Spleen

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The spleen is a secondary lymphoid organ present in all vertebrates. It is structurally complex and has a number of different functions such as immunological monitoring of bloodborne antigens, storage of blood and destruction of aged or abnormal blood cells. The importance of single functions and, in consequence, the microanatomy of the spleen vary among different species.

Introduction

The spleen is a secondary lymphoid organ present in all vertebrates. Its most primitive version is found in cyclostomes, where the splenic tissue is part of the gut wall. In higher vertebrates the organ parenchyma is divided into two large compartments, the white pulp and the red pulp, which are distinguished by colour in fresh organ sections at low magnification. The white pulp harbours dense and highly organized accumulations of B and T lymphocytes around arterioles, while most of the red pulp consists of blood-filled spaces. These red pulp spaces are composed of two different structures. First, the splenic sinuses represent a specialized part of the vasculature connecting arterioles and veins. They are not present in all species. Second, the splenic cords are strands of loose connective tissue without endothelium filled with all types of blood cells, macrophages and plasma cells. **See also**: Lymphatic system; Lymphoid system

In mammals, the spleen has three main functions: first, it represents a large mass of organized lymphatic tissue passed by recirculating lymphocytes, which are able to promptly elicit specific T or B lymphocyte-mediated immune reactions against antigens carried by the blood. Due to its 'open' type of circulation, bloodborne antigens have a more direct access to the splenic lymphatic tissue than to the tissue of other lymphatic organs. Second, the splenic red pulp has a filtering function for the blood. This function comprises the removal of material that can be phagocytosed by red pulp macrophages, including aged or abnormal red blood cells or microorganisms and leucocytes covered with immune complexes. Third, in some mammalian species, but not in humans, the spleen serves as a reservoir of erythrocytes, which are transfused into the circulation on sympathetic stimulation. In humans, only thrombocytes are normally pooled in the spleen. **See also**: Erythrocytes; Platelets

Histology

The microscopic structure of the spleen has been most thoroughly investigated in mice and rats. The nomenclature for different microanatomical compartments and different splenic macrophage populations was coined in rats. Thus, in the following paragraphs splenic microanatomy is first described in this species and then in humans. The overall morphology of mouse spleens is similar to that of rats. With respect to the so-called 'compartments' of the spleen, it has to be kept in mind that a large proportion of lymphocytes are migratory cells. Lymphocytes arrive via the blood, migrate into a compartment to settle down for some time and leave again into the blood. This process is called lymphocyte recirculation. It supports the detection of antigens and the spreading of immune responses in the body. Thus, the spleen is an organ of variable cellular composition. **See also**: Lymphocytes; Lymphocytes: recirculation

White pulp of rat spleen

The splenic white pulp (**Figure 1**) is divided into three compartments, where either T or B lymphocytes predominate. The T-cell zone is called the periarteriolar lymphatic sheath (PALS). There are two further compartments primarily inhabited by B lymphocytes, the follicles and the marginal zone (MZ). **See also**: B lymphocytes; T lymphocytes: helpers
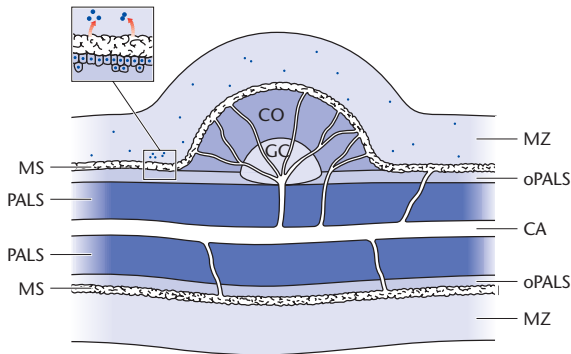
Periarteriolar lymphatic sheath

Different branches of the splenic artery enter the hilum of the spleen and give rise to 'central arterioles'. This name indicates that there is a thick, sleeve-like accumulation of small T lymphocytes around the arterioles, which is called the PALS. The T lymphocytes settle down in a meshwork of specialized fibroblasts, which exhibit an unusual phenotype. Most of the T cells in the PALS are CD4+, while CD8+ T cells form a smaller population. Besides T lymphocytes, the PALS harbours evenly distributed MHC class II+ bone marrow-derived dendritic cells with long cytoplasmic extensions. These cells are called the interdigitating dendritic cells (IDCs). It is assumed that they represent the decisive cells presenting peptide antigens for the induction of primary T-cell immune reactions. Dendritic cells and cells of the monocyte–macrophage system have a common ontogeny. However, the exact phenotype and function of IDCs have been a matter of recent debate.

Figure 1 Schematic drawing of a longitudinal section through the rat splenic white pulp. The central arteriole (CA) is accompanied by a broad periarteriolar lymphatic sheath (PALS) predominantly inhabited by T cells. The outer PALS (oPALS) also serves as a recirculation compartment for stimulated B cells. The germinal centre (GC), the corona/mantle zone (CO) and the marginal zone (MZ) are B-cell regions. PALS, germinal centre and corona are separated from the marginal zone by the marginal sinus (MS). The inset demonstrates that the inner wall of the marginal sinus is lined by marginal metallophilic macrophages while the outer wall is leaky and permits extravasation of particles and blood cells. The borderline separating the MZ from the red pulp does not exist and is only drawn for didactic reasons. Reproduced with permission from Steiniger and Barth (2000). Copyright © 2000 Springer.

