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Outer mantle zone of the follicle in the human spleen

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Summary. The follicular circumferential connective fibre reticulum, which is a continuation of the circumferencial reticulum (CR) of the periarterial lymphocyte sheaths, was visualized at the periphery of lymphoid follicles in the human spleen by means of anticartilaginous antiserum. If this CR is considered to be the borderline between the follicular and extrafollicular structures, then at the inner aspect of the CR and at the periphery of the fully developed follicle two mantle zones can be distinguished: an inner zone consisting of small lymphocytes, and an outer consisting of medium-sized lymphocytes. Both all groups are B-lymphocytes displaying positive surface IgM. The reticulum is the same in both mantle zones and it is completely different from the reticulum of the marginal zone. In some instances it is so sparse as to appear to be absent completely.

The newly delimitated outer mantle zone also has a specific cellular composition differing from the marginal zone. Most importantly, it does not possess that mixture of lymphocytes and other blood cells which is considered to be the defining feature of the marginal zone. The characteristic cellular components of the outer mantle zone are the densely accumulated medium-sized lymphocytes, with other cells interspersed very sporadically.

Key words: Human spleen – Follicles – Mantle zones – Marginal zone – Extracellular structures

Introduction

At the borderline between the white and red pulp, there is a zone which has been called by various names particularly in the perifollicular region (Weidenreich 1901; Strasser 1922; Krumbhaar 1948; Snook 1964; Millikin 1966; Müller-Hermelink and Lennert 1978; Blue and Weiss 1981; Saitoh et al. 1982) and which has been attributed to the white pulp (Nieuwenhuis

and Keuning 1974; Veerman and van Ewijk 1975), to the red pulp (Fujita and Kashimura 1981), or considered to be an individual zone (Blue and Weiss 1981; Saitoh et al. 1982; Pabst and Binns 1982). It has most frequently been referred to as the marginal zone and defined as a region distinguished by a characteristic population of cells comprising macrophages and a mixture of lymphocytes, red cells, platelets, granulocytes and monocytes (Blue and Weiss 1981). The very fine reticulin fibres in the marginal zone, evident by electron microscopical pictures, are not easily demonstrable in light microscopical preparations (Veerman and van Ewijk 1975).

In our studies of the human spleen, using anticartilaginous antiserum, we visualized the extracellular structures of the spleen very distinctly (Brozman et al. 1983; Brozman 1984). We have used this possibility to characterize the individual compartments of the spleen, and particularly in determining the borderlines between individual compartments and subcompartments; we have considered not only the cellular but also the extracellular structures. This enabled us to take a point of view on the confused and contradictory issues concerning the bordeline zone between the white and red pulp and to provide evidence of a structurally delimitated new zone at the periphery of the follicle in the human spleen.

Material and methods

We examined 25 human spleens from the current biopsy material of the Institute of Pathology of the Medical Faculty in Bratislava. The spleens had been removed for the clinical diagnosis of congenital spherocytic haemolytic anaemia or thrombocytopaenic purpura. We also examined 5 human spleens removed from patients who had sustained an injury and were immediately operated on. The majority of cases concerned children aged from 6 to 12 years, the rest were adults aged up to 55 years.

Small tissue blocks were quickly frozen in dry-ice-acetone mixture. Fifty serial cryostat sections of 5 mμ thick were prepared, air dryed and fixed in acetone or in 4% formaldehyde. The acetone-fixed sections were incubated with anti-cartilage antiserum, which was prepared by inoculating rabbits with collagenase-treated residue obtained after extraction of human knee or rib hyaline cartilage homogenate with 3.4 mol dm^{-3} NaCl and extraction with 0.05 mol dm^{-3} sodium citrate buffer at pH 3.2 (Brozman et al. 1983). The antiserum was diluted 1:160 to 1:320 in phosphate buffered saline (PBS). In the 2nd layer fluorescein isothiocyanate labeled swine antirabbit globulin (SwAR-FITC, SEVAC, Praha, CSSSR) diluted 1:20 in PBS was used. Besides, acetone-fixed sections were incubated with various antisera to human immunoglobulins (SEVAC, Praha, CSSR; Dako, Copenhagen, Denmark). Formaldehyde-fixed sections were stained with haematoxylin and eosin (HE) and adjacent serial acetone-fixed sections were treated mostly with anti-cartilage antiserum and with antiserum to IgM. The sections stained with HE were examined not only in visible but also in fluorescent light and in their combination with the microscope Univar (Reichert, Wien, Austria), equipped with an epi-illuminator. Adjacent serial sections were used for the nonspecific esterase reaction: substrate: l-naphthyl-acetate. Diazonium salt: hexazonium pararosaniline (Davis and Ornstein 1959). Incubation pH 7.2–7.4, room temperature, 10–20 min.

