Chapter 1

GENERAL INTRODUCTION

Partly based on: Timens W, Leemans R. Splenic autotransplantation and the immune system: adequate testing required for evaluation of effect.
1. HISTORICAL REVIEW

The spleen has been a mysterious organ for ages, and has often been a subject of study. In ancient times the spleen was thought to be related to the digestive system. Erasistratus believed that the spleen maintained the symmetry of the abdomen, but had no further function. Plato claimed that its function was to keep the liver "bright and shining". Hippocrates proposed a vital balance of four essential humours of the body: blood, phlegm, golden bile and black bile. In spite of the lack of anatomical and histologic knowledge in this period, he had described the anatomy of the spleen with remarkable accuracy. The description of function, however, was described different from that of today; the liver was supposed to be the source of golden bile and the spleen of black bile. Galen believed that "humours unsuitable for its nutriment are discharged by the spleen through a canal into the stomach". He called the spleen: "Splenum mysterii organon".

During the 17th and 18th century the main contributions to the study of the spleen consisted of careful anatomical dissections. In 1777 William Hewson recognized associations with the lymphatic system. Rudolph Virchow demonstrated in 1846 that the follicles in the spleen were related to the white blood cells and in 1885 Ponfick recognized the ability of the spleen to remove particles from the blood. About thirty years later, Morris and Bullock described the spleen as an important organ in the resistance to infections. O'Donnell reported a case of "acute septicemia" in a 6-year-old boy 2 years after splenectomy in 1926. The boy's father who had had a splenectomy in 1919 also died "of septic pneumonia, manifesting a similar lack of resistance to the disease". The role of the spleen in resistance against infections was discussed by Perla and Marmoston in 1935. It was only after the publication of King and Shumacker on postsplenectomy infections in 1952, that there was a rise in concern over the decrease in resistance against infections as a consequence of splenectomy. After this publication the immunological aspects of the spleen became increasingly the targets of scientific interest.

2. THE SPLEEN

Anatomy and histology

Gross

The spleen is a soft, vascular lymphatic organ with roughly the size of a clenched fist and with the shape of a bean. It contains the largest aggregation of lymphoid tissue in the body and it has a central position in the mainstream of the blood vascular system. The size of the spleen of an adult varies from 12 to 15 cm in length, 4 to 8 cm in width and 3 to 4 cm in thickness. The average weight is about 140 g. in the adult female and about 180 g. in the adult male. It lies in the shelter of the 9th to 11th rib at the left side of the abdominal cavity (fig. 1).
The spleen is soft in consistency and friable and is shaped by adjacent, firmer viscera. Together with the visceral peritoneum it forms strong suspensory attachments to the stomach (gastrosplenic ligament), diaphragm (phrenicosplenic ligament), kidney and pancreas (splenorenal or pancreaticosplenic ligament), colon (phrenicocolic ligament) and sometimes with a peritoneal fold to the abdominal wall on the left posterolateral aspect. A long fissure can be seen on the medial side of the spleen; this forms the hilus and is the site of the main entrance and exit for the blood vessels.

Around the spleen is a strong fibrous capsule with collagenous trabeculae extending into inside the pulp. The splenic parenchyma can be divided into white and red pulp as can be seen on fresh surgical specimens.

**Histology**

The spleen consists of two general components: the white pulp (± 5-20%) and the red pulp (± 85%), enclosed by a capsule and interspersed by trabeculae. 

![Anatomy of the upper abdomen with respect to the spleen.](image)
**Capsule**

The capsule is composed of dense connective tissue with a few smooth muscle cells. This reflects the minimal contractile capacity of this capsule in man (in dogs it is highly contractile). Serosa covers the capsule except at the hilus where vessels enter the spleen. From the inner surface of the capsule a branching network of trabeculae subdivides the spleen into communicating compartments. These trabeculae carry the blood and lymph vessels into the splenic pulp.

**White pulp**

The white pulp is composed of 3 major compartments that are easily recognized in routinely stained histological sections (fig. 2). These compartments are the periarteriolar lymphocyte sheet (PALS), the lymphoid follicle (LF) and the marginal zone (MZ)\textsuperscript{10,11}. The PALS is the T-lymphocyte compartment of the white pulp in which T-lymphocytes are interspersed in concentric layers of stromal cells around a central artery. The lymphocytes of the PALS are mostly recirculating cells. The PALS is a site of T-cell clonal expansion. A small percentage of the T-cells within the PALS are in an activated state, demonstrated by IL2-receptor (CD 25) expression. The other T-cells are in a resting state\textsuperscript{10,11}.

![Fig. 2](image.png)
The LF of the spleen are globular structures attached to the PALS with similar structure as lymphoid follicles in other lymphoid organs\textsuperscript{12}. They can be differentiated into primary and secondary LF. The primary LF consists of a homogeneous aggregate of small B-cells in an inactivated state. Upon activation the primary LF will become a secondary LF with a germinal centre (GC) surrounded by a small rim of remaining small B-cells, the lymphocyte corona (LC). This germinal centre of the secondary LF consists of differentiated B-cells (centroblasts and centrocytes), a few T-cells and follicular dendritic reticulum cells. In the lymphoid follicles a special type of dendritic cells (follicular dendritic cells) is found, which are able to bind immune complexes. They can maintain immune complexes on their cell surface for a long period without phagocytosis. This seems to play a role in the down-regulation of the production of plasma cells\textsuperscript{13}. Germinat centres provide a site for rapid proliferation of B-cells with isotype switching and affinity maturation. Upon maturation of the germinal centre, cell division stops and cells differentiate into memory cells or plasma cells, which acquire the ability to migrate out of the germinal centre\textsuperscript{14}. A specific type of mononuclear macrophages is present, that phagocytose defective lymphoid cells and debris in the germinal centre\textsuperscript{14}.

