Mechanisms of allograft rejection: the role of anti-endothelial antibodies

Prof. Kathryn Wood, Transplantation Research Immunology Group, Nuffield Department of Surgery, University of Oxford, UK

Prof. Wood briefly reviewed the mechanisms of allograft rejection in general and then focused on the potential of preformed antibodies reactive with endothelial cells to trigger rejection. Special attention was given to the role that B-cells might play in the rejection process.

Donor-derived antigen-presenting cells are often considered to be the trigger for rejection by initiating a CD4 positive T-cell population response. This leads to the development of a complex set of effector mechanisms, each of which can damage the graft, but which together lead to a profound response that if untreated will elicit rejection.

A similar set of reactions can be envisaged, but this time with recipient B-cells as the driving cell. For example, it is becoming increasingly clear that B-cells act not just as antibody-producing cells but also as very functional antigen-presenting cells. They can interact with T-cells and drive the response, either as initiators or amplifiers (Fig. 1). Both this ‘B-cell mechanism’ and the conventional cascade process should probably be seen as running side-by-side with the B-cells playing a more central role than previously considered.

Most of the treatment agents at our disposal currently target T-cells but newer ones are becoming available, notably against B-cells.

Grafts lost in 3% of recipients per year

Despite the use of extensive routine testing for HLA-reactive antibodies, antibody-mediated rejection remains a significant problem, and 3% of recipients per year lose their grafts – a not insignificant number.

Biopsies taken following acute antibody-mediated rejection have enhanced our view of this process, especially via staining for complement deposition. C4d deposition can clearly be seen on the capillary walls within a renal transplant associated with early rejection, for example. The endothelial cells (EC) lining the wall appear to be a primary target of rejection.

EC present a range of potential targets for recognition by antibodies. Which antigens are present on the cell surface is an ongoing area of research, but a number of potential targets can be considered, including HLA class I and II, Vascular EC antigens, MICA–MHC Class I-related gene A, vimentin and the angiotensin I receptor can also be listed as possible molecular targets of anti-endothelial cell antibodies (AECA). This probably represents a range of specificities rather than a single system of antibody-mediated reactivity, and a cell-based target expressing the range of potential antigens would therefore be preferable to a solid-phase assay from a predictive point of view to enable multiple antibody specificities to be detected if they are present in patients before transplantation.

Pathways of antibody-mediated damage to EC

The primary downstream effects triggered when antibodies bind to EC include complement activation and the eventual formation of a lysis-causing membrane attack complex (MAC) at the

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Fig 1. B-cells can drive the immune response and, if untreated, trigger rejection.
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Introduction

AbSorber is dedicated to developing and marketing products that will increase the numbers and overall success rate of transplantation. The meeting at Arlanda, Stockholm, was the 2nd Annual AbSorber Symposium designed to share recent findings in this area relevant to products currently under development and clinical testing. Experts from the Nordic countries and Europe presented interesting scientific and clinical data at this meeting, which was chaired by Professor Gunnar Tydén, Karolinska Institute, Huddinge.

XM-ONE®, a donor-specific endothelial cell crossmatch test, is the first AbSorber product available for clinical use by the transplant community. It will increase the available information for managing transplanted patients and provide physicians with an improved tool to enhance risk assessment.

Today, XM-ONE® is well on its way to being implemented at key European and US transplantation units; more than 50 centers are trained to perform the test. Scientific interest is very high and a number of leading groups will be involved in its continued development. In addition, several XM-ONE® clinical projects are currently ongoing to develop the product for new indications, and more projects will be started in 2009.

AbSorber also has additional products in the pipeline, including several intended to address the problems of AB0 incompatible transplantations. AB0 columns for removing antibodies are part of our portfolio, as are products designed to measure the presence of antibodies that increase the risk of rejection. Several symposium presentations focused on these areas.

We wish you pleasant reading.

Anders Karlsson
CEO, AbSorber
end of the classical pathway of the complement cascade. This view is, however, probably too simple. It is likely that the cascade releases other mediators that can be equally deleterious in terms of the immune response. The overall result will be recruitment of inflammatory leukocytes by complement fragments plus the deposition of complement fragments and binding of phagocytic cells through complement receptors.