Results

By means of an anticartilaginous antiserum, the periarterial circumferential reticulum (CR) was distinctly visualized in the white pulp, frequently exhibiting a lamellar character in its peripheral part (Fig. 1). The outer lamellae separed the periarterial lymphocyte sheaths (PALS) from the marginal zone

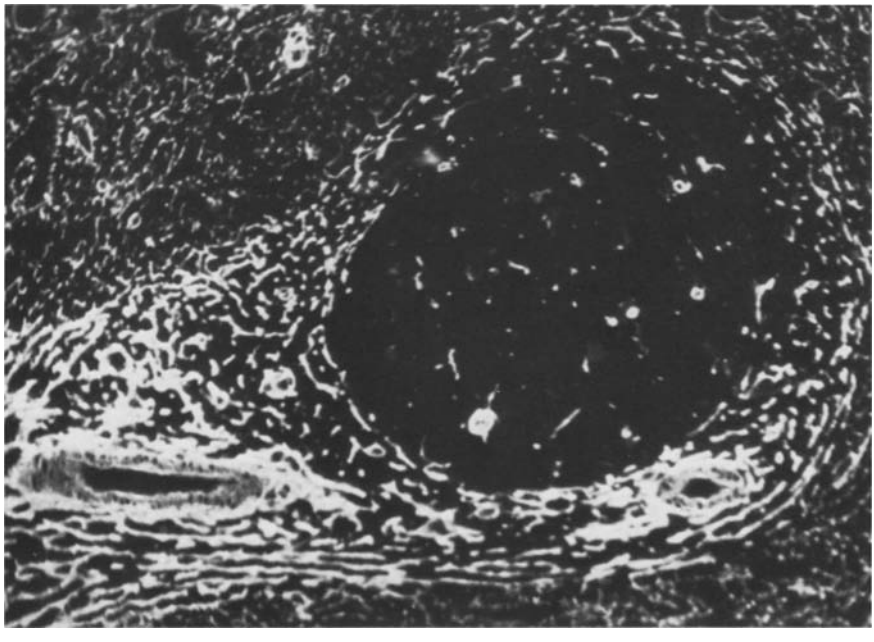

Fig. 1. Human spleen 282876 (female, 8-year-old): Intense immuno-fluorescence of the circumferential reticulum of the PALS *at the left* and follicle *at the right*. Anti-cartilage antiserum and SwAR-FITC. ×250

and were more or less distinctly seen to continue on the surface of the follicles as follicular CR. This follicular CR was sporadically found to be considerably narrowed and discontinuous, particularly at the peripheral pole of the follicle diametrically opposite the central artery. Yet in many instances it distinctly separated the whole follicle from the marginal zone (Fig. 2). Follicular capillaries were directed toward this CR (Fig. 3), passed into the marginal zone or disappeared in the CR. On using anti-IgM antibodies, cells displaying surface IgM (SIgM) were visualized on the periphery of the follicle, in the region of the follicular CR and in the perifollicular area (Fig. 4). In addition very intensive immunofluorescence appeared in the germinal centers as an intercellular IgM network. In the adjacent serial cryostat sections stained with HE (Fig. 5), the cells yielding a positive reaction to SIgM surrounded the germinal centre and formed an inner layer of small lymphocytes and an outer layer of medium sized lymphocytes. On examining the HE stained sections in fluorescent light, a variably distinct fluorescence appeared also in the follicular CR (Fig. 6). This autofluorescence virtually corresponded to the localization of the immunofluorescence seen on using anticartilaginous antiserum, providing further evidence for the identification of the follicular CR. When such a HE stained preparation was examined in a suitable combination of visible and fluorescent light, a relatively distinct fluorescence appeared in the follicular CR and cellular elements were simultaneously identified (Fig. 7).