The germinal centre is surrounded by a small border of small lymphocytes (in fact the pre-existent cells of the former primary follicles); this is called the corona or mantle zone. The corona in its turn is enclosed by medium-sized lymphocytes, mostly B-cells, and this is called the marginal zone (MZ). The MZ is an anatomical demarcation between the white pulp and the red pulp. The real border is formed by the perifollicular zone\textsuperscript{15}. A great part of the arterial circulation within the MZ terminates intercellularly and at the outer side of the MZ sinuses are present, which are smaller than the sinusoids of the red pulp. The MZ provides an environment, which by its low blood flow allows a prolonged and intimate contact between antigens in the bloodstream and the lymphocytic system\textsuperscript{11,14,16}.

Lymphoid cells in the MZ have been demonstrated to possess surface immunoglobulin (mainly IgM, in absence of IgD), as well as receptors for Fc-fragments and complement factors (C3b and C3d)\textsuperscript{11,14,16}. It is because of this low flow in combination with a specific type of B-cells that the MZ is supposed to have an important role in the primary immune response to T-cell independent antigens type 2 (TI-2 antigens) like the polysaccharide encapsulated bacteria e.g. pneumococci, meningococci and haemophilus influenzae. The marginal zone is a distinct anatomical lymphocyte compartment in the spleen with unique immunohistological features\textsuperscript{11}.

**Red pulp**

The red pulp consists of a loose reticular tissue rich in capillaries and venous sinusoids. These sinusoids comprise approximately 30% of the volume of the red pulp. They form a meshwork with many interconnections but also bulb-like extensions with blind ends projected into the cord tissue\textsuperscript{15}. The sinusoids have a unique endothelium of longitudinally arranged cells. These run parallel to the long axis of the sinusoids like the staves of a barrel and possess close junctional complexes at regular intervals along their lateral
surfaces to the white pulp veins. Slit-like spaces, which can be penetrated by cells flowing from the pulp cords, separate the endothelial cells. The basal membranes have been shown to contain actin and myosin which can probably contract to vary the tension in the endothelial cell and the dimensions of the interendothelial slits\textsuperscript{14}. The interendothelial slits are a critical point in the pathway of particulates through the spleen and in the filtration function. Part of the red pulp tissue has a reticuloendothelial nature with small aggregates of B- and T-lymphocytes and many mononuclear phagocytes. Morphometrically, the size of the lymphoid, non-filtering red pulp compartment seems to equal that of the white pulp\textsuperscript{17}. The macrophages are not simply phagocytic cells, but have also secretory capacities and enhance in this way the immunogenicity of antigens. They have the ability to produce components of complement factors, interferon, haematopoetic colony-stimulating factors and fibroblast stimulating factors. This whole system is part of the so-called mononuclear phagocytic system (MPS)\textsuperscript{18}.

Vasculation and innervation

As 5-10\% of the cardiac output at rest passes the spleen, the spleen has to be richly vascularized\textsuperscript{9,10}. The splenic artery is the largest of the three branches of the celiac artery, which originates from the abdominal aorta. After passing the upper body of the pancreas horizontally, giving a few branches to the stomach (left gastro-epiploic artery and short gastric artery) and pancreas (large pancreatic artery) the splenic artery divides into several branches about 3,5 cm before the spleen. These branches will divide further, into superior and inferior branches, subdividing into several smaller branches and finally enter the spleen in the hilus. Ramifications of the splenic arterial branches develop internally into trabecular arteries, which pass through the white pulp as central arteries, branches of which supply the lymphatic nodules in the white pulp. From the centre of the lymphatic node the artery can pass through to the red pulp or split into branches in the marginal zone. Via marginal zone sinuses the blood can also reach the red pulp\textsuperscript{6}.

The red pulp is assumed to have two systems for the blood circulation, which will be described under ”Microcirculation”. The venous drainage commences in the venous sinusoids, located in the red pulp, subsequently draining into trabecular veins. The trabecular veins terminate in branches that unite to form the splenic veins at the hilus of the spleen. The splenic vein passes along the dorsal and superior part of the pancreas and with the superior mesenteric vein becomes the portal vein. At a short distance before the superior mesenteric vein, the inferior mesenteric vein empties into the splenic vein\textsuperscript{6,7}. The lymphatic vessels in the spleen are few in number and not as extensively distributed as the blood vessels. Lymphatic capillaries originating in the splenic capsule and trabeculae converge in lymph nodes of the hilus outside the spleen and subsequently pass to lymph nodes along the splenic artery and the celiac axis\textsuperscript{6,7}.

The splenic nerve supply originates from the celiac plexus. It follows the splenic artery in the hilus and innervates the musculature of the branching vessels. Also preganglionic
parasympathetic fibres of the right vagal nerve follow the splenic artery into the spleen⁶.

**Microcirculation of the blood**

The microcirculation of the blood in the spleen is perhaps the most complex of any organ in the body. It contains blood with a packed cell volume twice that of arterial blood. Most studies of the microcirculation in the spleen have been performed in animals and the results were often extrapolated to the human spleen. It is not clear whether these results are sufficiently representative for the human situation, because the histology and subsequently the micro-anatomy of the human spleen seems to be different from spleens in animals¹¹,¹⁵,¹⁹. However, because of difficulties in investigation of the spleen in man, we have to rely on well designed animal experiments to provide useful hints in the elucidation of the complex mechanisms in the human spleen.

