"Subclasses of ABO isoagglutinins in ABO-incompatible kidney transplantation."

Latinne, Dominique ; Squifflet, Jean-Paul ; De Bruyère, Marc ; Pirson, Yves ; Gianello, Pierre ; Sokal, G. ; Alexandre, Guy P.

Subclasses of ABO Isoagglutinins in ABO-Incompatible Kidney Transplantation

D. Latinne, J.P. Squifflet, M. De Bruyere, Y. Pirson, P. Gianello, G. Sokal, and G.P.J. Alexandre

ABO BLOOD group incompatibility has always been considered a major obstacle to organ transplantation. However, previously reported results[1] of transplants performed across this barrier have modified this opinion. From 1982 to January 1988, 31 patients received ABO-incompatible kidneys. If three nonsplenectomized patients are excluded, the actuarial graft survival at 5 years is 73% ± 10 (unpublished data).

Hyperacute vascular rejection has been avoided by plasmapheresis, the process of removing the recipient's preformed natural antibodies before grafting. In most cases, splenectomy and immunosuppressive therapy allow long-term function without postoperative irreversible humoral rejection. Simultaneous presence of ABH antigens and specific antibodies is an immunological paradox that is still unexplained.

We analyzed the classes and subclasses of ABO-specific antibodies and searched for any correlation between the occurrence of vascular rejection and the production of specific immunoglobulins.

PATIENTS AND METHODS

Twenty-eight patients were included in our study. All received an ABO-incompatible kidney (16 A to O, 6 B to O, 3 A to B, 2 AB to B, and 1 AB to A). A or B preformed natural antibodies were eliminated before the graft by means of plasmapheresis and injection of soluble antigens. Titers of natural and immune isoagglutinins were monitored daily during plasmaphereses and after grafting. Natural isoagglutinins were identified by hemagglutination at 4°C (diluted serum and A or B red cells in saline solution). Immune antibodies were detected by hemagglutination after fixation at 37°C and elution at 56°C. Five patients had an acute vascular rejection (3 nonsplenectomized), 6 others responded to rejection therapy despite a high increase of their isoagglutinins titer, and 17 patients had a functioning graft after one or more rejection crises or remained in chronic rejection without an increase in isoagglutinins titers.

In order to analyze the classes and subclasses of these specific antibodies, sera from these 28 patients were studied retrospectively. Anti-A and anti-B isoagglutinins were identified by cytofluorometry with specific mouse antihuman monoclonal antibodies and A or B red cells.

Three samples of 100 μL 5% A or B red cells in suspension were incubated with the same volume of patient's serum: the first hour at RT, the second hour at 37°C, and the third hour at 4°C after preincubation with DTT 0,01M (30 min at 37°). Sequential samples of serum provided before and after the graft were analyzed in each patient. After washing, 100 μL of different mouse monoclonal antibodies was added (specific for human, IgM, IgA, IgG1, IgG2, IgG3, or IgG4, Southern Biotechnology). After incubation and washing, a fluoresceinated goat antimouse immunoglobulin (FITC GAM-Coulter) was added. New incubation at RT and washing were followed by flow cytometry analysis (Epics C Coulter). Results were expressed as the percentage of fixed red cells measured by fluorescence labeling.

RESULTS

Plasmaphereses reduced the ABO blood group antibodies from hemagglutination titers of 1/8 to 1/1,024 to titers below 1/4. Natural isoantibodies returned to their initial titers within a few days. Immune antibodies appeared in all patients, increasing their natural isoagglutinin levels. Hemagglutination titers were between 1/1 and 1/256. Five patients underwent transplant excision after irreversible acute vascular rejection crises. All had a significant increase in natural and immune isoagglutinin titers.

Classes and subclasses of immunoglobulins were analyzed in serum samples at different times after the graft. In the three patients without splenectomy, the percentage of Ig fixed cells increased—IgM + A, IgM + G1, or IgM + G1 + G2 + G3. The two other patients who rejected the graft showed an increase in both IgM and IgG2 specific immunoglobulins. Six patients had rejection crises with increased hemagglutination titers but responded to therapy. The percentage of IgM fixed cells increased in both cases: IgG1 and IgG2 in two cases, IgG1 and IgG3 in one case. The hemagglutination titer in one of the patients reached 1/65,000 without any evidence of irreversible rejection; IgM^+, $IgG1^+$, and IgG^+ fixed cells increased after the graft.

In the third group of patients, in whom no increase in hemagglutination titers was observed, the percentage of fixed red cells did not show great variation.

DISCUSSION

We attempted to explain why, on the one hand, most patients receiving an ABO-incompatible kidney preserve good renal function despite the presence of isoagglutinins (sometimes in high titers), while, on the other hand, some patients do not respond to rejection therapy and are unable to preserve graft function. Three factors may be implicated: (1) antigenic tissue distribution (qualitative and quantitative), (2) type of antibodies (classes or subclasses), and (3) interactions between antigen, antibody, and complement at different

From the Department of Transplantation (Squifflet, Pirson, Gianello, and Alexandre) and Immunohematology (Latinne, De Bruyere, and Sokal), University of Louvain, Brussels, Belgium.

Address reprint requests to D. Latinne, MD, University of Louvain, Clos Chapelle-Aux-Champs, 30 Brussels 1200, Belgium.

0041-1345/89/$3.00/+0

steps of the immune reaction. As was reported previously, the titer of serum A or B isoagglutinins does not seem to predict graft function.[2,3] However, a qualitative study of these antibodies may be helpful. The results obtained with specific monoclonal antibodies directed against different classes and subclasses of immunoglobulins do not show any specific correlation between an increase in complement-fixing antibodies (IgM, G1, G3) and the occurrence of vascular rejection. The graft survival in one patient with a very high titer of isoagglutinins (IgM, G1, and G2) was particularly impressive. These antibodies seem to circulate in the blood without fixing to tissue antigens or leading to a lytic process.

Are the antigens qualitatively (types of A or B saccharidic chains) or quantitatively (density of dispersion of antigenic sites) responsible for this apparent lack of reactivity?[4] Is the affinity or accessibility of antigen-antibody interaction, complement binding, or activation processes implicated in inhibiting the immune reaction?

These questions need to be tested experimentally and by means of functional analysis of the components of the immune cascade that lead to allograft destruction.

REFERENCES

1. Alexandre GPJ: Transplant Proc 19:4538-4542, 1987
2. Chopek NW: Transplant Proc 19:4553-4557, 1987
3. Reding R: Transplant Proc 19:1989, 1987
4. Breiner NE: Transplantation 42:88-90, 1986
5. Oriol R: Vox Sang 51:161-171, 1986