Virchows Archiv A Pathol Anat (1992) 421:41–45

Virchows Archiv A
Pathological Anatomy and Histopathology

Microscopic features of the regeneration of white pulp in autotransplanted spleens in rats

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Received October 29, 1991 / Received after revision February 4, 1992 / Accepted February 5, 1992

Summary. The process of regeneration of white pulp in autotransplanted splenic tissue, implanted into a pocket made by the greater omentum in rats, was investigated histologically and immunohistochemically. Three days after transplantation, almost all implants were necrotic. At day 7, viable splenic tissue had gradually regenerated at the periphery of the implants. At day 10, lymphocytes accumulated around the arterioles. The accumulations resembled the periarteriolar lymphoid sheath of normal spleens in structure, but consisted mainly of B-lymphocytes, with a few scattered T-lymphocytes. However, by week 2, the localization peculiar to T- and B-lymphocytes became definite, and follicular dendritic cells were simultaneously observed in the lymph follicles. This regeneration of the white pulp in the autotransplanted spleens differed from the formation of the white pulp during ontogeny and during recovery after spleen irradiation.

Key words: Spleen – Autotransplantation – White pulp – Regeneration – Rats

Introduction

The spleen plays an important role in the prevention of infections. Frequently, splenectomy must be performed in patients following trauma or in idiopathic thrombocytopenic purpura. These patients are then in danger of overwhelming post-splenectomy infection (OPSI), which has a high mortality during childhood (Diamond 1969).

Autotransplantation of splenic tissue has been proposed for the prevention of OPSI, based on the fact that ectopic splenic tissue can survive in tissues without vascular anastomosis (Fleming et al. 1976). The protective function of implants against bacterial infection has been reported in animals, in that autotransplanted rats had a better survival rate than splenectomized rats (Harding et al. 1987), and this procedure has already been performed clinically (Aigner et al. 1980; Mizrahi et al. 1989). In addition, splenic transplantation in animals is useful as an experimental model for investigating the ontogeny, structure, and function of the spleen. It has been confirmed histologically that the implants undergo almost complete necrosis immediately, and then regenerate to form splenic tissue which is indistinguishable from the original structure (Tavassoli et al. 1973). Studies on the histological process of this regeneration are few, and some differences in results have been reported (Tavassoli et al. 1973; Dijkstra and Langevoort 1982; Sasaki 1990). The present study focused on the histological process of regeneration of the white pulp using immunohistochemistry in autotransplanted splenic tissue.

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Materials and methods

Forty-five adult male rats of the Wistar strain, 8 weeks old and weighing 180–220 g, were used. They were kept under conventional conditions and fed with commercial pellets (Oriental Yeast, Tokyo, Japan) and tap-water ad libitum.

Animals were anaesthetized with intramuscular injections of 1 mg of sodium pentobarbitone per 20 g body weight. Splenectomy was performed via a medial abdominal incision. The spleen was removed, weighed, and then cut into 16 pieces. All pieces were implanted together in a pocket made by the greater omentum, and the abdominal wound was sutured. The implants were removed at 3, 7 and 10 days, and at 2, 3, 4, 8, 24 and 48 weeks after implantation. Five rats were examined at each time period.

Each implant was divided into two pieces. One was fixed in 10% buffered formalin solution for 3 days and embedded in paraffin after dehydration in a graded ethanol series. Sections cut at a thickness of 3 μm were stained with haematoxylin and eosin. The other piece was treated by a modified AMeX method (Sato et al. 1986); the tissue was fixed in acetone overnight at 4° C, dehydrated in acetone at room temperature for 15 min, cleared in methyl benzoate for 30 min and xylene for 30 min, then penetrated with paraffin at 60° C for 2 h in a vacuum evaporating embedder. Sections cut at a thickness of 3 μm were used for immunohistochemical staining.