The spleen constitutes the only organ specialised for the filtration of blood. It has been suggested that there is a fast and a slow pathway of the bloodstream in the red pulp of the spleen for which two compartments are assumed to exist for this bloodstream within the spleen. The first system is the closed circulation with direct connection via the sinusoids and collecting veins to the trabecular veins (fig. 3). The second (more important) system is the open circulation with arterial vessels ending blindly in the red pulp cord spaces. From the cords the blood runs intercellular and is subsequently collected in sinusoids from which it will be transported by pulp veins to trabecular veins. The fast compartment is intra-vascular, whereas the slow compartment is in the reticular meshwork¹⁷,²⁰.

Some arterial capillaries of the red pulp show cyclic changes in luminal calibre, with sometimes a very low to absent flow. Erythrocytes pass through interendothelial slits in venous sinus walls always from the reticular meshwork into the sinuses²¹.

![Fig. 3](image-url) Schematic cross section of the spleen with blood circulation; c: corona, *: cords of Billroth (With permission from: The Human Spleen, W.Timens²²).
**Lymphocyte circulation**

A unique feature of lymphocytes, in contrast to all other cells of the blood, is their continuous migration between lymphoid and non-lymphoid organs through the lymphatic and blood vessels. Granulocytes and monocytes mostly remain in organs once they have left the bloodstream, but lymphocytes may temporarily leave the bloodstream and return to it at later stage (lymphocyte recirculation). This recirculation of lymphocytes is important for the ability to recognise antigens throughout the body and for the interaction between accessory and lymphoid cells in initiating immune reaction\(^\text{15}\). The extent of lymphocyte recirculation in the spleen by the blood far outweighs the total number of lymphocytes using the classical route via lymph vessels and thoracic duct. In a young adult man about 2,5x10\(^{14}\) lymphocytes recirculate through the spleen per day; which is approximately 8 times more than through all lymph nodes\(^\text{23}\). The lymphocytes enter the spleen through the arterial bloodstream and migrate to several splenic compartments. T-lymphocytes rapidly enter the central part of the periarteriolar lymphatic sheaths (PALS), while B-lymphocytes persist in more peripheral parts of the PALS and by 24 hours are evenly distributed throughout the corona. A few migrating B-cells are found in the germinal centres, but no T-cells. It is unknown as yet whether the venous route or the lymphatic route is the most important outflow for lymphocytes of the spleen; probably the venous route is more important than that via lymphatic vessels.

The exact migratory mechanisms and routes of the lymphocyte subsets through all the splenic compartments are very complex and have not yet been clarified\(^\text{10,14}\).

**Functions of the spleen**

The spleen is a unique organ in the immune defence system of the body. It is the only organ which can clear low opsonized antigens from the bloodstream and it is the only organ which is specialised in producing antibodies in a short time after contact with antigens. Besides this, the spleen is a true lymphoid organ with several organised lymphoid compartments.

Because of the central position in the blood stream and the large blood supply of about 5 per cent of the blood volume per minute, the spleen represents an important meeting point between antigenic information transported by the blood and the immune system. It possesses a wide range of the immune cell repertoire and its specific architecture allows unique functions. Two major critical functions of the spleen can be recognized: it serves as a large phagocytic filter and it is a major antibody producing organ.

**Filtration**

Filtration of the blood is the best known and a (quantitatively) important function of the spleen. The reticular meshwork in the red pulp with the terminal arterial vessels and the venous sinusoid are specialised for filtration of the blood. When blood passes the
endothelial wall of the sinusoids, bloodcells have to pass through the interendothelial slits (fig. 4). These slits are only small in diameter, hence during this passage the blood cells have to deform, subsequently to regain their normal form. If the cells lose their deformation capacity or if the cell walls are too fragile, the cells cannot pass through this filtration system. Erythrocytes with intracellular inclusions (pittings, Howell-Jolly bodies, intra-cellular organisms as malaria, etc.) can be cleared of these inclusions during the passage without destroying the entire cell. The membrane of the cells reseals and the cells pass into the sinuses and the general circulation. The perisinusoidal phagocytic cells will clear the inclusions and the aged bloodcells\(^{18,24}\).

Fig. 4 Passage of an erythrocyte to a sinusoid (With permission from: Immuno-architecture of regenerated splenic and lymph node transplants, R.Pabst, J.Westermann, H.J.Rothkötter\(^{20}\)).
Phagocytosis of foreign particles can be promoted by interaction with opsonins, serum factors which enhance their uptake by specific phagocytosing cells. The spleen has a prominent role in the generation of opsonins. Splenic phagocytes, together with macrophages in the liver, synthesise the majority of components of the classical pathway of complement. Generation of specific antibody is primarily dependent upon the spleen. The role of the spleen in phagocytosis of foreign particles is particularly important for non- or badly opsonized particles. Whereas the mononuclear phagocyte system (MPS) in the liver is the main site of phagocytosis of opsonized particles, the spleen is the major organ for the phagocytosis of non-opsonized particles. In experiments in rabbits, the phagocytosing capacity for non-opsonized particles appeared to be sixty times as effective in spleen as in liver when corrected for weight (reviewed by Lockwood). The unique microvasculature of the spleen supposedly contributes to the specialised function of the spleen in the phagocytosis of insufficiently opsonized particles. The greatly retarded blood flow in the red pulp cords allows a very intimate and prolonged contact between antigens and phagocytes. Thus, particles can be ingested without specific ligand-receptor interactions. An important practical implication of this specialised phagocytosing capacity is that the spleen is the most important site of clearance in the early phase of bacterial invasion before sufficient amounts of specific antibody have been produced. This is particularly important for blood-borne, T-cell independent type 2 antigens like polysaccharide encapsulated micro-organisms (e.g. pneumococci).