The PALS is subdivided into an inner and an outer part. Although T cells prevail, the outer PALS is also populated by B lymphocytes, which pass this compartment during their migration from the MZ to the follicles. In addition, macrophages of a special phenotype are located in the outer PALS. It has to be expected that – besides IDCs – special macrophages also reside in the inner PALS, but this has not been investigated in detail up to now. **See also**: Dendritic cells (T lymphocyte stimulating); Major histocompatibility complex (MHC)

Follicles

Follicles are hemispherical accumulations of B lymphocytes, which are attached to the PALS at larger intervals. Primary follicles exhibit a uniform internal structure. They consist of small recirculating B lymphocytes expressing immunoglobulin M (IgM) and IgD. These B cells pass through a network of resident follicular dendritic cells (FDCs). **See also**: Follicular dendritic cells (B lymphocyte stimulating)

If the migratory B lymphocytes meet a specific antigen (i.e. the antigen recognized by their cell surface immunoglobulin), which is retained in the form of immune complexes on the surface of FDCs, they are arrested within the follicle. The specific B cells proliferate and after some time they are subjected to a hypermutation mechanism of their immunoglobulin genes and they then enter a differentiation and selection pathway. As these B cells are much larger than the recirculating B lymphocytes and have pale-staining nuclei and abundant cytoplasm, they give rise to a so-called 'germinal centre' within the follicle. The expanding germinal centre displaces the small, dark recirculating B lymphocytes to the periphery of the follicle. In sections, the recirculating B cells thus form a dark ring-like structure called the mantle zone or corona. Follicles with a germinal centre are termed secondary follicles. They only arise when specific B-cell immune reactions occur in the spleen and their existence is of limited duration. Germinal centre B cells are surface IgD−, while mantle zone B cells coexpress IgM and IgD. **See also**: Germinal centres; Somatic hypermutation in antibody evolution

A full-blown germinal centre comprises two zones, the dark zone and the light zone. In rat spleens these zones are more difficult to distinguish after routine haemalum-eosin staining than in human organs. The dark zone is oriented towards the PALS and harbours centroblasts, proliferating B cells with sparse cytoplasm and large nuclei. The light zone, oriented towards the red pulp, is populated by centrocytes. These cells have smaller nuclei, more cytoplasm, and are less densely arranged than centroblasts. They are supposed to be nondividing differentiating B cells arising from centroblasts, which are on their way to become plasma cells or memory B cells. **See also**: Immunological memory

Apart from B lymphocytes and capillaries, splenic primary and secondary follicles are composed of FDCs, macrophages, some ill-defined fibroblasts and CD4+ T cells. Most authors regard FDCs as sedentary cells arising from the local connective tissue. However, it is still debated whether FDCs immigrate from the bone marrow during early embryogenesis. FDCs have an extraordinary capacity to retain antigen–antibody complexes on their cell surface for prolonged times. They are assumed to attract migratory B cells towards primary follicles and they play a decisive role in B-cell differentiation within germinal centres. Macrophages are contained in every primary follicle. In secondary follicles these cells develop into large so-called 'tingible body macrophages'. Their cytoplasm is filled with the nuclear remnants of phagocytosed apoptotic B cells, which have not survived the mutation and selection process in the germinal centre. CD4+ T cells also occur evenly distributed within primary follicles. In secondary follicles CD4+ T cells tend to localize more densely at the circumference of the germinal centre bordering the mantle zone. **See also**: Macrophages

Marginal zone (MZ)

The PALS and the follicles are surrounded by the MZ, which separates both compartments from the splenic red pulp. The MZ is a broad region primarily occupied by relatively large memory B cells. It gives a light impression after routine staining, because memory B cells have paler nuclei and more cytoplasm than the T cells of the PALS or the B cells of primary follicles. The MZ is delimited from the PALS and the follicles by a very irregular capillary blood vessel called the marginal sinus. The endothelium of

the marginal sinus towards the MZ is rather leaky, permitting access of particulate materials, antigens, some red cells and recirculating lymphocytes to the MZ. The MZ memory B cells have a distinctive cell surface immunoglobulin phenotype by expressing IgM, but no or only minor amounts of IgD. **See also**: B lymphocytes

Besides memory B cells, two types of macrophages are associated with the MZ, the MZ macrophages and the marginal metallophilic macrophages. The MZ macrophages are scattered in the MZ, while the marginal metallophilic macrophages form a row of cells accompanying the marginal sinus. In mice, both types of macrophages can be distinguished by specific monoclonal antibodies. In rats, such a distinction is not possible up to now. A characteristic feature of MZ macrophages and marginal metallophilic macrophages in rats is that these cells strongly express a cell surface adherence molecule of the immunoglobulin superfamily named sialoadhesin. The structure of the MZ must be maintained by a framework of fibroblasts. However, nothing special is known about these cells. The MZ represents the entry compartment for antigens and migratory lymphocytes to the white pulp. Thus, recirculating T and B lymphocytes traversing the MZ on their way to the PALS or to the follicles, respectively, are always present. **See also**: Immunological adhesion and homing molecules

The MZ is clearly distinguished from the surrounding splenic red pulp due to its densely arranged lymphocytes, but there is no special border between both compartments. The spaces of the MZ are not lined by endothelial cells although they contain scattered red cells. Thus, similar to the cords of the splenic red pulp, the MZ may be regarded as a part of the 'open' circulation of the spleen. In contrast to the splenic cords, the MZ only allows a limited access of erythrocytes. Side branches of the central arterioles cross the MZ and reach the red pulp. These vessels are called 'MZ bridging channels'.