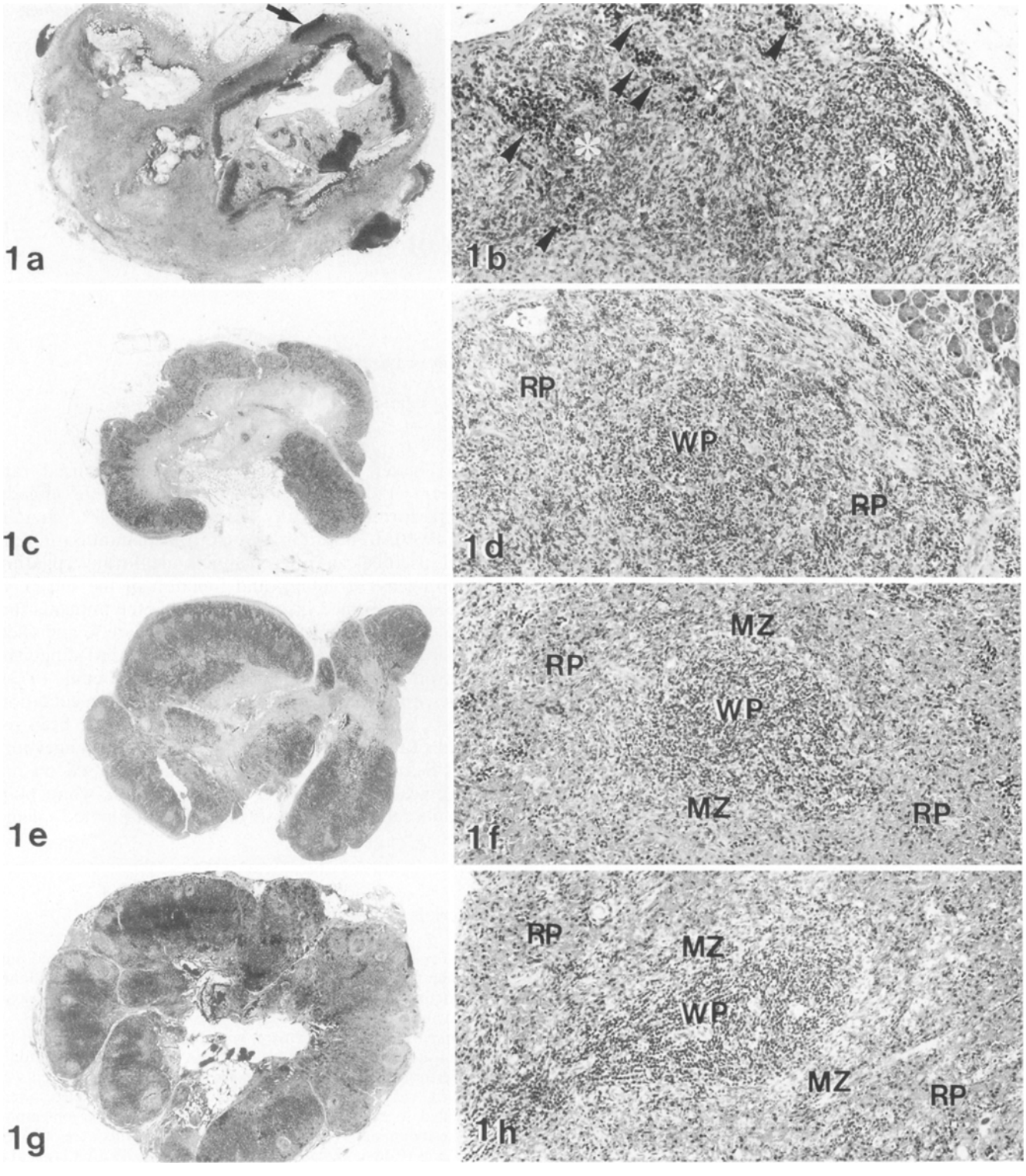

Fig. 1a–h. Microscopic features of regeneration in autotransplanted tissues. **a**, **b** Day 7. Viable tissue (*arrow*) is found beneath the serous membrane of the greater omentum making the pocket. Lymphocyte accumulations (*asterisks*) are seen in this viable tissue and the red pulp appears with extramedullary haematopoiesis (*arrowheads*). **a** ×7; **b** ×100. **c**, **d** Day 14. Splenic tissue is seen growing in the periphery, and the central necrotic area is replaced by granulation tissue. The white pulp (*WP*) and the red pulp (*RP*) are clearly distinguished. **c** ×7; **d** ×100. **e**, **f** Week 3. Splenic tissue is proliferating. The marginal zone (*MZ*) appears around the white pulp (*WP*). **e** ×7; **f** ×100. **g**, **h** Week 48. The splenic area is enlarged and shows a lobular structure. White pulps (*WP*) are distributed mainly in the peripheral part. The microscopic features of the splenic tissue in the implant are those of normal spleens. In the centre, fatty tissue with minute calcifications remains. **g** ×7; **h** ×100

Monoclonal antibodies against rat T-lymphocytes, B-lymphocytes, and thymus glycoprotein (Sera-Lab, Sussex, UK) were used in the first step. Anti-rat-thymus glycoprotein antibodies are specific to follicular dendritic cells (FDC), but not to the interdigitating cells in the T-dependent areas (Barclay 1981). These antibodies are also positive in endothelial cells of vessels, including the marginal sinus (Barclay 1981). Sections were deparaffinized with xylene, then immersed in acetone, and washed in phosphate-buffered saline (pH 7.4). Immunohistochemical staining was performed using the avidin-biotin-peroxidase complex (ABC) method, with an ABC kit (Vector, Burlingame, Calif., USA). Endogenous peroxidase activity was abolished with 99.6% methanol containing 0.3% hydrogen peroxide for 20 min. Non-specific binding of antibodies was blocked by incubation with normal horse serum for 20 min at room temperature. Sections reacted with the first-step antibodies overnight at 4° C, then incubated with biotinylated horse anti-mouse IgG antibodies containing 2% normal rat serum. This was followed by incubation with the ABC solution for 40 min at room temperature. Colour was developed in DAB solution (Wako, Osaka, Japan) to which 0.015% hydrogen peroxide had been added. The sections were counterstained with methyl green.