Another special feature of the spleen is the generation of tuftsin, originating from the Fc-fragment of IgG. This is a tetrapeptide reported to exert stimulatory effects on activity and migration responses of phagocytic cells.

The spleen also plays a part in the alternative complement pathway. Complement factors work synergistically with antibodies in promoting phagocytosis of bacteria. In the presence of the complement factor C3b the immunoglobulin (Ig) opsonization degree required for phagocytosis is decreased 100-fold. Moreover, lysis of bacteria can take place by complement factors only too. In the primary immune response to TI-2 antigens C3d is also an important factor and a high density of C3d-receptors is found in the marginal zone of the spleen.

The spleen is the site of IgM specific antibody generation very early after exposure to blood-borne antigen. The first contact of antigens entering the spleen via the blood and immunocompetent cells occurs in the marginal zone, a structure exclusively present in the spleen. This marginal zone is unique in its microvasculature, enabling a very low blood flow, in the presence of specialised macrophages antigen presenting cells and a subset of intermediate-sized B-cells with a specific phenotype: IgM+, IgD-, and strongly CD21+11,26,31,32. Although B-cells with this phenotype can be found in other lymphoid tissues, the splenic marginal zone contains the largest accumulation of this type of B-cells in the body. When the blood enters the marginal zone sinusoids, there is a considerable increase in flow area diameter, with a subsequent decrease in blood flow. Similar as in the
red pulp a sluggish flow results; this enables a close contact between antigens and phagocytes or lymphoid cells, and between different cell subtypes involved in the immune response.13,26

Because the spleen is a major lymphoid organ it also plays an important role in the primary humoral and secondary immune response. After encountering antigens in the MZ-sinuses, antigen-specific B-cells migrate to the lymphoid follicles.14 From here they can either differentiate to produce antibodies (plasmacells) or to B-memory cells. The primary immune response is very important and can provide antibody production within 6 hours after the first contact with an antigen. The MZ B-cells are particularly well equipped for rapid and easy activation in a primary immune response.32

The spleen is also of importance in the secondary immune response, because the formation of memory cells of B- and T-lymphocytes is especially promoted by the spleen.22,25,27

The spleen is specifically involved in the immune response to thymus-independent antigens type 2 (TI-2 antigens). These antigens, generally polysaccharides, are the antigenic component of encapsulated bacteria such as Streptococcus pneumoniae, Haemophilus influenzae and Neisseria meningitides. The immune response to TI-2 antigens is characterised by the need of T-cell produced factors, although it is independent of the actual presence of T-cells.33 After splenectomy this response is significantly decreased or even absent. The initiation of the response to polysaccharide antigens (TI-2 antigens) takes place in the splenic marginal zone. In rodents, TI-2 antigens were found to localise specifically on antigen-presenting cells in the marginal zone and the elimination of MZ cells abrogated the immune response to such antigens.35 As described above, marginal zone B-cells have a distinct immunophenotype. By their high expression of CD21, the receptor for complement fragment C3d, MZ B-cells play a specific role in the immune response to TI-2 antigens, as these antigens are able to bind C3d.26,29 In this way, TI-2 antigen-C3d complexes can bind to - and activate marginal zone B-cells.26,29

Other functions

The spleen has a reservoir function for a large number of all kinds of blood cells by means of a process that is not yet understood. This storage function is mainly for thrombocytes, but also for erythrocytes and lymphocytes. Of all thrombocytes in the body about 30% may be stored in the spleen. The spleen is the largest lymphoid organ with 25% of all white blood cells of the body, mainly lymphocytes. Only 5% of the red cells are supposed to be stored in the spleen.

The number of blood cells in the spleen at a given time depends on the presence or absence of pathology in the spleen and/or of the blood cells. In the reticular meshwork the haematocrit of the blood is twice that of arterial blood. The spleen appears to function as a "nursery" for reticulocytes after their release from the bone marrow,21 and is supposed to play a role in final maturation. Reticulocytes have a reduced negative surface charge, are less flexible, contain unneeded organelles and are bigger than mature red cells. The reticulocytes will be sequestered in the red pulp for two days because of these properties.
thus allowing them to mature. After maturation the erythrocytes will be remodelled and then emerge into the circulation\textsuperscript{37}.

Additional known functions of the spleen are haematopoiesis during fetal life, a positive effect on factor VIII serum levels, inhibition of serum angiotensin-converting enzyme activity, and participation in reutilization of iron from destroyed erythrocytes\textsuperscript{27,36}.

It is even suggested that the spleen has a role in lipid metabolism, with lower HDL-cholesterol and higher triglyceride serum levels after splenectomy\textsuperscript{37}.

A summary of the functions of the spleen is given in table I.

### Table I Functions of the spleen\textsuperscript{16,27,38}

<table>
<thead>
<tr>
<th>White Pulp</th>
<th>Red Pulp</th>
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</thead>
<tbody>
<tr>
<td>- Antibody synthesis</td>
<td>- Filter function</td>
</tr>
<tr>
<td>- Initiation of humoral response</td>
<td>- Phagocytosis (in particular)</td>
</tr>
<tr>
<td>- to -TI2 antigens badly opsonized particles)</td>
<td>- Reservoir of thrombocytes and immature erythrocytes</td>
</tr>
<tr>
<td>- Reservoir of lymphocytes</td>
<td>- Haematopoiesis (fetal life)</td>
</tr>
<tr>
<td></td>
<td>- Tuftsin production</td>
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<tr>
<td></td>
<td>- Role in alternative complement pathway</td>
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<tr>
<td></td>
<td>- Positive effect on factor VIII</td>
</tr>
<tr>
<td></td>
<td>- Reutilization of iron</td>
</tr>
<tr>
<td></td>
<td>- Inhibition of angiotensin-converting enzyme</td>
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</tbody>
</table>

### 3. CONSEQUENCES OF SPLENECTOMY

Although incidental reports mentioned a relationship between splenectomy and infection, it was not until 1952 that a causative association was reported between splenectomy (for congenital haemolytic anaemia) and the occurrence of meningitis with sepsis\textsuperscript{5}. Since then the increased risk of infection and septicemia directly related to splenectomy has been well defined in the literature. Such infections are now generally termed “Overwhelming Post Splenectomy Infections” (OPSI).