Red pulp of rat spleen

The splenic red pulp consists of the splenic cords and the splenic sinuses (also named sinusoids). The cords represent the 'open' part of the splenic circulation, which is formed by strands of loose connective tissue occupied by lymphocytes, plasma cells, macrophages, granulocytes, red cells and thrombocytes. Terminal arterioles of the red pulp arising from the central arterioles, directly open into the splenic cords. In the cords the blood moves in highly irregular spaces, which lack endothelial cells, to finally enter the splenic sinuses from the outside. A proportion of the blood may also directly enter the open beginnings of red pulp veins. The splenic sinuses are specialized vessels, which are assumed to be connected to other terminal branches of central arterioles. They are regarded as the 'closed' part of the splenic vasculature. However, the walls

of splenic sinuses are lined by endothelia with intercellular slits permitting entry of red and white cells from the cords. These specialized endothelia exhibit a phenotype different from all other endothelia in the body. In addition, the sinus-lining cells are not attached to an ordinary basement membrane. Instead, they stick to strands (also called ring fibres) of basement membrane-like material, which are oriented like 'hoops' around a barrel. This construction necessitates a very special arrangement of microfilaments within the sinus endothelial cells. The splenic sinuses finally deliver their contents into the trabecular veins of the spleen. In contrast to humans and other species, the splenic capsule and the trabeculae, which branch from the capsule into the red pulp, are not very prominent in rats. Both structures consist of specialized fibroblasts. **See also**: Circulation in vertebrates

A large number of macrophages and T lymphocytes are evenly distributed all over the splenic red pulp. B lymphocytes, however, tend to accumulate around small red pulp arterioles.

White pulp of human spleen

Periarteriolar lymphatic sheath

Humans exhibit much more interindividual variation in the size of different white pulp compartments than inbred laboratory rats (**Figure 2**). This may be due to 'outbred' genetic background, age, health condition, medical intervention
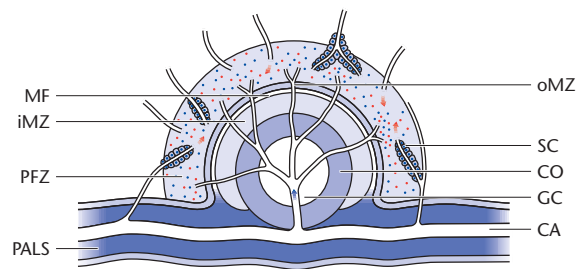
Figure 2 Schematic drawing of a longitudinal section through the human splenic white pulp. The central arteriole (CA) is accompanied by a relatively sparse periarteriolar lymphatic sheath (PALS). Germinal centre (GC), corona/mantle zone (CO) and marginal zone (MZ) represent B-cell regions. The marginal zone is subdivided by myofibroblasts (MF) into an inner marginal zone (iMZ) and an outer marginal zone (oMZ). Recent results indicate that the iMZ may be a recirculation compartment similar to the corona/mantle zone. The stippled area at the junction of fibroblasts and PALS indicates that CD4+ T cells may accompany the fibroblasts and that the fibroblasts continue into the outer PALS. The vascular structures in the perifollicular zone (PFZ) are hypothetical: sheathed capillaries (SC) and capillaries from the germinal centre may deliver their blood into open spaces. Distinct borders between corona and marginal zone and between marginal zone and perifollicular zone are absent due to lymphocyte migration. Borderlines are only depicted for didactic reasons. Capillaries and zones of the follicle are not drawn to scale. The 'central arteriole' may also traverse the corona/mantle zone or the germinal centre. Reproduced with permission from Steiniger and Barth (2000). Copyright © 2000 Springer.

before removal of the organ and other causes. There have been very few comprehensive investigations of human splenic microarchitecture using immunohistological techniques. The findings mentioned below primarily relate to spleens removed from young patients aged 18–35 years, who were splenectomized because of severe trauma in accidents.

In contrast to rats, the arterial tree in human spleens is more highly branched. Larger arterioles are associated with a broad PALS, while the smaller vessels only have a very thin layer of T cells. The most conspicuous compartment of the white pulp are the follicles and not the PALS, as found in rats. The term 'central arteriole' is very difficult to apply in humans, because the PALS may be totally interrupted by follicles. Thus, arterioles may lose their T-cell sheath, run across germinal centres or follicular mantle zones and then regain a sheath (**Figure 3d**).