Results

Three days after implantation, all implants were almost completely necrotic and no viable splenic tissue could be identified. At day 7, the necrotic area had decreased slightly, and granulation tissue appeared. In 2 of 5 implants at day 7, tiny amounts of viable splenic tissue accompaning a few accumulations of lymphocytes and haematopoiesis were found in the periphery of the implants (Fig. 1a, b). At week 2, viable splenic tissue was proliferating in the periphery of the implants, between the central granulation tissue and the surrounding greater omentum. The white pulp tended to be distributed in the periphery of the regenerated tissue, and the white and red pulps became distinguishable in all implants (Fig. 1c, d). The marginal zone (MZ) was observed clearly at week 3 (Fig. 1e, f); thus all structural compartments of the spleen were complete in all implants (Table 1). The central necrotic area was replaced by fibrous tissue. After week 3, no essential changes were observed in the histological structure (Table 1). At weeks 24 and 48, all implants revealed a lobular structure (Fig. 1g, h).

The lymphocytes forming the tiny accumulations at day 7 were of B-cell type. At day 10, several tiny accumulations of lymphocytes were observed around the arterioles in the splenic tissue regenerating in the peripheral area of the implants (Fig. 2a). There were 4–12 of these periarteriolar lymphocyte sheath (PALS)-like accumulations in each section (mean, 6.6 per section). The accumulations, however, consisted mainly of B-lymphocytes, with a few scattered T-lymphocytes (Fig. 2b–e). In 2 of 5 implants, there was a slight predominance of T-lymphocytes in the central area and of B-lymphocytes in the peripheral area in some accumulations. At week 2, localization, which was peculiar to T- and B-lymphocytes in the PALS and lymph follicle (LF), was observed in all implants. However, a few intermingled accumulations of T- and B-lymphocytes still remained in 2 implants. At that time FDC, the processes of which branched between lymphocytes, were also observed in the LF. Before week 2, no FDC were found. After week 3, intermingling of T- and B-lymphocytes disappeared in the accumulations, and localization peculiar to T- and B-lymphocytes was confirmed in all implants (Fig. 3a–c).