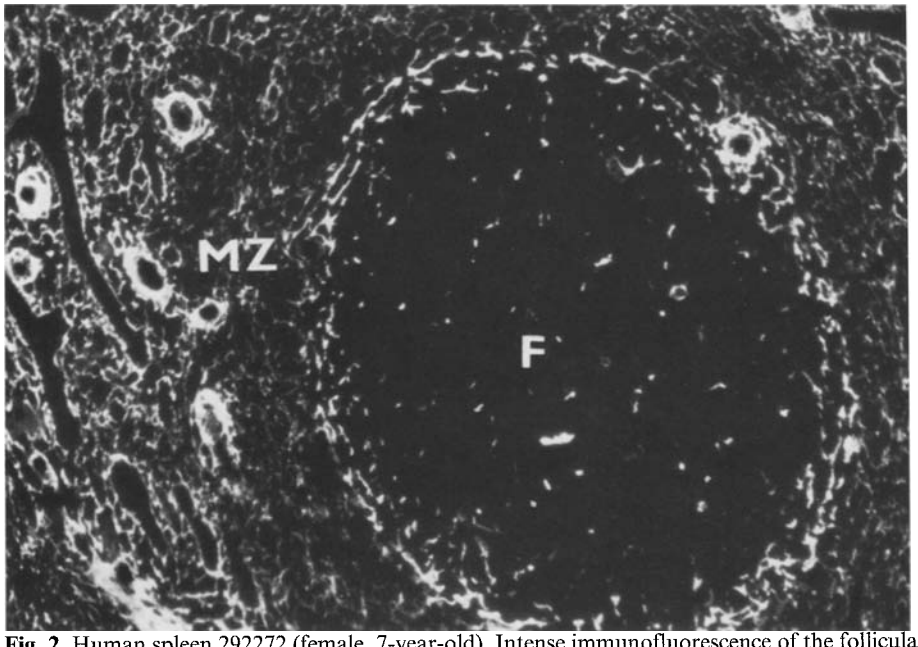

Fig. 2. Human spleen 292272 (female, 7-year-old). Intense immunofluorescence of the follicular circumferential reticulum and of the pericapillary spongy sheaths *at the left*. Circumferential reticulum between marginal zone (*MZ*) and follicle (*F*). Stained as in Fig. 1. ×250

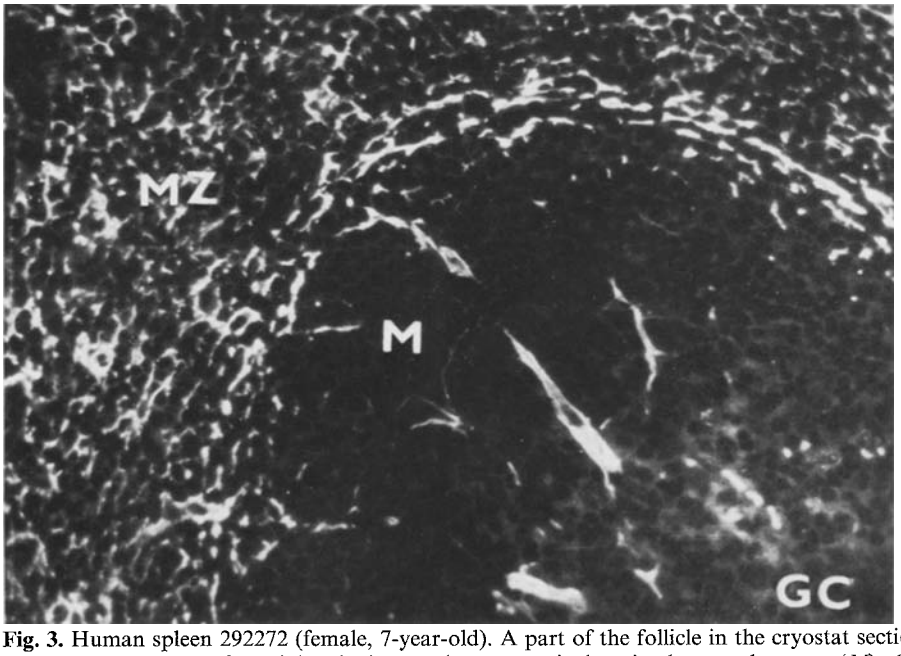

Fig. 3. Human spleen 292272 (female, 7-year-old). A part of the follicle in the cryostat section 5 with distinct circumferential reticulum and sparse reticulum in the mantle zones (*M*). *GC* germinal center. Stained as in Fig. 1. ×400