In most cases the OPSI syndrome is caused by one of the following micro-organisms: Streptococcus pneumoniae (50%), Neisseria meningitides (12%), Escherichia coli (11%), Haemophilus influenzae (8%) and Staphylococcus aureus (8%), but also by mycobacteria, viruses and parasites\textsuperscript{39,40,41}.

The frequency of OPSI is dependent on age and the cause of splenectomy. The highest frequencies were found after thalassaemia and Hodgkin’s disease and the lowest
frequencies after trauma. Singer came to an overall frequency of 4.25% with a mortality of 2.52%. In patients who had had a splenectomy for traumatic splenic rupture the mortality due to sepsis was 0.58%, after thalassaemia however it was 11.0%. In the total population the incidence of mortality due to sepsis was 0.01%. In a more recent review about OPSI in 12514 postsplenectomy patients (with 5902 sufficient reports), under 16 years of age an OPSI frequency of 4.4% was found with a mortality of 2.2%, but for adults these figures were 0.9% and 0.8% respectively. Overall there was 3.6% morbidity and 1.8% mortality.

The highest incidence of OPSI is generally found in infancy and childhood. Patients who have undergone splenectomy for haematologic diseases, reticuloendothelial diseases or portal hypertension have a higher incidence than those undergoing splenectomy for trauma.

Most frustrating is the high overall mortality rate of OPSI of about 50%. As indicated above after-splenectomy there is a significant decrease in the primary immune response to bacterial capsular polysaccharide antigens. These antigens belong to the group of TI-2 antigens, and other antigens of this type also give a similar decreased immune response after splenectomy. Another cause for the increased risk of OPSI is a decrease in phagocytic activity, in particular with respect to phagocytosis of poorly- or non-opsonized antigens. After a splenectomy the phagocytic function will be partly taken over by the liver. However, the liver needs a higher level of antigen opsonization. This may present an important problem especially with respect to thymus independent type 2 antigens like encapsulated bacteria which are badly opsonized, in particular because also the spleen dependent specific TI-2 antibody response is hampered. A lower phagocytic activity also results from decreased tuftsin concentrations after splenectomy. The general ability to generate a specific antibody response after the first contact with a blood born antigen, the primary immune response, is also reduced. This is consistent with a low production of IgM after splenectomy.

The alternative complement pathway also seems to be reduced after splenectomy, with normal functioning of the classical pathway.

After splenectomy the ability of the body to filter the blood will be reduced which results in an increase of erythrocytes with inclusions like vacuoles and Howell-Jolly bodies, and with surface pits. The ability to remove intracellular organisms such as malaria and bartonella is also reduced. The loss of splenic maturation for reticulocytes causes a high percentage of immature erythrocytes and reticulocytes in the bloodstream.

Another, less important impairment that can have consequences is a decreased reservoir function for blood cells. There will be an increase of thrombocytes and a prolonged residence time of lymphocytes in the blood shortly after splenectomy. However, after a few months the thrombocytosis seems to be reduced to normal.

The effects of splenectomy in humans and animals are summarised in table II.
4. PRESERVATION OF SPLENIC FUNCTIONS

Spleen salvage techniques

From the time the OPSI syndrome was recognized to be related to splenectomy, it still took several years before attempts were made to diminish the risk of infection. One approach has been the introduction of spleen-saving techniques. Several techniques have been described concerning the management of the various degrees of splenic rupture. For an evaluation of this techniques Shackford et al. published a grading system which was modified for clinical use\textsuperscript{53,54,55}. Later on the grading of the American Association for the Surgery of Trauma (AAST) came in use\textsuperscript{56} (table III).

There is a wide variety of techniques aimed at splenic preservation\textsuperscript{57}, being summarised in table IV and figure 5.

First of all the non-operative treatment with close observation should be considered. This is only possible in haemodynamically stable patients who are conscious and without associated abdominal injury. Mostly these patients are kept on the intensive care with frequent checking the circulatory parameters. Blood samples are taken regularly for evaluation of haemoglobin and haematocrit and sonography or CT-scan of the abdomen should be performed. This regime can be applied quite safely in patients with a capsular tear (grade I or II rupture). Traub, Wiig and Pearl et al. described good results of this therapy\textsuperscript{58,59,60}. Cogbill et al. published a multi-centre study involving 112 splenic injuries, treated by nonoperative management\textsuperscript{61}. In 13 cases a laparotomy was needed (5 splenectomies and 8 splenic salvage procedures) without mortality. Based on this experience they extended their criteria for selective nonoperative management of blunt splenic injuries even to class III.