**Clinical relevance of non-HLA AECA**
Most of the published data are relatively old, but suggest that non-HLA AECA are clinically relevant. Irreversible vascular rejection has occurred in HLA-identical sibling transplants and in the absence of anti-HLA antibodies, for example. Furthermore, the AECA were present pre-transplant. AECA have also been eluted from rejected heart and kidney grafts.

**Summary**
The role of B-cells in determining the outcome of the immune response in allograft rejection is increasingly recognised. While we generally lack therapeutic agents to combat their effects, better diagnostic tools for characterizing antibodies are emerging and these will be of great help both pre-operatively and post-operatively. In the future, we would like to monitor what happens to antibodies post-transplant and have better tools for following the complex interactions that occur in tissues.

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**ABO-incompatible kidney transplantations using antigen-specific immunoadsorption and rituximab**
Prof. Gunnar Tydén, Department of Transplantation Surgery, Karolinska University Hospital, Stockholm, Sweden

The increasing discrepancy between the numbers of available deceased donor organs and patients on the waiting list plus the superior results obtained with living-donor transplants help explain today’s interest in performing more ABO-incompatible kidney transplantations. Prof. Tydén reviewed current progress towards this goal.

At the Dept. of Transplantation Surgery, Karolinska University Hospital in Stockholm, long-term outcomes of living donor transplantation are superior to deceased donors; 10-year living-donor graft and patient survival rates are 70% and 85% respectively compared to 40% and 55% for deceased donors. Although these transplantations take place under highly optimized conditions, there are plenty of reasons to increase the number of living-donor transplants in general.

Several European centers have been instrumental in building up a considerable body of experience in ABO-incompatible kidney transplantations, starting with Gothenburg in 1974. Already in a key publication from 1987 on experiences with 26 ABO-incompatible living-donor renal allografts, a group in Brussels showed that the procedure was feasible, albeit at a high price (Alexandre GP et al. Transplant Proc 1987; 19 (6): 4538-42). Donor-specific platelet transfusions, plasma exchange, splenectomy, massive amounts of immunosuppressive drugs and graft irradiation were commonly used, for example.

The ABO blood group system (Fig. 1) illustrates the main transplantation possibilities available today. Adding the incompatible constellations together gives an increase of 35.75%. Transplanting against incompatible blood groups could thus in theory increase the number of living-donor procedures by almost 40%.

**Renewed interest in living-donor transplants**
Recent years have seen a renewed interest in ABO-incompatible living-donor transplantations... potential to increase living-donor transplants by almost 40 %

![Fig. 1. The ABO blood group system. Transplanting against incompatible blood groups could increase the number of living-donor procedures by almost 40% (figures based on a European population).](image-url)
transplants, partly due to the introduction of an antigen-specific immunoadsorption column (Glycosorb) that removes only existing AB0 antibodies, thereby avoiding the morbidity associated with plasma exchange (Tydén G et al. Transplantation 2003; 76 (4): 730–1).

This protocol, which also includes measures to prevent AB0 antibody rebound (Prograf/Cellcept/prednisolone, Mabthera [rituximab] and Gammagard) is the one generally used in Europe today. Figure 2 illustrates the plasma perfusion arrangement. Running the adsorption on 4 consecutive days prior to transplant is usually sufficient. Pre-emptive, post-operation adsorption is also performed. In standard patients, rebound of antibodies does not occur.

Impressive results but high costs

Initial results (Sept. 2001– Aug. 2005) were very good. In 19 AB0-incompatible transplants, actual graft survival was 95% with 0% rejection episodes. In 115 compatible transplants, equivalent figures were 96% and 16%. The use of rituximab in the former protocol is the only real difference between the two. This very good outcome was not a ‘center-effect’ as similar results were seen in Freiburg and Uppsala, although the antibody titers do vary quite widely.

Today, the procedure is used in 40 centers worldwide, but a high and rather unpredictable cost hinders its more widespread use. Nevertheless, it is more cost-effective than long-term dialysis.

Summary

Antigen-specific immunoadsorption plus the use of rituximab instead of splenectomy are the key features that have contributed to the success of the new AB0-incompatible kidney transplantation protocol.

The biochemistry of the AB0 blood group system

Assoc. Prof. Jan Holgersson, Dept. of Clinical Immunology, Karolinska University Hospital, Huddinge, Sweden

ABH antigens, the red blood cell molecules that define the blood groups A, B, AB and 0, are carbohydrate antigens carried by proteins or lipids present on the surface of most cells. Assoc. Prof. Holgersson reviewed structural modifications relevant to the ABH histo-blood group barrier in transfusion and transplantation.