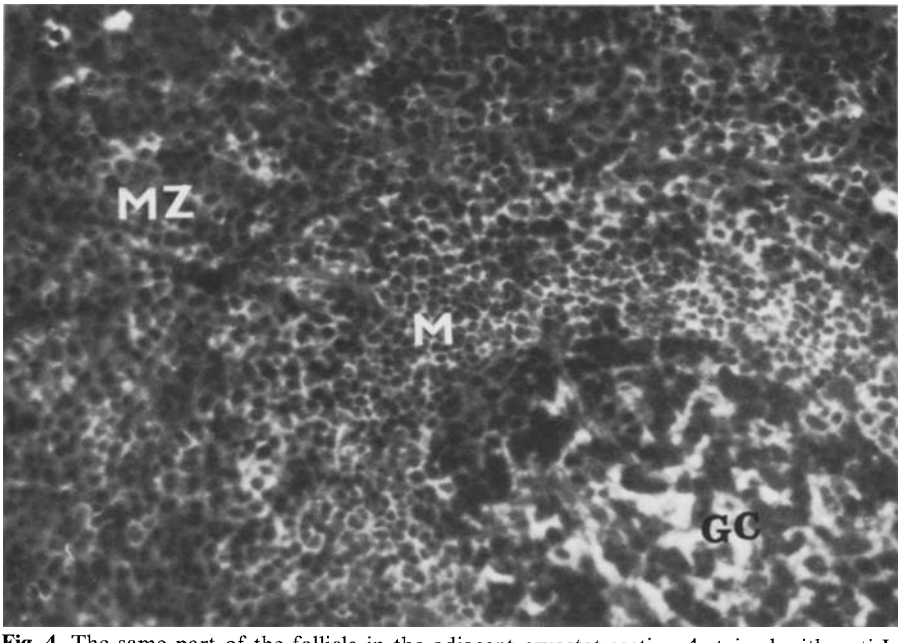

Fig. 4. The same part of the follicle in the adjacent cryostat section 4 stained with anti-IgM and FITC. Surface IgM on small and medium-sized lymphocytes in mantle zones (*M*) and marginal zone (*MZ*) Intercellular IgM network pattern in the germinal center (*GC*). × 400

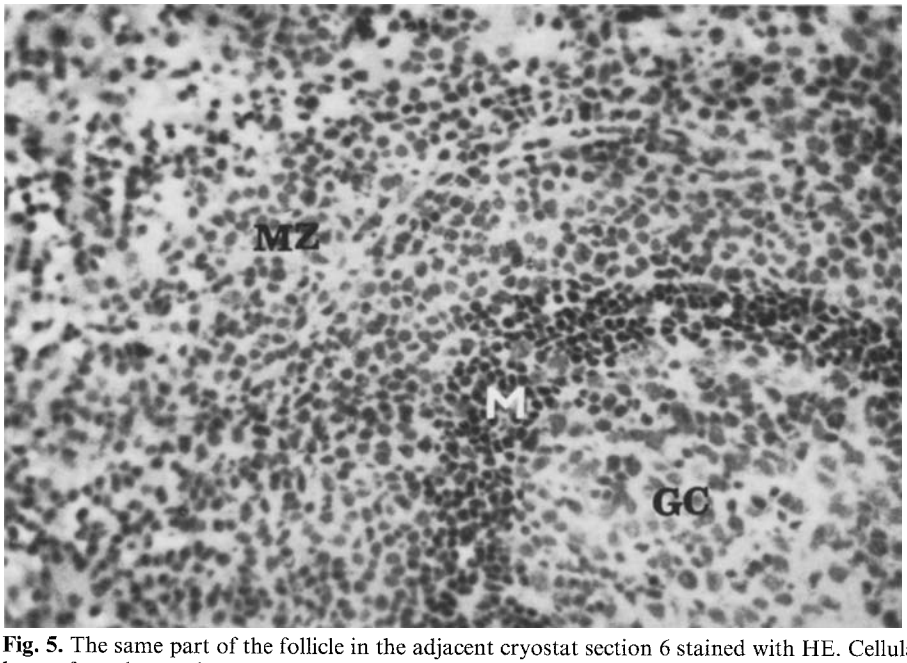

Fig. 5. The same part of the follicle in the adjacent cryostat section 6 stained with HE. Cellular layers from lower right to upper left: Large and medium-sized lymphocytes in the germinal center (GC). Small lymphocytes with dark nuclei of the inner mantle zone (*M*); medium-sized lymphocytes of the outer mantle zone and marginal zone (*MZ*). Visible light. × 400