### Table II: Effects of splenectomy

<table>
<thead>
<tr>
<th>Immunological</th>
<th>Non-immunological</th>
</tr>
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<tbody>
<tr>
<td>- Reduced phagocytic activity of badly opsonized antigens</td>
<td>- Reduced filter function</td>
</tr>
<tr>
<td>- Decreased tuftsin formation</td>
<td>- Increase of reticulocytes</td>
</tr>
<tr>
<td>- Lower IgM serum level</td>
<td>- Increase of platelets</td>
</tr>
<tr>
<td>- Prolonged residence time of lymphocytes in blood</td>
<td></td>
</tr>
<tr>
<td>- Reduced alternative complement pathway activity</td>
<td></td>
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<tr>
<td>- Increased auto-antibody activity</td>
<td></td>
</tr>
<tr>
<td>- Diminished numbers T-suppressor cells</td>
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CHAPTER 1

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Table III. Modified grading system of splenic ruptures according to Shackford and the American Association for the Surgery of Trauma.

<table>
<thead>
<tr>
<th>GRADE</th>
<th>SHACKFORD</th>
<th>AAST</th>
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<tbody>
<tr>
<td>I</td>
<td>Localised capsular rupture without significant parenchymal injury.</td>
<td>Haematoma subcapsular &lt; 10% surface. Laceration capsular &lt; 1 cm deep.</td>
</tr>
<tr>
<td>II</td>
<td>Localised capsular rupture with local parenchymal injury.</td>
<td>Haematoma subcapsular 10-50% surface or parenchymal &lt; 5 cm diameter. Laceration parenchymal 1-3 cm deep.</td>
</tr>
<tr>
<td>III</td>
<td>Parenchymal injury not extending into the hilus or involving major vessels.</td>
<td>Haematoma subcapsular &gt;50% or parenchymal &gt; 5cm. Laceration parenchymal &gt; 3cm or involving trabecular vessels.</td>
</tr>
<tr>
<td>IV</td>
<td>Severe parenchymal injury extending into the hilus or involving major vessels.</td>
<td>Laceration involving segmental or hilar vessels.</td>
</tr>
<tr>
<td>V</td>
<td>Completely shattered or fragmented spleen or separation from the blood supply.</td>
<td>Completely shattered spleen or devascularisation.</td>
</tr>
</tbody>
</table>

Table IV. Therapeutic strategies in the management of ruptured spleen.

- non-operative: observation.
- haemostatic agents: thrombin, gelatine foam, collagen cyanoacrylate adhesive.
- arterial ligation: main trunk, segmental vessels.
- splenorrhaphy: mattress sutures, omental wrap, absorbable net.
- partial splenectomy: stapling, laser, sutures.
- total splenectomy: autotransplantation, (splenosis).
Later on the nonoperative management of splenic injury has been described with a high success rate even in patients with other (neurologic) injuries. In the last 5 years the nonoperative management of splenic injury has become more accepted and got a place in the techniques of treatment. At present this approach is still a matter of debate because of the risk of delayed complications of nonoperative management of the splenic injuries.


For the operative treatment of spleen rupture various techniques can be used alone or in combination (see table IV page 25).

The use of haemostatic agents is one of the possibilities for treatment of splenic injuries. Coln et al. described a trial with Gelfoam®, Avitene®, Surgicel® and Collastat® in rabbits and the best results were archived with the use of Collastat®. In 1990 Krar et al. and Ochsner et al. used fibrin glue in patients with splenic and hepatic injuries, also with good results. Because of the risk of clot displacement this therapy seems to be suitable only
for minor injuries of the spleen or in combination with other techniques such as splenorrhaphy. It is suggested that there may be a place for fibrin glue in the laparoscopic approach of abdominal trauma. Although in a number of publications the laparoscopic approach was considered as valuable, this technique is still under discussion. Successful use of an argon beam coagulator in splenic injury has been described in an animal experiment with pigs, this technique being more effective in treating splenic injuries than the use of other conventional surgical techniques such as sutures, electrocautery, digital pressure and application of hemostatic agents. This technique seems favourable when combined with laparoscopy. Clamping the splenic arteries during the operation may provide a temporary arrest of bleeding. Ligation of the splenic artery is feasible in arterial bleeding, when this is not amenable to direct suture. Because the spleen is also nourished by the short gastric and left gastro-epiploic arteries, total necrosis of the spleen will be prevented. Ligation of the main artery is feasible and may permit splenic conservation, but because of reduction of the blood flow through the spleen the phagocytosis of badly opsonized particles will be diminished. This may lead to an increased risk of OPSI. Division of the main artery into several branches occurs outside the spleen and usually only the affected branches need to be ligated. Arterial embolisation has also been described as a technique to treat splenic rupture.

Suturing of the spleen can be performed by mattress sutures with or without an omental flap or haemostatic material. To avoid the danger of tearing, Teflon or other patches can be helpful. This technique is most satisfactory in children because they have a strong capsule, but can also be performed in adults. However, it remains a risky form of treatment, because of the fragility of the splenic tissue.

An elegant treatment of splenic injuries is that of splenorrhaphy by wrapping the spleen in an absorbable net. It is safe and can be performed together with the use of haemostatic agents. Although good results have been described by several authors, drawbacks do exist. These aspects will be further described and discussed in chapter 2 of this thesis. In selected cases it is possible to perform a partial splenectomy, for example in case of lesions in the lower pole of the spleen. This technique has been described with good results (also in own experience). Partial splenectomy is enabled by the segmental blood supply of the spleen. Most individuals have two primary lobar intrasplenic arterial branches, so upper or lower pole resection can be performed by the finger-fracture technique or with the use of a laser. Haemostasis of the section can be carried out by mattress sutures, stapling and CO₂ laser. Local haemostasis can also be obtained after ligation of upper- or lower pole arteries.

Whenever the above-described techniques fail to stop the bleeding of the ruptured spleen or when the rupture is too serious (grade V) a splenectomy has to be performed. This may be combined with autotransplantation of spleen tissue, as will be discussed later. If there are accessory spleens it is advisable to leave them in situ because it is very well possible
that accessory spleens can compensate for some of the impaired functions after splenectomy (discussed in chapter 6 of this thesis).

