The human spleen after trauma
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Chapter 7

SUMMARY, DISCUSSION AND CONCLUSIONS
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In the introduction of this thesis it has already been pointed out that the spleen is more important than initially was thought. The spleen has an important role in the defence system of the body against blood-borne antigens, and is essential in blood clearance of poorly opsonized antigens. Consequently the spleen is capable of the clearance of thymus independent type 2 (TI-2) antigens, such as polysaccharide encapsulated bacteria like Streptococcus Pneumoniae, which are generally poorly opsonized. The spleen has an important role in the primary and secondary antigen-specific immune response to blood born antigens. After a splenectomy changes in the immunological defence system are known and this can result in the overwhelming post splenectomy infection (OPSI) syndrome, especially in children, with a high mortality. It is therefore important to preserve the immune function of the spleen. The best way to do this is by preserving the spleen itself or part of it, or alternatively, if this is not possible, by preserving some splenic tissue, providing an alternative blood supply. The latter is the aim of spleen autotransplantation after splenic trauma.

In chapter 1 an overview is given of the historical background of the role of the human spleen, the role of the spleen in the immune system, the consequences of splenectomy, and the aims of this thesis.

In chapter 2 a description is given of a technique of wrapping an absorbable net around a ruptured spleen. In a study of 31 patients with severe traumatic rupture of the spleen good results were achieved by this technique. However, it is difficult to operate on the spleen in an emergency situation and it is also difficult to decide which therapy is the best in the given situation. The general opinion at present is that this operation should be viewed as a speciality manoeuvre that requires considerable experience. In the addendum of this thesis an algorithm is given. One has to keep in mind that the risks of the procedures used for preserving the spleen should not exceed the overall risks of a splenectomy.

In chapter 3 the mononuclear phagocyte system (MPS) function was studied using the Fc-receptor test. In most studies after an autotransplantation of splenic tissue (AST) where a scintigram was made, 99mTc colloid scans were used to evaluate the MPS function. We used, 99mTc-labeled rhesus-positive erythrocytes coated at low density with anti-rhesus antibody. This test is considered to provide a parameter for splenic MPS Fc-receptor function, allowing capture of low-opsonized particles/antigens. This was done in 24 patients who underwent splenectomy, 10 of whom underwent splenic autotransplantation. All patients undergoing AST showed a hot spot at the site of splenic implantation indicating the presence of Fc-receptor-bearing (phagocyte) cells. In 8 of the non-transplanted patients a hot spot was also found and considered to be ectopic splenic tissue. The kinetic of the Fc-receptor test is normally bi-exponential, but in this study it showed a delayed and monoexponential blood clearance in all patients. There were no significant
differences between the patient groups. The results of the analyses of the kinetics of the Fc-receptor-bearing cells were not characteristic of adequate restoration of the overall splenic Fc-receptor function. Based on this study, autotransplantation of a small amount of splenic tissue after splenectomy cannot be considered sufficient to restore splenic MPS function, especially in relation to low-opsonized antigens and is inadequate for blood clearance. To achieve this effect an adequate blood flow through the autotransplant is needed as well as a rather large volume of tissue.

In chapter 4 we studied the restoration of the humoral immune response to pneumococcal capsular polysaccharides and the phagocytic function of granulocytes in 10 human subjects undergoing autologous spleen transplantation (AST) after splenectomy compared to 14 subjects after splenectomy only. The phagocyte activity showed normal results in relation to the reference values, and no significant differences between the patient groups. To evaluate the antibody responses we measured the cell wall polysaccharide antigens without distinguishing between antibodies directed against capsular polysaccharides or cell wall polysaccharide antigens. A specific ELISA was used with an absorption step to remove antibodies against cell wall polysaccharide antigens. We used capsular antigens and this showed a surprising effect of AST after splenectomy on specific antibody responses after pneumococcal vaccination.