The cellular composition of the PALS is not much different in humans and rats. The PALS contains mostly CD4+ T lymphocytes and less CD8+ cells. Large MHC class II+ IDCs are also present. A meshwork of specialized fibroblasts (formerly called 'fibroblastic reticulum cells') forms a major part of the internal structure of the PALS, especially in the outer PALS. These cells produce adhesion molcules and chemoattractants (so-called chemokines) for recirculating B and T lymphocytes. Interestingly, the fibroblasts continue into the MZ of the splenic follicles (see below, **Figure 3b**). **See also**: T lymphocytes: cytotoxic; T lymphocytes: helpers

Follicles

Human splenic follicles have a globular egg-shaped form instead of the hemispheric arrangement seen in rats (**Figure 3a**–**d**). There are two major differences in comparison to rats. First, in humans memory B cells are predominantly associated with the follicles; only a very thin row of B cells, which may be absent altogether, accompanies the PALS (**Figure 2**). Second, there is no marginal sinus in humans. Thus, the different follicular compartments are less distinct in humans than in rats.

In human spleens of the age described above, follicles often occur as primary follicles. Secondary follicles may be found, but in most of these the germinal centres are small and have a uniform interior without dark or light zones. In such cases the mantle zone is rather broad (**Figure 3b**). Full-blown germinal centres exhibiting both zones and an asymmetric mantle zone are mostly detected in children or only on special occasions. Such germinal centres are only associated with a small mantle zone (**Figure 3a** and **c**). Similar to rats, CD4+ T lymphocytes, FDCs and tingible body macrophages are regular components of germinal centres. **See also**: Germinal centres

There are several phenotypic differences among the B lymphocytes in the germinal centre and in the mantle zone. For example, germinal centre B cells are positive for CD38, but negative for IgD. IgM expression by germinal centre B cells is low, but this is difficult to distinguish due to IgM immune complexes retained on FDCs. In contrast, mantle zone B lymphocytes coexpress IgD, IgM and CD23, but are negative for CD38. **See also**: B lymphocytes

In humans, there is no clearcut border between the mantle zone and the MZ, because a marginal sinus is absent and marginal metallophilic macrophages do not occur. Thus, small recirculating mantle zone-type B lymphocytes (IgD$^+$, IgM$^+$, CD27$^-$) intermingle for some distance with memory phenotype B cells (IgD$^{+/-}$, IgM$^+$, CD27$^-$), which are located more superficially (**Figure 3a**–**d**). This may represent immigration or emmigration of recirculating testing B lyphocytes into or out of the mantle zone via the follicular surface. Thus, it is not clear, whether a MZ does exist in human spleens at all. The majority of the B cells at the surface of the mantle zone express IgM, but no or only minor amounts of IgD. They are larger than typical mantle zone B cells and their nuclei stain more lightly. In addition, strongly IgD+ B lymphocytes may also be found in the outermost follicular periphery and extend along the surface of the PALS. Whether these cells correspond to mantle zone-type recirculating B cells needs to be further investigated. The surface of many follicles is divided into an inner and an outer part by a thin layer of specialized fibroblasts arising from the PALS and exhibiting a specialized phenotype (**Figure 3b**). Interestingly, a row of CD4+ T lymphocytes often accompanies this fibroblast layer. Thus, in humans the follicles appear to be embedded in an extension of the PALS. As this phenomenon is individually variable, it may represent a consequence of CD4+ T cell migration from the follicular periphery to the PALS or vice versa. Interestingly, CD8+ lymphocytes are more or less absent from the splenic follicles. Macrophages are present at the follicular surface in humans, but in contrast to rat and mouse MZ macrophages they do not express sialoadhesin.