Discussion

According to Dijkstra and Langevoort (1982) and Sasaki (1990), the PALS formed first, followed by the LF. Other reports suggest the PALS formed later than the LF in part (Sasaki 1990). Our immunohistochemical observations showed that regeneration of the white pulp began in the form of intermingled lymphocyte accumulations of T- and B-lymphocytes around the arterioles at day 10. These accumulations resembled PALS in structure, but most lymphocytes in the accumulations were B-lymphocytes, with a few T-lymphocytes. The PALS and LF were, however, simultaneously formed at week 2. This finding suggests a transition from the intermingled accumulation of T- and B-lymphocytes to the separate localization of lymphocytes in the PALS or LF. Dijkstra et al. (1983) also reported the intermingled accumulation of T- and B-lymphocytes, which was postulated at a transitional stage during the first days after transplantation.

In studies on the ontogeny of white pulp (Veerman and van Ewijk 1975; Dijkstra and Döpp 1983) and its recovery after irradiation (Rozing et al. 1978; Satoh 1991), no intermingled lymphocyte accumulations, as

Table 1. Regeneration of compartments in the autotransplanted splenic tissues

Time after transplantation	*n*	Necrosis	Granulation	PALS	LF	GC	MZ	RP
3 days	5	5	3	0	0	0	0	0
7 days	5	5	5	0	0	0	0	2
10 days	5	5	5	0	0	0	0	5
2 weeks	5	5	0	5	5	1	2	5
3 weeks	5	2	0	5	5	1	5	5
4 weeks	5	1	0	5	5	1	5	5
8 weeks	5	0	0	5	5	0	5	5
24 weeks	5	0	0	5	5	2	5	5
48 weeks	5	0	0	5	5	0	5	5

PALS, Periarteriolar lymphoid sheath; LF, lymph follicle; MZ, marginal zone; GC; germinal centre; RP, red pulp