Significant antibody titre rises were found for both IgM and IgG in the AST patients as compared with the patients without splenic regrowth. A partial improvement was seen in patients with splenosis (accidental seeding of splenic tissue in the abdominal cavity) and no rise was seen in the non-transplanted group without splenosis. Considering this significant antipneumococcal antibody increase, after splenectomy AST can be expected to improve the specific humoral response to pneumococcal infections and presumably also to other TI-2 antigens. This study showed also that there is a strong possibility that "born-again" or accessory spleens will provide immune protection after splenectomy. However, failure of ectopic splenic tissue to prevent OPSI after splenectomy has been described.

Although it is not yet clear whether complete protection against all pneumococcal subtypes can be obtained, AST may be expected to help limit the risk of the OPSI syndrome. This demonstrates that AST can play a role in the management of severe splenic injury in which splenectomy is inevitable, particularly when, at an appropriate time point, followed by vaccination with polyvalent pneumococcal vaccine.

In chapter 5 the evaluation is described of the restoration of the humoral immune response after splenic autotransplantation in a rat model. The rats were divided into 3 groups; splenectomy, splenectomy with AST and sham operation, and all vaccinated with 23-valent pneumococcal vaccine. In this study the type specific anti pneumococcal polysaccharide ELISA with absorption of antibodies to cell wall polysaccharide antigens was used. The results of this study support our findings in humans as described in chapter 4. Significant antibody titre rises of IgM and IgG were found in the autotransplanted rats,
comparable to sham-operated rats for most types without significant differences. Splenectomized rats showed significantly lower increase in Ig-levels. The titres were highest 3 days after vaccination.

The architecture of the autotransplants demonstrated fully regenerated splenic fragments with a normal white pulp. Immunohistochemical studies demonstrated structurally functional autotransplants including an intact marginal zone with B lymphocytes and macrophages. It appears that, with respect to both architecture and immune capacity, the autotransplants are capable of inducing a primary immune response against TI-2 antigens.

This study showed that even small amounts of regenerated splenic autotransplants can provide an adequate humoral response to several pneumococcal capsular polysaccharides, and can be expected to give improved protection against OPSI, especially in combination with vaccination.

From our and other animal studies it should be concluded that to obtain the best results of vaccination after spleen autotransplantation, the vaccination should be given at a time-point at which complete splenic regrowth can be expected. Keeping the limitations of an animal model (including strain and species differences) in mind, it should be advised to give the vaccination not earlier than three months, but most likely better at 6 months after autotransplantation.

In chapter 6 the examination of accessory spleens of 10 patients is described and compared with normal spleens. The histological as well as the functional (immunohistological) structure of the accessory spleens was found similar to that of the normal spleens. This includes the capacity to bind pneumococcal polysaccharides. These findings, in combination with our findings reported in chapter 2 to 4, imply that after removal of the orthotopic spleen, accessory spleens can be expected to take over the most important humoral immune function. This includes the spleen specific ability to initiate a primary humoral response to encapsulated bacteria like pneumococcs. Consequently preservation of accessory spleens would be a valuable way of preventing infection and sepsis after splenectomy because of trauma.

Conclusions

The following conclusions can be drawn from this thesis:

1. A severely injured spleen can often be saved by wrapping in an absorbable net. This operation should be considered a speciality manoeuvre that requires considerable experience.

2. Spleen autotransplantation after splenectomy provides some mononuclear phagocyte system activity but is inadequate for blood clearance.

3. The spleen has an important and unique role in the initiation of the specific immune response, especially to the T-cell independent type 2 antigens such as pneumococcs.
4 Autotransplanted splenic tissue is capable of at least partial restoration of specific anti-pneumococcal humoral immune function after splenectomy.

5 Spleen autotransplants have a normal white pulp lymphoid compartment, including a functionally intact marginal zone.

6 Autotransplantation of splenic tissue after splenectomy, especially in combination with (pneumococcal) vaccination, can be expected to improve protection against the OPSI syndrome.

7 The functional anatomical compartments of accessory spleens do not differ from normal spleens and therefore can be expected to perform normal splenic immune functions. Consequently, when accessory spleens are encountered during routine surgical procedures, not involving spleen pathology, it is strongly advised to leave them in situ.