Perifollicular zone

In humans, there is an additional compartment surrounding the surface of primary or secondary follicles, the perifollicular zone. This zone is not found in rats. It might compensate for the lack of a marginal sinus as an entry compartment to the white pulp. The perifollicular zone is composed of small blood-filled spaces without endothelia. It may thus be attributed to the red pulp. However, there are some distinctive features of the perifollicular zone: it contains much more conspicuous accumulations of erythrocytes, granulocytes and monocytes than the remainder of the red pulp (**Figure 3d**). In addition, it harbours sheathed capillaries, a type of vessel that is lacking in rats or mice. The capillary sheaths in this zone consist of one or two layers of macrophages, which strongly express sialoadhesin and CD68 (**Figure 3c**). Additional capillary sheaths may occasionally occur in the red pulp, but these sheaths are
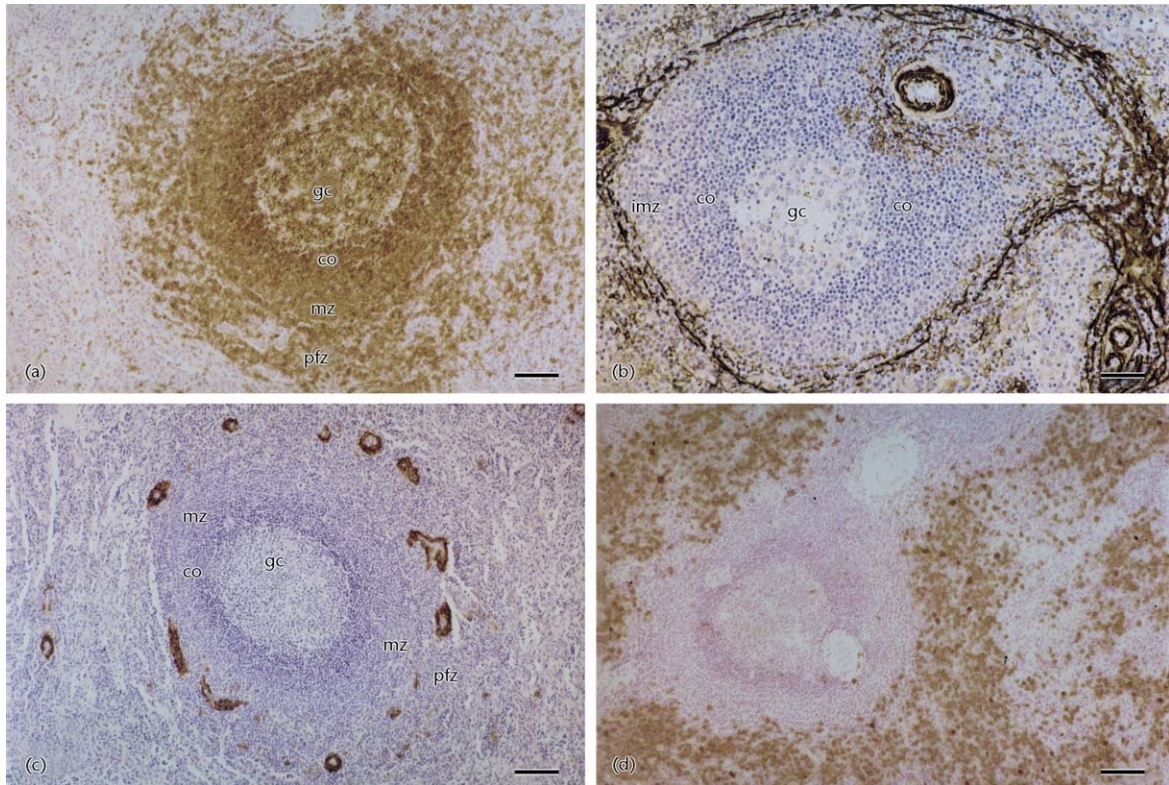
Figure 3 Peculiarities of the different zones of a secondary follicle in the human spleen. (a) Detection of B lymphocytes by staining of the CD20 surface antigen with monoclonal antibody (mAb) L26. Four distinct compartments are revealed: the germinal centre (gc) appears light due to the presence of unstained CD4+ T cells and follicular dendritic cells. The corona/mantle zone (co) is populated by smaller cells and stains more darkly because of the nuclear counterstain. The marginal zone (mz) is densely populated by larger CD20+ memory B cells with more cytoplasm and thus gives a paler impression. This area may not represent a separate compartment. The perifollicular zone (pfz) in this particular specimen harbours many CD20+ B cells intermingled with CD20− monocytes and neutrophils. The large unstained areas in the perifollicular zone represent capillary sheaths. A 15-year-old girl with splenic trauma. Avidin–biotin complex (ABC) technique on paraffin section, diaminobenzidine (DAB) chromogen. Bar = 80 μm. (b) Detection of smooth muscle α actin by mAb asm-1 in a paraffin section. A row of actin-positive fibroblasts divides the inner marginal zone (imz) from the outer marginal zone. Typical appearance of a secondary follicle in healthy adults with a remnant of a germinal centre (gc) and a broad corona/mantle zone (co). The myofibroblasts continue into the outer PALS at the right margin of the figure. A 29-year-old female patient with splenic trauma. ABC technique, DAB chromogen. Bar = 50 μm. (c) Sheathed capillaries demonstrated in the perifollicular zone (pfz) by strong staining with mAb HSN1 against human sialoadhesin in a paraffin section. The capillary sheaths are closely associated with primary and secondary follicles and clearly occur outside the marginal zone (mz). They surround capillaries seemingly approaching the follicle from the red pulp, which branch in the perifollicular zone. (The staining in frozen sections is more widespread and also shows weak reactivity with red pulp macrophages.) gc and co indicate germinal centre and corona/mantle zone. Same specimen as in (a). ABC technique, DAB chromogen. Bar = 80 μm. (d) Expression of CD15 in the perifollicular zone demonstrated by mAb 28. Monocytes and granulocytes are positive. These cells accumulate outside the clear marginal zone. The staining in the red pulp (right part of figure) is less dense. ABC technique on cryostat section. A 53-year-old female patient with gastric malignancy. DAB chromogen. Bar = 100 μm. Reproduced with permission from Steiniger and Barth (2000). Copyright © 2000 Springer.

difficult to distinguish, because they are negative for sialoadhesin. Examination of a large number of human spleens gives the impression that the sheath macrophages are motile and that capillary sheaths may dissolve and be reconstituted. Direct proof of this process is lacking, but in some spleens strongly sialoadhesin-positive macrophages are found scattered all over the perifollicular zone, while sheaths cannot be detected. The function of capillary sheaths in humans is unknown. The perifollicular zone may also be occupied by major numbers of memory phenotype B lymphocytes ($IgD^{+/-}$ IgM^+ $CD27^{+/-}$) which resemble phenotypically the B lymphocytes of the (**Figure 3a**).

