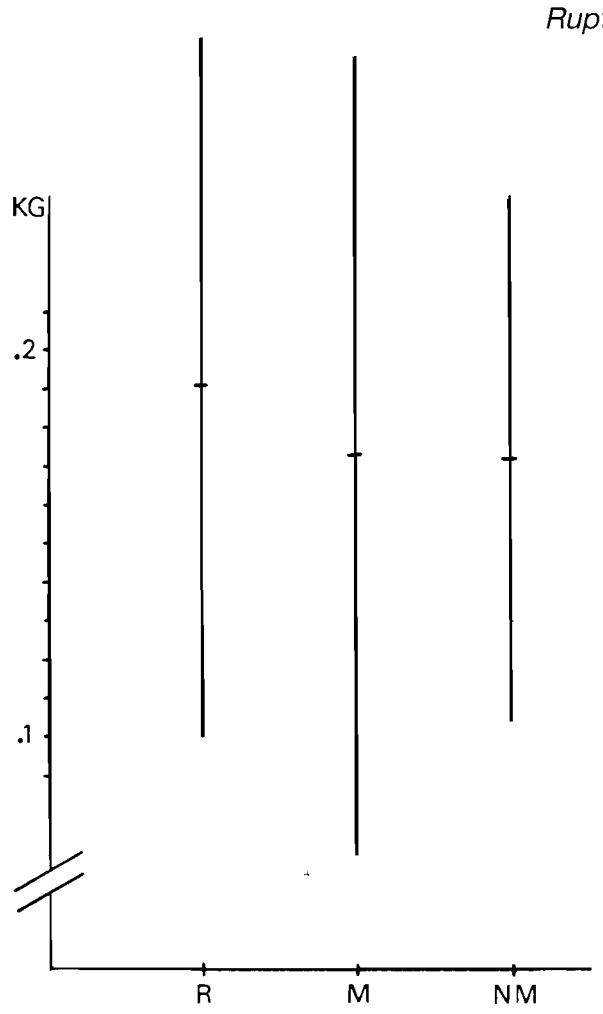

Fig. 6. Weight of the spleen, mean and standard deviation, of the three groups studied.

R - ruptured group (mean weight: 0.191 kg)
M - malignant group (mean weight: 0.173 kg)
NM - non-malignant group (mean weight: 0.172 kg)

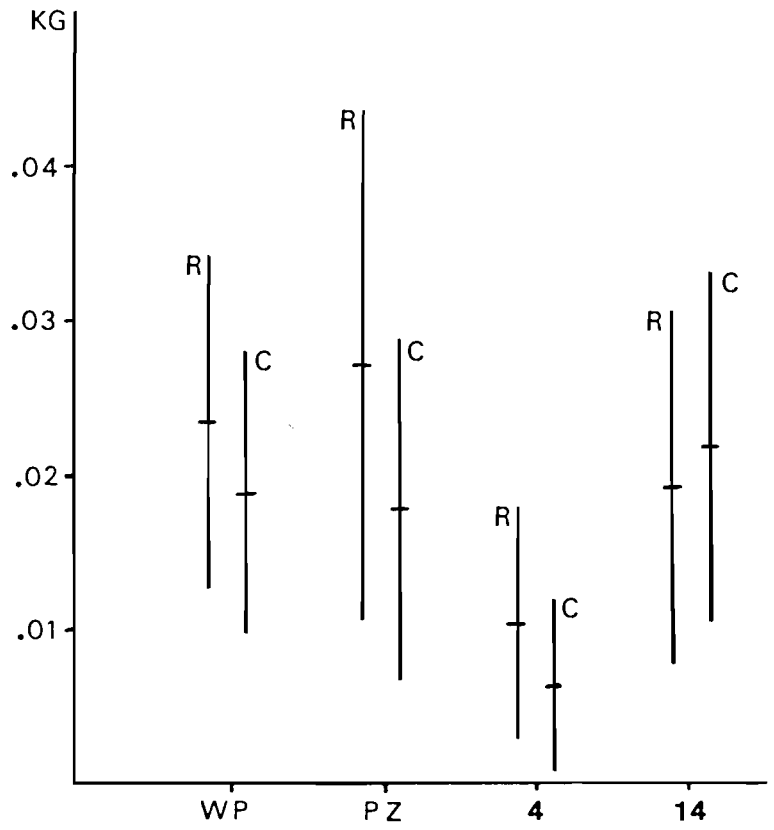

Fig. 7. Summary of morphometrical results. Comparison of ruptured and non-ruptured spleens regarding mean and standard deviation of the amount of white pulp, perifollicular zone, Leu 4-positive areas, and Leu 14-positive areas.

R - ruptured group
C - combined malignant and non-malignant group

WP - white pulp: mean R: 0.023 kg
mean C: 0.067 kg

PZ - perifollicular zone: mean R: 0.027 kg
mean C: 0.018 kg

4 - LEU 4-positive: mean R: 0.010 kg
mean C: 0.006 kg

14 - LEU 14-positive: mean R: 0.019 kg
mean C: 0.022 kg

the amount of B-cell follicles is not altered, the composition changes, with more mantle zone cells and less marginal zone cells (van Krieken et al., 1989). In the red pulp there are less CD8 (suppressor/cytotoxic) lymphocytes.

It is difficult in a system as complex as the immune system to relate number of cells to activity and cause, especially in man in which experiments on this point are impossible. There are, only to our knowledge, data on the composition of the lymphoid tissue of the spleen in autoimmune disorders (van Krieken and Te Velde, 1986; van Krieken et al., 1988), Hodgkin's disease (Timens et al., 1982) and some other malignancies. The findings in ruptured spleens are completely different from those data and are difficult to interpret. Anyway, the present results point to an activation of the lymphoid system and fit into the hypothesis that spleens that rupture are predisposed to the event due to (subclinical) infections in a similar way as is already well known in infectious mononucleosis. In immunological studies on the human spleen one has to be cautious when using ruptured spleens as controls.

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