Final modification of the A, B and H structures takes place in the Golgi apparatus of the cell where several glycosyltransferases add monosaccharides to a core carbohydrate. The presence or absence of the A-gene encoding an α1,3-N-acetylgalactosaminyltransferase and the B-gene encoding an α1,3-galactosyltransferase determine the ABH histo-blood group of an individual. These glycosyltransferases will add N-acetylgalactosamine (GalNAc) or galactose (Gal) respectively in an α1,3 linkage to the terminal galactose of the H antigen (Fuc α1,2Galβ-R). This results in either the A (GalNAcα1,3[Fucα1,2]Galβ-R) or B (Galα1,3[Fucα1,2]Galβ-R) determinant, or both, present on cells of A, B and AB individuals. Individuals serologically typed as blood group O carry the unmodified H antigen on the cell surface.

Different types of core saccharides act as acceptors for the H and Se gene α1,2-fucosyltransferases that add fucose (Fuc) to the terminal Gal residue. The ABH antigens can be structurally divided into subtypes depending on the core saccharide structure; type 1 is based on the Galβ1,3GlcNAc sequence, type 2 on the Galβ1,4GlcNAc, type 3 on the Galβ1,3GlcNAc, type 4 on the Galβ1,3GlcNAcβ and type 6 on the Galβ1,4Glc sequence.

All humans, except those of blood group AB, have preformed or natu-
r al anti-A or anti-B antibodies directed against structures that are not present in that individual (Fig. 1). This is what creates the ABH histo-blood group barrier in blood transfusion and transplantation.

Natural anti-A and anti-B antibodies were long thought to be only of the IgM isotype, but have been shown to be present as isotypes IgM, IgG and IgA in healthy individuals. The IgM antibody is often of lower affinity, but because of its pentameric structure it usually binds antigen present in several copies on the cell surface with high avidity. It is further a strong activator of complement. The IgG isotype is generally the most abundant class in human plasma and is divided into subclasses IgG1, IgG2, IgG3 and IgG4, distinguished by differences in function, structure of the heavy chain and decreasing serum concentration. IgG1 and IgG3, and to a lesser extent IgG2, are also effective in activating complement, although not as effective as IgM. In normal, healthy individuals, anti-A and -B antibodies of the IgG isotype have been found to be mostly of the IgG1 and IgG2 subclasses.

Donor-reactive anti-endothelial cell antibodies in LXM positive and negative patients evaluated for living donor kidney transplantation

Dr. Mats Alheim, Dept. of Clinical Immunology, Karolinska University Hospital, Huddinge, Sweden

Kidney transplant rejections still occur despite negative lymphocyte crossmatch test results. Anti-endothelial cell antibodies specific for non-HLA and not detectable by the crossmatch test may cause this graft rejection. Dr. Alheim described new test results that help shed light on this important issue.

<table>
<thead>
<tr>
<th></th>
<th>Group A</th>
<th>Group B</th>
<th>Group AB</th>
<th>Group 0</th>
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<td>Red blood cell type</td>
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<td>B</td>
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</tr>
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<td>Anti-A and Anti-B</td>
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<td>Antigens present</td>
<td>A antigen</td>
<td>B antigen</td>
<td>A and B antigens</td>
<td>No antigens</td>
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Preformed donor-reactive antibodies detected in lymphocyte crossmatch (LXM) tests and donor-specific HLA class I and II antibodies identified in solid-phase assays negatively affect transplantation outcome. Early rejections nevertheless still occur even when LXM tests prove negative. Anti-endothelial cell antibodies specific for non-HLA and not detectable in the LXM may be the cause of this kidney graft rejection.

To further investigate the possibility of preventing this type of rejection, sera from 106 patients undergoing evaluation for living donor kidney transplantation were crossmatch tested against
TIE-2 positive donor cells using the XM-ONE® kit. This rapidly detects any specific anti-endothelial cell antibody plus antibodies to HLA class I and II antigens also expressed on endothelial cells.

Patient sera were also tested in cytotoxic T and B and flow cytometric T LXM (T FXM) tests. The percentage of panel reactive antibodies (PRA) present in patient sera was determined by the FlowPRA® test.