Perspectives

Splenic trauma does not occur very frequently. Therefore, a patient with splenic trauma ideally should be admitted to a hospital with a surgical department with special attention for or specialised in trauma surgery and sufficient knowledge of and experience with spleen salvaging techniques, such as use of an absorbable mesh. Trauma surgeons should realise that the outcome of use of the mesh-wrapping technique is dependent on their particular attention to the procedure1,2.

The steps to perform after a patient with a rupture of the spleen has been admitted to hospital are shown in a flow-chart in table I. In short the following steps are advised3,4,5. The first priority has to be to save the spleen. This can be tried without an operation when the patient is clinically stable (stable vital functions without signs or threats of splenic bleeding). In case of an unstable patient a laparotomy has to be performed. When considered feasible, attempts should be made to wrap the spleen in an absorbable net. When a splenectomy is needed, autotransplantation of splenic tissue should be performed in the omentum, as in this way vascular supply and ingrowth of vessels is most easily enabled. Autotransplanted spleen tissue should not be put through a mesh, but preferably should be cut in particles of 5 to 10 mm³ to preserve microarchitecture. This intact splenic microarchitecture seems essential for repopulation and restoration of specific splenic lymphoid compartments6. Although the ideal amount of tissue to be transplanted is unclear, most likely this should be at least 20-50 gram.

After autotransplantation, anti pneumococcal vaccination is advised with a time interval of at least three months, or when feasible six months. As this time interval between autotransplantation and vaccination still is deduced from animal experiments6, future prospective human studies, with an increased number of patients, should provide information about the optimal vaccination time point.
The use of a batch of specific spleen-dependent antigens, such as different pneumococcal polysaccharide subtypes, can provide a procedure for testing functional splenic immune response capacity in man. This can be done after spleen autotransplantation, but also after other spleen operations or in case of other spleen pathology. In such a "test-vaccine" it is important to include a sufficiently wide range of antigenic variety of epitopes. Within an individual patient, the use of a test-vaccine could provide information about the success of the spleen saving procedure. In addition this would provide information about
humoral response failures to specific antigens in the vaccine. The latter is of importance as in this way information is obtained that this patient likely is not able to respond properly to one or more specific subtypes of microbial species. In this case, when such a patient would be admitted to the hospital with a possible diagnosis of infection, serology may not provide adequate information, as the patient apparently was not capable to rise a humoral response to some antigens! In such case the diagnostic approach should be directed to direct detection of the (microbial) antigen.

When, by using the test-vaccine, insufficient protection by the spleen-saving procedure or partial humoral response failure to some antigens is demonstrated, the use of protein-conjugate vaccines should be considered. Vaccines consisting of a protein (like tetanus toxoid) conjugated to bacterial polysaccharides can be expected to be able to initiate an adequate response to the polysaccharide, without the need for presence of a functional spleen8,9,10. Despite this the use of protein-conjugate vaccines should not be considered a replacement for spleen preserving procedures. It is clear that spleen preserving procedures like spleen autotransplantation have the advantage that potentially the spleen-specific humoral response capacity to theoretically any encountered TI-2 antigen would be restored, whereas the use of conjugate vaccines only gives protection to the antigens used in the vaccine.

Whereas the role of the spleen in the immune system has been recognized in recent years, it was not readily clear whether preservation of the spleen in case of trauma would add anything to outcome with respect to morbidity and mortality. With the results presented in this thesis the importance of spleen preservation has been supported. The spleen autotransplantation studies have shown that this procedure significantly improved the immune response capacity against relevant bacteria, related to postsplenectomy morbidity.

Future studies should aim at further improving the effects of autotransplantation by optimizing the outgrowth of the amount of spleen tissue and determination of optimal vaccination time point.

When splenectomy is unavoidable in a patient with a splenic trauma, spleen autotransplantation combined with anti pneumococcal vaccination should be considered a valid option to reduce the risk of subsequent morbidity.
References