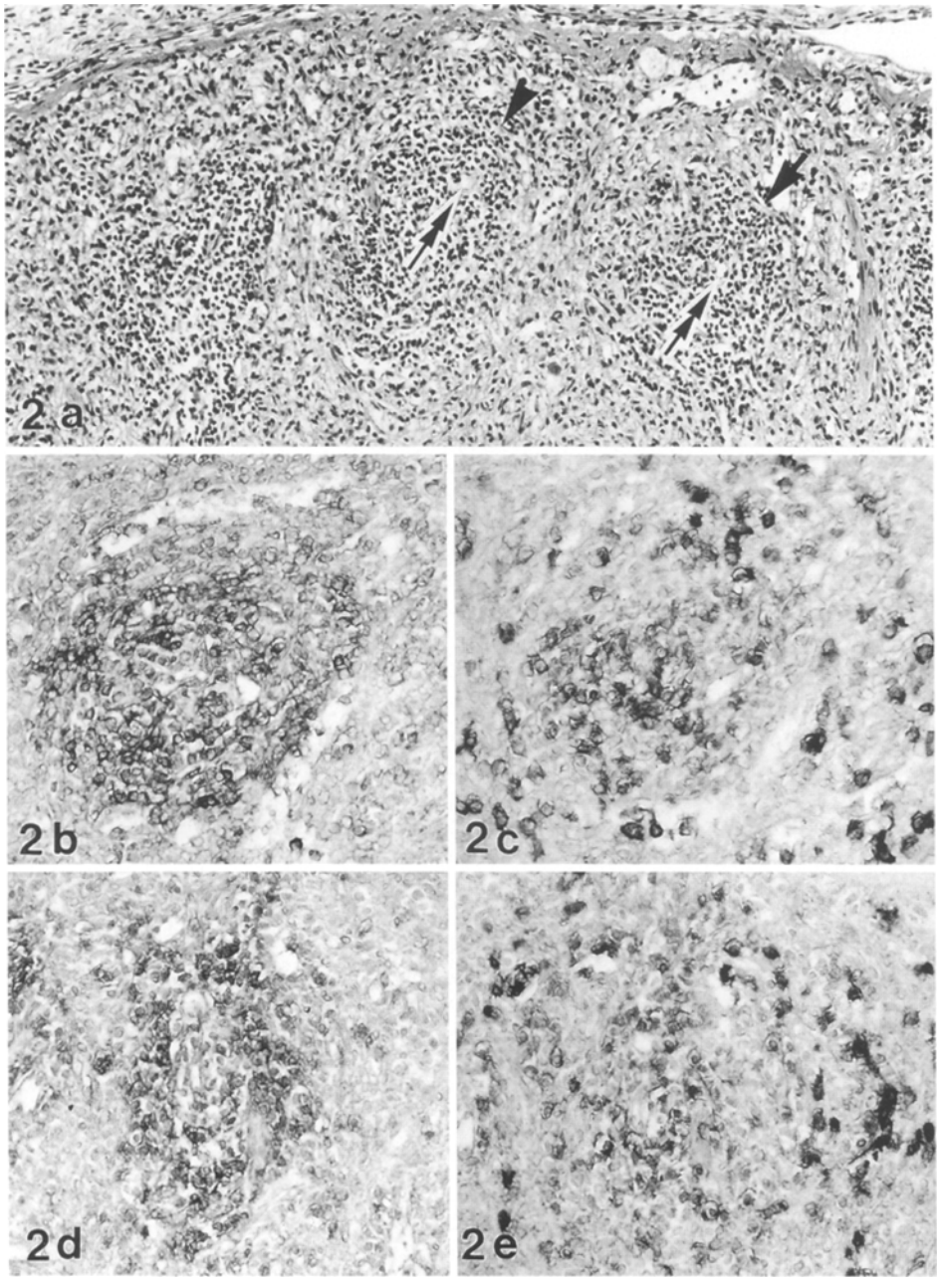

Fig. 2a–e. Lymphocyte accumulations around the arterioles in an implant at day 10. **a** Lymphocyte accumulations (*arrow* and *arrowhead*) are seen around the arterioles (*double arrow*) in the periphery of the implant. ×120. **b** Immunostaining for B-lymphocytes. The accumulation indicated by an *arrow* in **a** mainly consists of B-lymphocytes. ×250. **c** Immunostaining for T-lymphocytes. A few T-lymphocytes are scattered in the accumulation shown in **b**. ×250. **d** Immunostaining for B-lymphocytes. B-lymphocytes are distributed predominantly in the peripheral area of the lymphocyte accumulation indicated by an *arrowhead* in **a**. ×250. **e** Immunostaining for T-lymphocytes. T-lymphocytes are distributed predominantly in the central area of the accumulation shown in **d**. ×250

seen in the present study, were reported. These accumulations are accordingly considered to be characteristic of the early stage of regeneration of autotransplanted spleens with differences in the order of appearance of T- and B-lymphocytes due to differences in the microenvironment. According to the results of Dijkstra and Langevoort (1982) in their investigation of ontogenetic development, regeneration of implants takes place in a mature environment. After transplantation, in contrast to development after irradiation, all cellular elements, including radioresistant non-lymphoid cells, undergo necrosis to the same degree. The microenvironment for the homing of T- and B-lymphocytes was almost completely destroyed after transplantation and no localization peculiar to T- and B-lymphocytes resulted until 4 days after the intermingled lymphocyte accumulations occurred, when the microenvironment necessary for localization peculiar to T- and B-lymphocytes might have been completed.