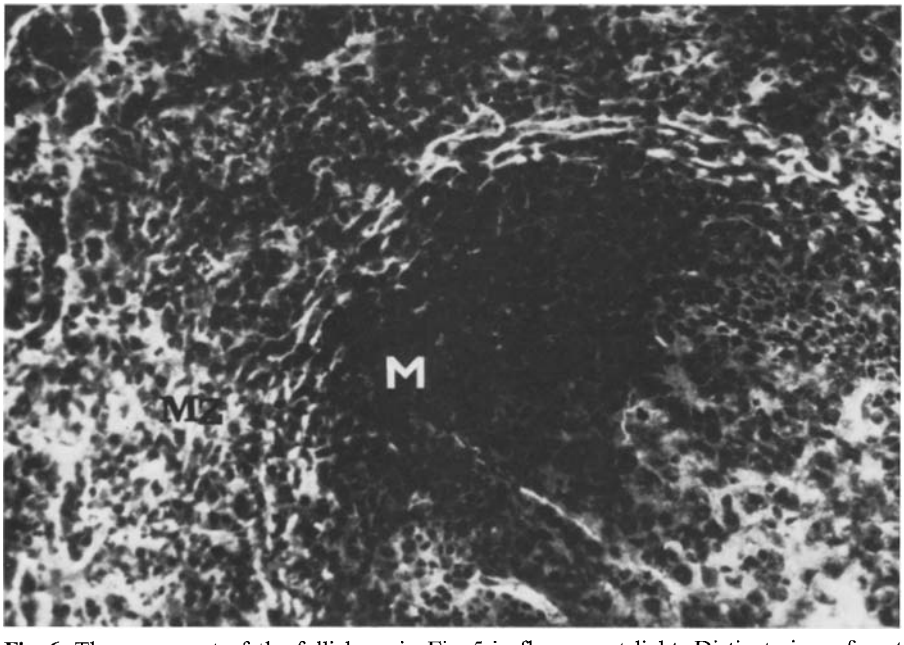

Fig. 6. The same part of the follicle as in Fig. 5 in fluorescent light. Distinct circumferential reticulum of the follicle between marginal zone (*MZ*) and mantle zones (*M*). ×400

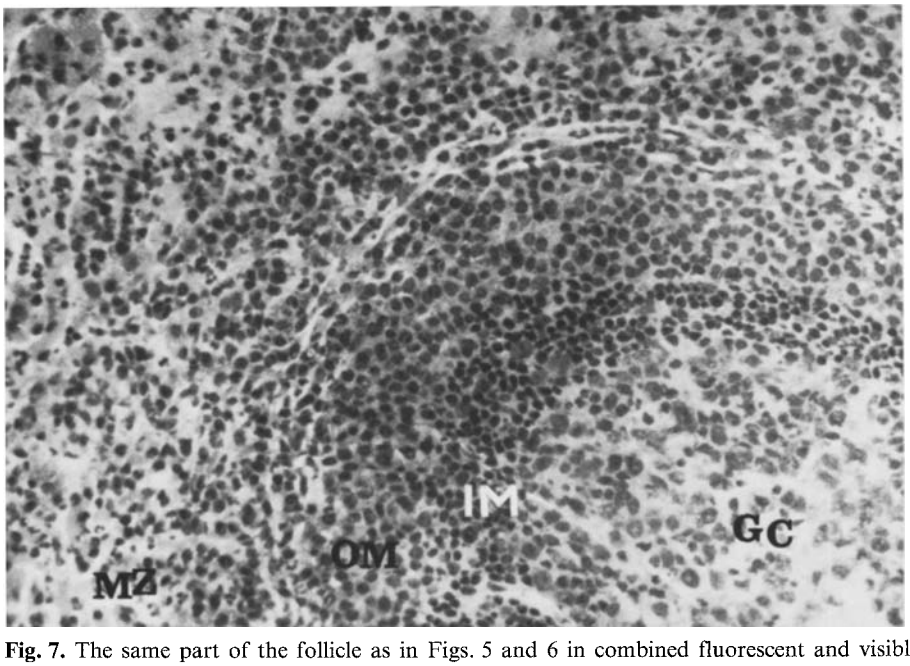

Fig. 7. The same part of the follicle as in Figs. 5 and 6 in combined fluorescent and visible. Distinct circumferential reticulum between marginal zone (*MZ*) and outer mantle zone (*OM*). *IM* inner mantle zone. *GC* germinal center. ×400