**Test results**

It was concluded that 17% of patients undergoing evaluation for living donor kidney transplantation had IgG binding to donor endothelial cells and 29% had IgM binding. Of the patients negative in conventional LXM tests, 12% were found to have IgG binding to donor endothelial cells.

**ABO in transplantation**

Dr. Per Grufman, Head of Development, AbSorber AB, Stockholm, Sweden

Current methods for measuring antibodies against blood group antigens in ABO-incompatible transplantation have a number of drawbacks. Dr. Grufman highlighted how improved diagnostic methods and columns for removing blood group antibodies can increase the number of transplantations performed.

ABO-incompatible transplantation is today performed at multiple centers around Europe and the USA, although only a few of them have developed larger programs. Current diagnostic methods for measuring titers of antibodies against blood group antigens are, however, far from ideal. They display poor reproducibility and the results are difficult to compare between centers, for example. In addition, the acceptable antibody titer before transplantation and the immunosuppressive protocol used varies between centers.

Developing better diagnostic methods that can be reproduced between different centers and thereby allow exchange of experiences is needed if ABO-incompatible transplantation is to become more common. As well as achieving this goal, the diagnostic methods developed by AbSorber will also allow detection of antibodies against different types of A and B antigens, thereby providing more detailed information than has previously been available. Fig. 1 shows data from two Elisa experiments with serum from donors of different blood groups (indicated in brackets) against A trisaccharides and A type-2 tetrasaccharides. Interestingly, one of the patients only has IgG2 antibodies against the tetrasaccharide and not against the trisaccharide.

**Extracorporal immuno-adsorption columns**

AbSorber is also developing extracorporeal immuno-adsorption columns for removal of blood group antibodies. Like AbSorber’s ABO diagnostic methods, these columns utilize the different types of blood group antigens to enable more efficient removal of anti-blood group antibodies (Fig. 2). Their use of tetrasaccharides and multivalent epitopes enhances performance considerably.

Introducing standardized diagnostic methods for measuring ABO antibodies as well as efficient methods for removing these antibodies will make it possible to increase the number of ABO-incompatible transplantations and thereby the total number of patients that can benefit from kidney transplantation.
XM-ONE® evaluation in Oxford: study outline and current experience

Prof. Kathryn Wood*, Dept. of Transplant Immunology, University of Oxford, UK

The Oxford BART Study utilizes biomarker analysis to detect/predict rejection after kidney or kidney/pancreas transplantation. Prof. Wood described Phase 1, an observational study on living donor renal transplant recipients, and presented the initial findings.

The XM-ONE® cross-matching test has been used as part of a larger panel of assays on kidney transplant patients. About 150 transplants are performed annually in the Oxford Unit, of which about 30% are living donors. All living donors are analysed as part of the pre-transplant routine with the XM-ONE® test using a protocol optimized to take account of clinical routines used in the unit. The FACS scatter profiles generated for IgG and IgM are typical for the test in general. Negative shifts have been observed in some patients, but running a 1/50 dilution seems to resolve this in most cases.

Two patient groups

The Phase 1 study comprises two groups of patients. Group 1 had blood samples taken on the day of transplant. For the XM-ONE® assay, 2 sera were used: day of transplant (current) and final crossmatch (historic). Each sample was analyzed in duplicate. A total of 9 patients enrolled but one did not proceed to transplant.

Group 1 results (Fig. 1) show a number of positive XM-ONE® assays, even in this small group of patients, and these will be correlated with clinical outcome. PRA status indicates that the assay is working well and generating useful data.

In Group 2, where 13 patients proceeded to transplant, the current sample is replaced by one taken 2 weeks pre-transplant. An autologous control set was included and XM-ONE® assays were again performed in duplicate and analyzed neat and at a 1/50 dilution. Figure 2 shows the results, including a comparison of the allogeneic and autologous assay results. PRA data show that both sets of results generally follow a similar pattern.

Summary

The XM-ONE® assay can detect the presence of IgM and IgG AECA and thus provides useful pre-transplant risk evaluation data. The Oxford Group now use it routinely. The most interesting data will, however, come when the clinical codes are broken. As the enrollment target is 190 patients, this will not happen for several years.