In the present study, FDC, which relate to B-cell homing (Dijkstra and Langevoort 1982), are assumed to have been transformed from reticulum cells. Although the appearance of FDC in implants has been identified functionally by the trapping of immune complexes (Dijkstra and Langevoort 1982) and morphologically by electron microscopy (Sasaki 1990), no monoclonal antibody techniques have been applied for this purpose. In the present study, FDC were observed immunohistochemically only in the LF and only from week 2, and no FDC were observed at other sites or before week 2. Imazeki et al. (1989) reported that, in an allograft model with different haplotypes of MHC class I antigen in

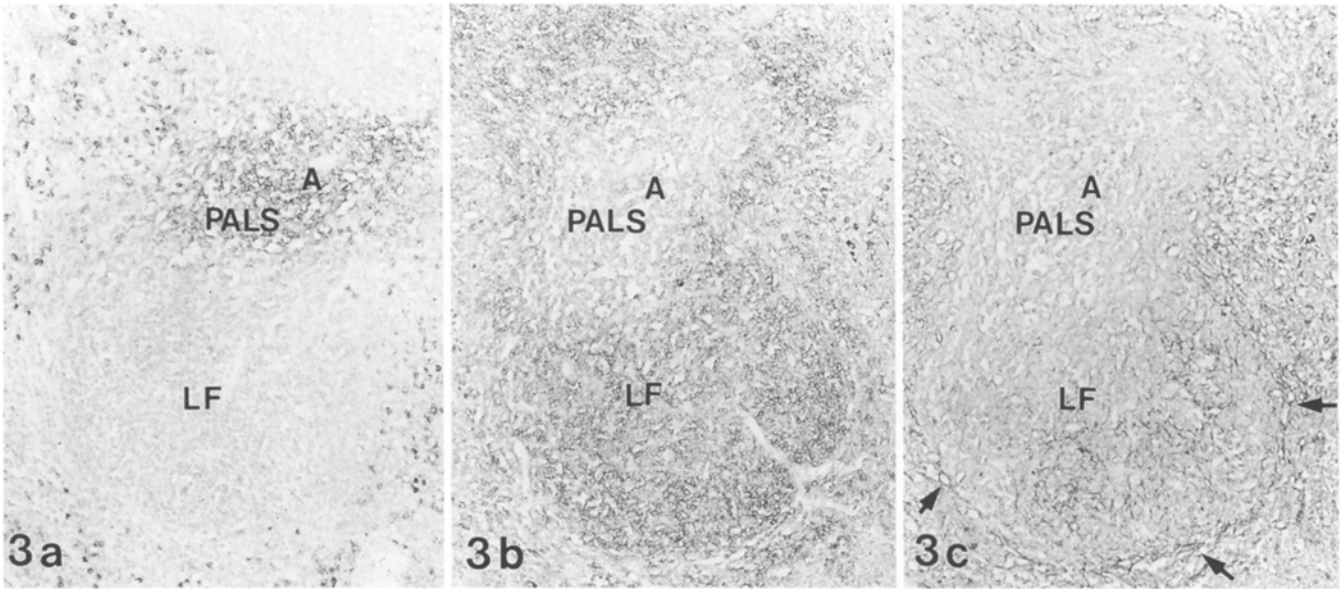

Fig. 3a–c. Week 2. Immunohistochemical staining of the white pulp. **a** Immunostaining for T-lymphocytes. T-lymphocytes accumulate around an arteriole (*A*) and form a periarteriolar lymphoid sheath (*PALS*). ×100. **b** Immunostaining for B-lymphocytes. B-lymphocytes accumulate and form a lymph follicle (*LF*). ×100. **c** Immunostaining for thymus glycoprotein. The processes of follicular dendritic cells are branched in the LF. Marginal sinusal endothelial cells (*arrows*) are also positive in a part of the surrounding of the LF. ×100

mice, FDC did not originate from the recipient bone marrow but originated from the residual stromal cells of the implant.

The MZ was observed in the implants 1 week after the appearance of the white pulp, and all splenic compartments, the red pulp, PALS, LF and MZ, were present at week 3 after transplantation.

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