Red pulp of human spleen

The facts mentioned for the splenic red pulp of rats also apply to humans. In humans, the phenotype of the sinus endothelial cells is also very special. For example, these cells express the α chain of CD8. The functional significance of this phenomenon, which is not found in rats, is unknown.

Function of Splenic Compartments

The function of splenic white pulp compartments during immune reactions has almost exclusively been investigated

in rats and mice. Thus, it is unknown how far the results may be extrapolated to humans. In rats and mice the most thoroughly examined function of the spleen is the antibody response against 'T-cell dependent' protein antigens, which is primarily described below.

Recirculation

The recirculation of lymphocytes forms the basis of splenic immune function. In rodents, recirculating B and T lymphocytes first enter the MZ. Whether this is accomplished by adhesion to the outer endothelium of the marginal sinus or by other mechanisms, is not clear. From the MZ the B lymphocytes move into the outer PALS and then into the mantle zone of secondary follicles. If antigen is not encountered, the B cells leave the spleen again via the red pulp. This passage may take more than 18 h in rats. T lymphocytes, on the other hand, directly head for the PALS, where they settle for some time before leaving via the red pulp. The passage of recirculating T lymphocytes through the spleen is much faster than that of B lymphocytes and takes about 4–5 h. Thus, immune reactions may spread to the spleen from other sites in the body by recirculation of primed T cells. This fact is implicit in the sequence of events described and not considered separately. **See also**: Lymphocytes: recirculation

Priming of T cells in the PALS

The first step during a primary antibody response against a protein antigen in the spleen is the 'priming' (i.e. the first stimulation) of naive CD4+ T lymphocytes by antigen-presenting MHC class II+ dendritic cells in the PALS. Until recently, IDCs were considered to fulfil this function, but there is now some controversy about the exact phenotype of the presenting cells. Ligation of the antigen-specific T-cell receptors of CD4+ T cells by MHC class II molecules carrying bound peptides on dendritic cells, ligation of the CD4 coreceptor molecules and of additional costimulatory molecules on the T-lymphocyte surface produce signals which in concert activate the T-lymphocytes and lead to their proliferation. **See also**: Antigen-presenting cells; Major histocompatibility complex: human; Major histocompatibility complex: interaction with peptides; T-lymphocyte responses: development

Formation of extrafollicular foci in the outer PALS

T lymphocytes activated by dendritic cells move to the outer PALS. At this site they encounter preactivated recirculating B lymphocytes, which may have bound and internalized their specific antigen in the blood (or somewhere else) via their surface immunoglobulin. Besides dendritic cells, B cells are also able to present antigen to CD4+ T lymphocytes, but this is only effective if the T cells have already been primed. Thus, activated B and T lymphocytes are arrested in the outer PALS, if they recognize the same antigen. A mutual stimulation via costimulatory molecules and cytokines ensues, which is summarized as T-cell 'help'. Adequate help from T cells leads to B-lymphocyte proliferation and differentiation into short-lived antibody-secreting cells in the outer PALS and around the MZ bridging channels. These accumulations of B cells are called 'extrafollicular foci'. The B cells persist for only a short period of about 10 days after a single antigen injection and then die by apoptosis. The immunoglobulin produced at extrafollicular foci has a low affinity for the antigen. It forms a decisive prerequisite for the subsequent development of germinal centres.

Formation of germinal centres

Antigen–antibody complexes formed by the immunoglobulin produced at extrafollicular foci and other extrafollicular locations localize on the surface of FDCs in primary follicles. FDCs capture such complexes by help of Fc and complement receptors and retain them for months or even years. Primed B cells passing through the follicle on recirculation are arrested when they meet their cognate antigen. They proliferate and start a hypermutation process of those parts of their immunoglobulin genes that code for the antigen-binding portions of their surface immunoglobulin. This process may lead to improved binding affinity for antigen and to positive selection of the respective B cells in the follicle. The hypermutation of immunoglobulin genes is thought to be confined to germinal centres. Thus, hypermutated immunoglobulin can be used to identify B cells having passed a germinal centre. **See also**: Affinity of antigen–antibody interactions; Follicular dendritic cells (B lymphocyte stimulating); Somatic hypermutation in antibody evolution