Relevance of anti-endothelial crossmatching in renal transplantation

Prof. Ilias Doxiadis, Department of Immunohematology and Blood Transfusion, Leiden University Medical Center, Leiden, The Netherlands

The use of XM-ONE® as an endothelial crossmatching method for solid organ transplantation was one component of the 15th International Histocompatibility and Immunogenetics Workshop, held in Buzios, Brazil. Although the data from the endothelial crossmatch Study (XM-ONE®) are still very preliminary, Prof. Doxiadis was able to point out one key trend.

The main goal of the crossmatching study was to define the relevance of the XM-ONE® assay with respect to early rejection and/or graft loss. So far, 5 centers have participated (Aarhus, Athens, Berlin, Prague and Leiden) and more...
...the isolation of Tie-2 positive cells functioned well in all reported cases

have applied to send in their data before the study closes.

Study goals and details
The study had both retrospective and prospective aspects, and the aim was to include a total of 50 patients with rejection episodes. Key goals included whether the antibodies defined with the XM-ONE® test are relevant for solid organ transplantation in a multi-center setting or if further fine-tuning is needed.

All centers received material to perform the XM-ONE® assay and were requested to carry out the tests and report back results. They were specifically asked to report both the HLA typings of donor and recipient, the specificity of antibodies directed against HLA (A, B, C, DR, DQ and, if possible, MICA), as well as the results of the XM-ONE® crossmatch in terms of both IgM and IgG.

The centers themselves decided if the XM-ONE® results they reported back were positive, negative or uncertain. This latter point was considered important for the study as its aim was to gather results rather than compare different curves produced using different types of equipment, for example. The number of rejection episodes and type were assessed. Some, but not all, centers tested for the presence of autoantibodies in the case of positive XM-ONE® results.

The XM-ONE® manufacturer’s recommendations in terms of assay and analysis were followed, and the isolation of Tie-2 positive cells termed as pre-endothelial cells seemed to function in all reported cases. Living cells are required for the retrospective testing and the use of frozen cells was a big help in this respect. Note that in some European countries, the approval of a medical ethics committee is required if the donor needs to be contacted for a retrospective analysis.

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<table>
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<td>1</td>
</tr>
<tr>
<td>no</td>
<td>1</td>
<td>7</td>
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</table>

*2 patients were MICA positive

Initial findings
Some study participants experienced a need for an endothelial positive control, and the range between the negative and positive control was in some cases not as clear as would be liked.

Figure 1 shows a good example of a flow cytometric analysis result and Figure 2 summarizes preliminary study data for pre-transplant anti-donor endothelial antibodies.

In total, 16 individual patient samples were analyzed (one was not used because of unclear diagnosis at testing). In the few cases tested, no autoantibodies could be detected.

The IgM results were not conclusive since some patients showed positive results with no signs of rejection. Among the IgM positive patients who had had a rejection episode, 2 were MICA positive.

The IgG results were better. In this preliminary analysis, IgG antibodies directed towards Tie-2 had only one false positive and negative result while all others reacted as hypothesized, i.e. positive in the case of rejection and negative for no rejection. Once again, 2 of the positive patients who had had a rejection episode were MICA positive.

Summary
The data gathered so far are very preliminary since the study is still ongoing and material remains to be collected. In addition, a number of centers in the US have expressed interest in contributing new data. It is thus too early to make any direct conclusion, but a trend that correlates predicting rejection with IgG antibodies against Tie-2 positive cells can be observed.

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Screening for HLA antibodies: clinical relevance of different antibody types

Prof. Ilias Doxiadis, Section of Immunogenetics and Transplantation Immunology, Eurotransplant Reference Laboratory, Leiden University Medical Center, Leiden, The Netherlands

Transplanting patients requires that many factors be taken into account, some of which may appear obvious while others may not. Prof. Doxiadis reviewed the clinical relevance of different antibody types against this background.

Transplantation history constitutes a sound starting point for assessment. This should clearly include patient history with respect to obvious sensitization towards HLA antigens via transfusion, pregnancy and/or transplantation, as well as to infections and/or vaccinations. Donor history is also important. Aspects to review include brain death, diseases, infections and tumors. The role of different cells acting in the transplant should not be restricted to T and B-cells. NK cells are also relevant, for example, and their effect can lead to graft loss even in perfectly matched organs.