Several analyses have been reported about the decision processes when facing a ruptured spleen, resulting in decision algorithms96,97,98.

**Autotransplantation after splenectomy**

Splenosis peritonei is the outgrowth of small splenic particles everywhere in the peritoneal cavity due to dispersion of spleen particles in traumatic or iatrogenic rupture of the spleen. Griffini and Tizzoni described as early as 1883 areas of spontaneous splenic regeneration in the peritoneum of dogs that had undergone splenectomy99. A few years later it was also described in man, but incorrectly called accessory spleens100. Kuttner and Faltin considered this phenomenon to be the result of seeding of particles of the ruptured spleen101. This hypothesis was proven by Von Stubenrauch and Kreuter who deliberately sowed splenic pulp in the peritoneal cavity, resulting in a large number of small spleen implants which ultimately grew larger than the original particles100,102.

The condition of splenosis peritonei may have been the stimulus to study the possibilities and therapeutic benefits autotransplantation of splenic tissue. The expression “splenic autotransplantation” in this thesis represents the transplantation of a part (or all) of someone’s own spleen to a site in the body without formal direct connections to the vascular system, in this way distinct from the technique of vascularised autotransplantation103,104,105.

“Accessory spleen” means the presence of a congenital extra spleen somewhere in the peritoneal cavity. The frequency of this condition is thought to be about 18%106.

The term "splenosis" was first suggested by Buchbinder and Kiphoff in 1939, to describe areas of spontaneous splenic regeneration in the peritoneum after splenectomy for trauma107.

Pearson et al. reported a reduced percentage of "pitted" red cells in 13 of 22 children after splenectomy for trauma, suggesting a return of splenic function by splenosis108. Nielsen described a positive correlation between a low percentage of vacuolated erythrocytes and the presence of ectopic splenic tissue detected by Tc-scanning109.

Histological and immunohistochemical studies of splenosis suggested a normal structure of splenic tissue, nearly indistinguishable from normal splenic tissue110. It was even advised that spleen tissue resulting from splenosis should not be removed without a specific indication111.

This splenosis could explain the lower incidence of OPSI after splenectomy for trauma when compared with splenectomy for other reasons108. This led to the hypothesis that an autotransplantation at the time of splenectomy might restore at least part of splenic immune function. Experiments in this field were started already by Marine and Manley in 1920. Further studies were performed in animals, e.g. rats, mice, dogs and pigs and later in men to evaluate whether or not autotransplantation might provide (some) protection against OPSI113,114,115.

Studies were also performed with respect to the ideal site for transplantation (peritoneum, omentum, subcutis) and the quantity of splenic tissue that was needed (a few grams up to
a complete spleen) to reach optimal results\textsuperscript{113,114}.

Histological studies in rats have shown that regeneration of autotransplanted splenic tissue occurs in phases. First necrosis of the autotransplant will occur. Within a few hours after transplantation the fragments become necrotic, except for a small rim of reticular cells underneath the capsule. All lymphocytes in the transplant die, and only remnants of reticular cells and erythrocytes remains. In the course of the following days, capillaries and reticular fibres grow out to form a subcapsular vascular space. After a week the regenerating tissue differentiates into an outer and an inner zone, with reticulum cells and sinus like spaces in the outer zone. Lymphocyte immigration starts and the red pulp is formed about two weeks after transplantation. In the following weeks the typical white pulp

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{fig6.png}
\caption{Schematic drawing of autotransplant regeneration with approximate time points. CO: corona; F: follicle; GC: germinal centre; lym.acc.: lymphocyte accumulation; MZ: marginal zone; PALS: periarteriolar lymphatic sheath. (With permission from: Immunoarchitecture of regenerated splenic and lymph node transplants, R.Pabst, J.Westermann, H.J.Rothkötter\textsuperscript{24})}
\end{figure}
compartments appear in approximately the same order as during ontogeny. First the periarteriolar lymphocyte sheaths (PALS) are formed, followed by the follicles and the marginal zones.

About 3-6 months after implantation the autotransplants have attained their final structure with germinal centres, suggesting a functional capacity of the white pulp (see also fig. 6). The histology of the autotransplants after regeneration resembles that of normal spleens but this is misleading in that the relative size of the splenic compartments is different from that of the normal spleen. The PALS and the marginal zone are relatively reduced to 50% of that in the normal spleen and areas of fibrotic tissue do occur, mainly in the central parts of the transplants.

Although splenic tissue possesses the possibility of regeneration, it is not known which factors regulate this regeneration. Orthotopic spleen seems to have a suppressive effect on regeneration of autotransplants, but the suppressive or stimulatory factors are unknown. Pabst suggested a stimulating factor by increased workload to the splenic compartments.

The site of transplantation also seems to affect the results of the transplantation. Splenic fragments have been implanted in many different sites, e.g. subcutaneous, intramuscular, subperitoneal, mesentery, small intestines, kidney, and portal vein, but most often in a pouch in the omentum. The best site for implantation seems to be intraperitoneal, especially in the omentum because of the extensive vascular supply. Although some authors suggested implantation of at least 20-30 cm² of splenic tissue the mass of the implanted tissue seems not to influence the result. The technique of transplantation is important. The best result is obtained after implantation of slices or cubes of spleen tissue with fragments of capsule and cords. In this way the chance of survival of the white pulp is the highest.

The age of the patient may influence splenic regeneration: the regenerative capacity and capacity of ingrowing vessels can be expected to be better in young patients than at older age.