The first step of germinal centre formation is the accumulation of antigen-specific B-cell blasts, which displace the recirculating nonspecific B cells to the periphery of the follicle, where they form the mantle zone or corona. Usually there are 3–6 'founder B cells', i.e. germinal centres arise oligoclonally. At some stage of this process CD4+ T cells specific for the same antigen are attracted into the arising germinal centre from the outer PALS. The germinal centre further develops a dark zone with dividing centroblasts and a light zone with nondividing centrocytes and FDCs. Centroblasts which have survived in the dark zone move into the light zone and differentiate into centrocytes. Survival of centrocytes is only possible when these cells are positively selected according to the affinity of their surface immunoglobulin for antigen. A part of this process may occur by the release of membrane fragments carrying immune complexes from FDCs. These fragments are taken up by centrocytes via their surface immunoglobulin and

the antigen is intracellularly processed into peptides. Display of antigenic peptides in the context of centrocyte cell surface MHC class II molecules activates the CD4+ antigen-specific T lymphocytes in the light zone, which then provide help for the B cells. There is a competition for T-cell help by the centrocytes and only high-affinity immunoglobulin-producing B cells will present enough peptides to receive positive signals from the T cells. Besides cytokines and other costimulatory signals, engagement of two receptor–ligand pairs, CD28 (on T cells) and CD80 or CD86 (on centrocytes) and, in addition, CD154 (on T cells) and CD40 (on B cells) decides about survival or death of centrocytes. Interactions of these and other costimulatory molecules also support B-cell isotype switching and determine the further differentiation of centrocytes. **See also**: Antibody responses: development

B-lymphocyte differentiation towards plasma cells or memory cells

Positively selected centrocytes have two alternative choices: they differentiate either into plasma cells or into memory B cells. Long-lived plasma cells (as opposed to the short-lived antibody-secreting cells in the extrafollicular foci) arise after preprogrammed centrocytes have left the germinal centres via the blood and settled down in the tissues. In the spleen such plasma cells reside in the red pulp. The majority of the preprogrammed centrocytes from the spleen will, however, migrate to the bone marrow to accomplish terminal plasma cell differentiation. **See also**: Immunological memory; Lymphocyte development

Memory B cells have a lower threshold for activation and differentiation to plasma cells and a longer lifespan than other B cells. In addition, the antigen-binding hypervariable regions of their surface immunoglobulin exhibit an increased affinity for antigen due to accumulated mutations in comparison to germline-encoded immunoglobulin. A certain proportion of the B cells preprogrammed towards memory leave the germinal centres and join the pool of recirculating B cells. A decisive interaction in the light zone that predetermines a centrocyte for memory cell differentiation and prevents plasma cell development is the binding of CD154 (CD40 ligand) to CD40 on the B cells. It is likely that centrocytes repetitively pass through the compartments of germinal centres. Thus, the structure of germinal centres may often be less obvious than described above. In long-standing germinal centres, dark and light zones are often no longer distinguishable.

Localization of memory B cells to the MZ

The MZ of the spleen is a rather intricate compartment, because in rodents it harbours at least three different types of B lymphocytes either defined by phenotype or by specificity. First, memory B cells arising in T-lymphocyte-dependent immune reactions localize to the MZ. In rodents and humans memory cells have hypermutated surface immunoglobulin and most of them have switched to expression of IgG, IgE or IgA. Second, in mice and humans a special memory B-cell population continues expressing IgM with hypermutated V-regions and does not switch to other immunoglobulins. These B lymphocytes are sometimes called 'MZ-phenotype B cells' or 'IgM-only memory B cells'. In human spleen sections the expression of IgD on these cells is variable, while in the blood they may be either IgD+ or IgD−. Rodent memory cells recirculate in the blood for some time and then preferentially settle down in the splenic MZ. In rats and mice they are rather sessile after having arrived in this compartment. It is under debate whether this is also true for humans. In humans CD27, a member of the tumour necrosis factor receptor family, is the most widely used surface antigen for identification of memory B cells. CD27+IgM+IgD+ lymphocytes represent about 15% of all blood B cells, while CD27+IgM+IgD− cells amount to about 2%. It remains to be investigated whether – in contrast to rodents – human memory B cells recirculate through the surface of splenic follicles. Finally, the third MZ B-cell population is defined by its specificity. These cells also express IgM and recognize, among other antigens, polysaccharides of bacterial capsules, so-called T-cell-independent type 2 (TI-2) antigens. Whether these cells represent memory B cells is under debate. B cells specific for TI-2 antigens are responsible for certain 'natural' or polyreactive antibodies, which are sometimes auto-reactive. TI-2 antigens consist of a backbone of polysaccharide with repetitive recognition sites (epitopes) for immunoglobulin. Why antibody responses against TI-2 antigens proceed with only minimal T-cell help is not entirely known. Non-T cells, for example macrophages or dendritic cells, may provide costimulatory signals for plasma cell differentiation. On the other hand, bacterial TI-2 antigens differ from T-dependent antigens by directly activating complement and may thus be additionally bound by complement receptors on MZ B cells. **See also**: Antibody classes; Antigens: thymus-dependent; Antigens: thymus-independent

In rodents all types of MZ B cells may be driven into fast and almost simultaneous plasma cell differentiation during emergencies caused by a systemic rise in specific antigen or by less specific agents such as lipopolysaccharide, certain cytokines or drugs. In this case, the B cells leave the MZ, migrate to the outer PALS, turn into plasmablasts and finally accumulate in the red pulp or in the bone marrow as plasma cells. Whether additional passages through germinal centres are necessary before memory B cells for T-cell-dependent antigens start plasma cell differentiation, is still controversial. Anyhow, MZ B cells and their human equivalent thus represent a decisive cell population for the sepsis-protective effect of the spleen.