Donor-specific antibodies in transplantation

Regarding the role of donor-specific antibodies in transplantation, the trend has been from broad home-made HLA antibody screening to commercial kits with single targets. As always, however, the key is how the results are interpreted rather than just the results themselves. The general opinion is not to transplant if complement-fixing antibodies are present in the current serum sample. If they are found in the historical sample, on the other hand, the decision tends to vary from center to center. For antibodies in post-transplant serum, treatment is the standard approach, but even if this is unsuccessful, the graft can still be retained for many years.

Data reveal that some transplantation centers show no differences in graft survival for sensitized and non-sensitized patients, while others do. For example, a center using only current sera may experience a large difference in graft survival at 2 years, whereas another that uses historical and current serum may not see a significant difference.

Study on decision-making effect on graft survival

To follow this aspect further, a study on graft transplantation was carried out at 24 centers performing a first deceased-donor kidney transplantation, including more than 34,000 procedures from 1982 to 2000. Patient groups were defined within each center as Group I (sensitized patients); 1,208 transplants with 50+ % cPRA, or as Group III (non-sensitized); 28,037 transplants with <10% cPRA. (Average graft survival at 2 years was 69% for Group I and 78% for Group III.) This difference was plotted against the policy of crossmatching (POC) of the individual laboratory serving the center. Historical crossmatches (hxm) were considered at 5 centers, 11 centers relied only on current serum samples (cxm) while 8 had no clear policy (unknown).

Antibody isotype switch

The fact that antibodies can switch from IgM should not be forgotten, nor that this switch occurs in one direction only. In a recent study on donor-specific antibodies that are not complement activated, a wide range of antibody classes and sub-classes were found in patients who had had a transplant rejection and been returned to the waiting list (Arnold ML et al. Tissue Antigens 2008; 72 (1): 60–6).

Summary

In general, ‘current’ antibodies, especially if they fix complement, suggest that we should not transplant. In historical serum, however, they allow this possibility. Of the antibody types, complement-activating IgG1 and IgG3 generally speak against transplantation. We should, however, never take our results for granted!
A flow cytometric crossmatch test for simultaneous detection of antibodies against donor lymphocytes and endothelial cells

Assoc. Prof. Jan Holgersson, Dept. of Clinical Immunology, Karolinska University Hospital, Huddinge, Sweden

Simultaneous detection of both donor lymphocyte and endothelial cell-reactive antibodies in a single-tube assay would offer a number of advantages. Assoc. Prof. Holgersson reviewed current progress towards achieving this goal.

Complement-dependent cytotoxicity or flow cytometry-based crossmatch tests using donor T and/or B-lymphocytes are adequate for detecting donor HLA class I and II-specific antibodies, but may fail to detect clinically significant antibodies against non-HLA.

To help overcome this drawback, a flow cytometric endothelial cell crossmatch (ECXM) test (XM-ONE®) that detects antibodies against donor endothelial cells as defined by Tie-2 expression has been developed. If lymphocytes that co-purified with endothelial cells could also be used as target cells in lymphocyte crossmatch (LXM) tests, simultaneous detection of antibodies reactive with donor endothelial cells and lymphocytes should be achievable. This possibility has been investigated.

Lymphocytes isolated with Tie-2 antibody-coupled magnetic beads were phenotyped by flow cytometry. Sera from ten patients, in which the percentage panel-reactive antibodies were determined, were tested on cells from nine donors using conventional flow cytometric LXM and ECXM tests. Channel shifts obtained on non-selected T and B-lymphocytes, T and B-lymphocytes and endothelial cells isolated with Tie-2 antibody-carrying beads were compared.

**Correlation results**

Cytotoxic and helper T lymphocytes, B lymphocytes, natural killer and natural killer-T cells were present among lymphocytes isolated with Tie-2 beads. HLA class I and II antigen expression was slightly higher on lymphocytes isolated with Tie-2 beads than on non-separated T and B-lymphocytes. Figure 1 shows channel shift data from the study. Very good correlation (r²=0.94) between the channel-shift values obtained on non-selected T-lymphocytes and T-lymphocytes isolated on beads carrying anti-Tie-2 antibodies was observed, while that between the channel-shift values obtained on non-selected and anti-Tie-2 antibody-selected B-lymphocytes was lower (r²=0.71).