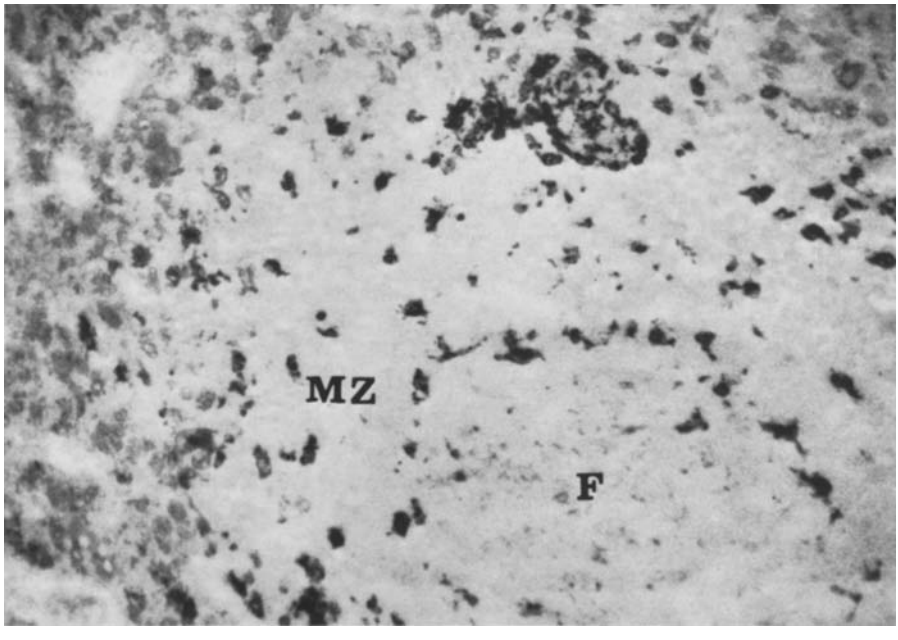

Fig. 8. Human spleen 292272 (female, 7-year-old). Strong activity of nonspecific esterase in individual and sheath cells in the marginal zone (*MZ*). F follicle. ×400

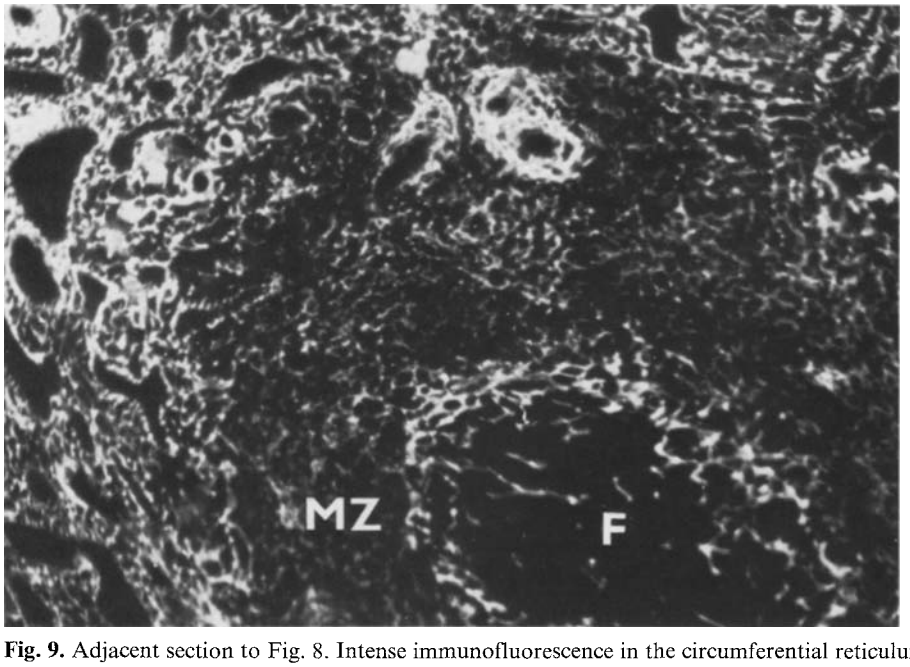

Fig. 9. Adjacent section to Fig. 8. Intense immunofluorescence in the circumferential reticulum between marginal zone (*MZ*) and follicle (*F*). Stained as in Fig. 1. ×400

The medium-sized lymphocytes were found to occur on both sides of the CR, more densely on the periphery of the follicle and less around it. Hence the "ring" of medium sized lymphocytes of the fully developed follicle is split into an inner layer belonging to the follicle and an outer "layer" belonging to the marginal zone.