Function of the red pulp

The open part of the splenic red pulp circulation, i.e. the splenic cords, form a large area where red or white blood cells and thrombocytes, but also bloodborne microorganisms or foreign material come into direct contact with macrophages, because there is no endothelial barrier. Thus, any cell or particle exhibiting an altered or foreign surface will be removed, if the surface is able to initiate phagocytosis. Especially cells or material covered by immune complexes will be bound and engulfed with the help of macrophage Fc and complement receptors. In addition, macrophage mannose or scavenger surface receptors may also play a role. In humans, macrophage activation and proliferation may contribute to a condition called hypersplenism, which leads to a massive enlargement of the splenic red pulp with sequestration of red cells, leucocytes and thrombocytes. Blockade of cell passage through the open circulation pathways by abnormal red cells, as found in spherocytosis, malaria or sickle cell disease, may also provoke hypersplenism. Hypersplenism also occurs when passage through the red pulp is impeded for other reasons, for example as a consequence of increased pressure in the portal vein (portal hypertension) or as a consequence of leukaemia, lymphoma and inherited metabolic diseases. Hypersplenism may lead to a severe and untreatable reduction of all types of cellular elements of the blood (pancytopenia) necessitating partial splenic embolization or splenectomy. **See also**: Phagocytosis

The surroundings of splenic red pulp arterioles are somehow involved in B-lymphocyte differentiation. In addition, the red pulp harbours plasma cells and large numbers of diffusely arranged natural killer cells and T lymphocytes. Knowledge about red pulp function with respect to these cell types is, however, almost nonexistent.

Consequences of Splenectomy in Humans

Total splenectomy should be avoided whenever possible, because there are several severe consequences of an asplenic state. Thus, partial splenectomy or partial arterial embolization of the spleen has been advocated. **See also**: Spleen: consequences of lack of function

Total splenectomy may lead to an increase in leucocyte and thrombocyte blood counts to more than normal values in some, but not in all patients, depending on the indication for organ removal. This is especially relevant in hypersplenic states with increased thrombocytopoiesis, because overt thrombocytosis and a risk of thromboembolic complications may ensue. In addition, loss of the spleen provokes a defect in IgM antibody production against TI-2 antigens such as capsular polysaccharides of bacteria. In addition, there are reports that CD27 + IgM + IgD + /−

memory B cells are severely reduced in the blood of asplenic children, but this has not been an unequivocal finding in all studies. The current controversy concerns the question whether (and which) human memory B cells have to spend a compulsory 'transitional' stage of development in the spleen or whether they also differentiate outside this organ. There are indications that human IgM + memory B cells are a unique population which does not even need germinal centres or T cells for differentiation and hypermutation of surface immunoglobulin. These cells may receive differentiation signals by a previous sojourn in the gut-associated lymphatic tissue. Such a hypothesis is supported by the detection of MAdCAM-1 in human splenic fibroblasts mentioned above, because MAdCAM-1 is a decisive adhesion molecule for lymphocyte recirculation through mucosal tissues.

In Western countries splenectomy produces an increased risk of sudden overwhelming sepsis caused by *Streptococcus pneumoniae*, *Neisseria meningitidis* and *Haemophilus influenzae*, especially in children. However, *Escherichia coli* also plays a role. There are several explanations for the overwhelming postsplenectomy infection (OPSI) syndrome. First, in terms of quantity, the splenic marginal zones are the most important sites in the body harbouring B lymphocytes specific for bacterial capsular polysaccharides. These B cells may also occur in other secondary lymphatic organs, but in smaller numbers. Thus, antibody responses against encapsulated bacteria tend to be reduced, but not totally absent after splenectomy. Second, the spleen provides a unique and direct contact of bloodborne antigens with memory B cells via the perifollicular zone. Third, removal of the spleen abolishes one of the largest compartments for phagocytosis of opsonized bloodborne bacteria (bacteria covered with immune complexes and/or complement).

At present, international or national guidelines for prophylaxis of the OPSI syndrome do not exist. In Germany, a review published in 1997 recommends polyvalent vaccination against pneumococci for adults either before splenectomy or afterwards in cases of emergency operations, with a booster after 5–6 years. In high-risk patients vaccination against *H. influenzae* is also advisable. Long-term prophylaxis with antibiotics is not used in adults. In children, vaccination against pneumococci and haemophilus is also recommended. However, there is a natural defect in antibody production against encapsulated bacteria under the age of 2 years which is explained by incomplete development of splenic MZs. Thus, in children long-term antibiotic treatment with penicillin should be given until the age of 5 years or longer, depending on the disease necessitating splenectomy. Vaccination against meningococci is optional. The practice in North America has been recently summarized and is almost identical. Although pneumococcal polysaccharide vaccines are apparently effective in splenectomized patients, so-called conjugate vaccines are more recommendable, because they

provoke T-cell-dependent antibody production. OPSI may, however, occur at all ages in spite of vaccination and antibiotic prophylaxis. Thus, adequate information is important for splenectomized patients and their relatives in how to behave in case of febrile infection. **See also**: Antimicrobials against streptococci, pneumococci and enterococci

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