The results suggest that T and B-lymphocytes co-purified with endothelial cells can be used in LXM tests to allow simultaneous detection of both donor lymphocyte and endothelial cell-reactive antibodies in a single-tube assay. Such an assay will facilitate investigations on the clinical significance of non-HLA specific antibodies in allograft rejection. It should also save material and labour.

**Fig. 1.** The channel shift values obtained on unselected T- (A) or B-lymphocytes (B; 256 scale) were plotted against the channel shift values obtained on the Tie-2+ T- (A) or B-lymphocytes (B; 1024 scale) for each of the serum–donor cell combinations analyzed. A linear regression analysis was performed on the two data sets (A and B), and the equations describing the curves determined. (Oral presentation, The XXII International Congress of The Transplantation Society, Sydney, Australia, August, 2008).

**Antibody-mediated graft failure after allogeneic hematopoietic stem cell transplantation**

Prof. Suchitra Holgersson*, Dept. of Transplantation Surgery, Sahlgrenska University Hospital, Göteborg, Sweden

Allogeneic hematopoietic stem cell transplantation is an effective therapy for patients with hematological malignancies. However, the introduction of reduced-intensity conditioning and the use of T-cell depleted bone marrow have increased the frequency of rejection following treatment. Prof. Holgersson reviewed recent findings in this field.

Current data clearly demonstrate that the presence of preformed donor-reactive antibodies is a dominant barrier for bone marrow (BM) engraftment in allo sensitized recipients. Hematopoietic stem cell transplantation (HSCT) is performed by infusion of donor BM/
Peripheral blood cells after myeloablation or reduced-intensity conditioning pre-treatment of the recipients. Reconstitution of hematopoiesis after HSCT is achieved by a constant replenishment from primitive, quiescent hematopoietic stem cells.

Clinical relevance of HSC antibodies

It has recently been indicated that CD34+/VEGFR-2+ cells from adult BM or cord blood may generate both hematopoietic and endothelial cells in vitro. Prof. S. Holgersson’s group has tested the hypothesis that antibodies to the hematopoietic stem cell population may be associated with rejections after HSCT. The tests focused on the relevance of antibodies directed against cells that express both the hematopoietic stem cell marker CD34 and the endothelial receptor VEGFR-2, as CD34+/VEGFR-2+ positive cells seem to be of major importance for hematopoiesis and thus engraftment after HSCT. Figure 1 suggests possible mechanisms for antibody-mediated rejection.

The study included 30 patients without post-HSCT rejection, 11 with rejection and 20 non-transplanted healthy individuals. In order to investigate the presence of donor CD34+/VEGFR-2+ cell-specific antibodies, 93 sera taken pre and post-transplantation from patients receiving HSCT were studied. Results showed that significantly more patients with rejection (9/11, 81%) had antibodies against donor CD34+/VEGFR-2+ cells but not CD34-/VEGFR-2- cells than patients without rejection (1/30, 3%) (P=0.001). In 8 transplantations, antibodies against donor CD34+/VEGFR-2+ cells were detected in advance of transplantation. Purified IgG fractions from patients with rejections significantly decreased the ability of these cells to form hematopoietic and endothelial colonies. Fractions from controls did not display this effect. In multivariate analysis, antibodies against CD34+/VEGFR-2+ cells proved to be the most significant risk factor for rejection.

Summary

These novel findings document a high rate of rejections in patients with donor-specific antibodies to CD34+/VEGFR-2+ HSC. These antibodies may significantly and commonly contribute to HSCT rejections. The present results open a new area of investigation for studying stem cell populations as targets for antibody-mediated destruction when using stem cell therapy for various disorders.

Fig. 1. Possible mechanisms of antibody-mediated rejection following stem cell transplantation (Nordlander A et al. Transplantation 2008; 86: 686–96).

1. Effector cells kill ADCC or macrophages
2. Complement cascade activation
3. Functional inhibition of hematopoietic proliferation (colony-forming capacity)
4. Immune complexes are formed as a result of HLA or non-HLA antigen shedding and antibody binding. Such immune complexes are efficiently taken up by antigen presenting cells (APC) via Fc- or complement receptors, which in turn boosts cellular immunity.
5. CTL
6. Th1
7. IL-2
8. IFN-g
9. APC
10. MAC
11. c1q

Prof. Suchitra Holgersson
Sahlgrenska University Hospital
Göteborg, Sweden

*Presented by Jan Holgersson