"Marginal zone macrophages", giving in the human spleen a very intensive reaction to nonspecific esterase, were mostly seen only outside the follicular CR (Fig. 8), sometimes forming a kind of margin around the follicle, which could be clearly identified on adjacent sections submitted to immunofluorescent staining by means of anticartilaginous antiserum (Fig. 9) and by demonstration of nonspecific esterase. Such a pronounced activity of nonspecific esterase was exhibited not only by individual "marginal zone macrophages" but also by enzyme positive cells accumulated in pericapillary sheaths. In the intrafollicular zone of medium-sized lymphocytes such findings were exceptional.

Cells containing cytoplasmic Ig, particularly IgM, were also identified. These occurred very rarely in the intrafollicular layer of medium-sized lymphocytes, and somewhat more frequently, yet still sporadically, in the extrafollicular area. Erythrocytes were seen in the former layer exceptionally, but in the latter they were numerous.

Discussion

At the periphery of the lymphatic follicles of the human spleen, follicular CR was visualized by means of anticartilaginous antiserum and was found to be the direct continuation of the CR of the periphery of PALS. In man, the outer lamellae of this reticulum are considered to be the borderline between PALS and the marginal zone. We propose that everything bounded by the CR could be considered to be a component of the follicle (Fig. 10).

In fully developed follicles two basic parts have so far been commonly distinguished: the germinal center and the mantle zone. The hitherto conceived mantle zone consists of small lymphocytes and is surrounded on the periphery by the marginal zone, in which medium-sized lymphocytes are considered to be the main cellular component (Veerman and van Ewijk 1975). These so-called marginal-zone cells are said to represent antibody forming cell precursors (B_2 cells) involved in the formation of plasma cells (Nieuwenhuis and Keuning 1974; Müller-Hermelink and Lennert 1978). At is this zone, containing medium-sized lymphocytes, that has been shown to be divided into two parts by the follicular CR. In the part that remained intrafollicularly, medium-sized lymphocytes were found almost exclusively and these were densely accumulated, with other cell elements occurring only singly among them. In this intrafollicular part erythrocytes, also identifiable in normal histological preparations, were rare or absent.

The term of mantle zone given to the layer of small lymphocytes shoud be extented to the layer of medium sized lymphocytes located inside the CR. Hence the mantle of fully developed follicle consists of two layers

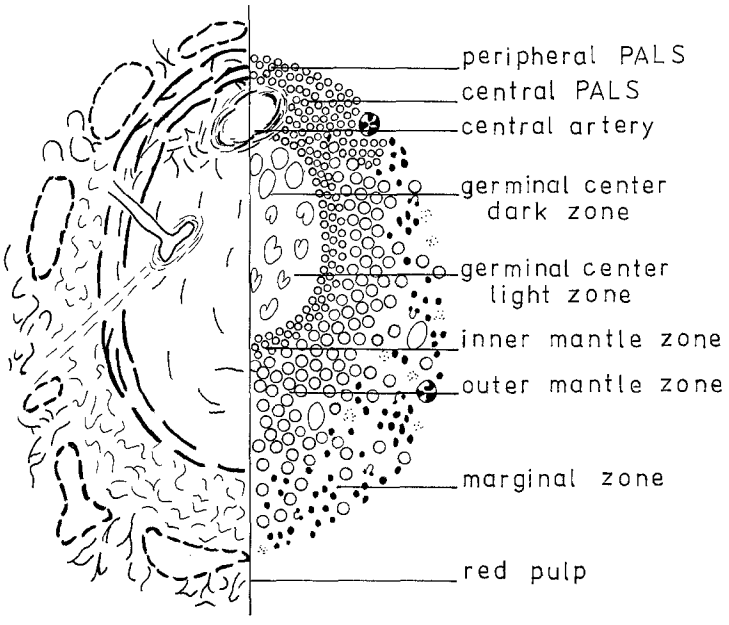

Fig. 10. Diagram of the fully developed follicle in the human spleen. *On the left:* visualization of extracellular structures. *On the right:* the main cellular (lymphoid) components: In the central PALS T-lymphocytes, in the peripheral PALS T- and B-lymphocytes, in the dark zone of the germinal center centroblasts, in the light zone centrocytes, in the inner mantle zone small B-lymphocytes, in the outer mantle zone medium-sized B-lymphocytes, in the marginal zone mixture of lymphoid and other blood cells