To test the function of autotransplanted splenic tissue and to evaluate whether or not autotransplantation is useful, several studies have been performed, mostly in animals. There are however histological and functional differences between the spleen of animals and man. It is not possible to perform the same tests in humans as in animals. Besides that, many different animal species, different autotransplantation techniques and different sites of transplantation have been used. This makes comparison and extrapolation to the human situation difficult. Some conclusions can be drawn. Because the blood flow in the autotransplants was mostly no more than 10% of normal splenic blood flow, a reduced clearance function is to be expected. Horton and Pabst already demonstrated the importance of the splenic blood flow in clearing organisms out of the blood stream. In rats, rabbits and dogs the blood clearance was significantly reduced. In man a reduction of circulating Howell-Jolly bodies was found after autotransplantation. In several studies resistance to induced pneumococcal sepsis in animals after splenectomy was evaluated by injecting bacteria intravenously. The results showed a better resistance in animals with autotransplantation than in those without.
Other positive aspects of autotransplantation have also been described e.g. improved alveolar macrophage function\textsuperscript{139}, improved phagocyte function in peripheral blood\textsuperscript{140}, correction of IgM levels\textsuperscript{141} and increased pneumococcal antibody titres after vaccination\textsuperscript{142}. The effect of autotransplantation on changes in lymphocyte subsets, immunoglobulin levels and complement levels is still a subject of discussion\textsuperscript{24,113}. On the other hand: in spite of the presence of some splenic tissue, a number of fatal cases because of OPSI have been published\textsuperscript{113,143,144}. Also complications of autotransplantations and of splenosis after splenectomy have been described, such as haemorrhage, abscess and ileus\textsuperscript{120,145,146,147,148,149}.

Despite all the studies performed so far it is still not completely clear whether and to what extent autotransplantation can give protection against OPSI, although positive arguments have been found.

As appears, reports on the effects of auto-transplantation of spleen fragments are controversial\textsuperscript{46,150}. Although beneficial effects have been reported\textsuperscript{128,135,151}, several other studies observed no significant differences compared to splenectomized patients without splenic regrowth\textsuperscript{129,143,144}. Several factors may account for this. First, the total amount of blood that is filtered is low, despite an acceptable vascularisation. Second, the micro-anatomy of the splenic fragments is probably not suited for the specific local low flow that is characteristic for the normal spleen and is essential for the close contact between antigen, and phagocytes and immune responsive cells. Third, for testing of the immune function of the autotransplanted spleen fragments two items have to be evaluated: phagocytosing capacity, with special attention to non- or badly opsonized antigens; and (humoral) immune response capability, with particular attention to TI-2 polysaccharide antigens. With respect to these items the presently used tests of the function of the autotransplanted spleen fragments may not be adequate for evaluation of the ability of the fragments to perform real ”splenic” immune functions.

5. AIM OF THIS STUDY

The spleen is an important organ of the immune system and splenectomy will have a negative effect on the immune functions, especially on the primary immune response to bacterial capsular polysaccharide (TI-2) antigens. The question is whether, and in what way, these functions can be preserved after splenic trauma, often followed by splenectomy. The first attempt to preserve splenic function should be to maintain the spleen itself with its own vasculature. In chapter 2 a study is described presenting the results of the use of a new splenic salvage technique with an absorbable net of Vicryl\textsuperscript{®}.

If splenectomy is inevitable, autologous transplantation of parts of the ruptured spleen into the greater omentum is another option to consider to preserve (at least part of) the immunological function of the spleen. Despite the studies performed, there has been a lot of controversy about the benefits of autotransplantation, especially in man\textsuperscript{152,153}. In 1984 we started with autotransplantation of splenic tissue after splenectomy for severe
traumatic rupture of the spleen in cases in which the spleen could not be saved. Along with this procedure, a study was started to evaluate whether or not an autotransplantation of splenic tissue in the omentum would have a positive effect on the immunological defence after splenectomy. Splenectomized patients that underwent a spleen autotransplantation were compared with splenectomized patients that did not undergo this procedure.

As in many studies attention has been focussed on the filter and phagocytosis function of the autotransplanted spleen, several tests of these functional capacities were performed. In chapter 3 a study determining the selective splenic Fc-receptor function is described, as a test of the mononuclear phagocyte system capacity of the autotransplants.

The general immune response capacity and the phagocytic activity of patients with and without autotransplants after splenectomy are described in chapter 4. With respect to immunological defence against postsplenectomy (bacterial) infections, special attention was paid to the capability of autotransplanted spleen tissue to mount a specific humoral immune response. An adequate humoral response would enable other non-splenic parts of the mononuclear phagocyte system to clear the opsonized pathogenic bacteria. In chapter 4 we have also included a test of the specific humoral response capacity of the above-described patient groups against pneumococcal polysaccharides, as present in the Pneumovax vaccine.

Consequent on the study involving human subjects, a similar prospective autotransplantation study has been performed in rats with tests of the primary humoral immune response against different pneumococcal polysaccharides, combined with evaluation of the immuno-architecture of the autotransplanted splenic fragments. The results are reported and discussed in chapter 5.

After accidental splenectomy, accessory spleens may function as a spare spleen, maintaining some of the immune functions. A basic condition to be able to perform adequate splenic immune functions is that the basic architecture of accessory spleens is similar to that of a normal spleen, including spleen-specific lymphoid compartments, like the marginal zone. A morphological and immunohistological study comparing human accessory spleens with their normal counterparts is described in chapter 6.

In chapter 7 the findings described in this thesis are summarized and discussed, including final conclusions. Based on the main findings, a perspective is given, with suggestions for further research.

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References


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