of different cells located in the same intra follicular reticulum and limited by the CR. We propose to call these two layers the inner mantle zone characterized by small lymphocytes and the outer mantle zone, characterised by medium sized lymphocytes. Symmers (1978) has coined a similar terminology concerning the lymph nodes, stating that the fully developed lymphoid follicle consists of the germinal center and one or more mantle zones.

In the human spleen, Saitoh et al. (1982) divided the marginal zone into two parts. They found that the inner half layer of the perifollicular region outside the mantle zone of the lymph follicle was composed of medium-sized lymphocytes, with a small number of reticular cells interspersed. In agreement with our results, they failed to find the mixture of lymphocytes and other blood cells, which is characteristic for the marginal zone (Blue and Weiss 1981), yet, they considered this inner half layer as part of the marginal zone. The outer half layer was composed of a reticular cell meshwork containing blood cells in wessels, which communicated with the splenic cords of the red pulp. Intermittent rows of reticular cells distinguished the outer from the inner half layer (Saitoh et al. 1982).

The denomination inner marginal zone and outer marginal zone was used before by Nieuwenhuis and Keuning (1974) in their studies of the germinal centers of the rabbit, in which they attributed both zones to the

follicular structures. They reported that 24 or 48 h after the intravenous administration of a booster dose of antigen large numbers of lightly labeled large pyroninophilic cells were observed in the spleen in the outer marginal zone. Yet this finding was suggestive of the functional differences between the inner and outer marginal zone. Functional differences can also be inferred from the analysis of labelled lymphocyte distribution within the marginal zone and PALS (Brelińska and Pilgrim 1983). These data indicate that labelled lymphocytes within the marginal zone accumulate asymmetrically in two subcompartments: in the internal layer in contact with PALS and in the external layer in contact with the red pulp.

Studies of nonspecific esterase activity have shown that the highest activity is displaced by "marginal zone macrophages". These were found only outside the CR in the zone corresponding to the outer half layer of the boundary zone of Saitoh et al. (1982) in the human spleen and to the outer margina zone of Nieuwenhuis and Keuning (1975) in rabbits. Enzyme positive cells were few in both the inner and the outer mantle zone. The differences between the inner and outer layer of the marginal zone indicates a different involvement of each layer in the migration of lymphocytes and in the processing of foreign material by macrophages and or other cells in the spleen.

Our investigations show that the inner layer of the classical marginal zone indicate that it does not belong to the marginal zone but to the follicle, forming its outer mantle zone, whereas the marginal zone proper consists only of the outer layer of the classical marginal zone. The borderline between the follicle and the marginal zone is formed by the outermost fibres of the follicular CR.

The newly delimitated marginal zone is characterized by a delicate uniform, histologically but slightly distinct reticulum, resembling more the reticulum of the red than that of the white pulp, and mixture of lymphocytes and other blood cells, thus meeting the requirements for the cellular characteristic of the marginal zone (Blue and Weiss 1981). The newly delimitated outer mantle zone is characterized by densely accumulated medium-sized lymphocytes and by a very sparse, coarse reticulum, resembling the reticulum of the other parts of the lymphatic follicle (Fig. 10).

The outer mantle zone of the follicle surrounds the inner mantle zone consisting of densely accumulated small lymphocytes, and from the outside it is bounded by the follicular CR which is the continuation of the peripheral PALS. The follicular CR is well marked in the region of the "arterial" pole where follicular arteriole enters, yet at the opposite pole it can be narrowed to 1–2 discontinous layers. However, even in these instances there can be but little doubt concerning the outer boundary of the follicle and thus the peripheral delimitation of the outer mantle zone.

Evidently, when the outer mantle zone is not developed, the follicle has only one mantle zone consisting of small lymphocytes, which corresponds to the hitherto accepted notion of the mantle zone of the follicle and the marginal zone.

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