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Brief communication

Interference of therapeutic antibodies used in desensitization protocols on lymphocytotoxicity crossmatch results

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ABSTRACT

Background: Therapeutic antibodies used to desensitize patients awaiting a human leukocyte antigen (HLA) or ABO-mismatched graft are suspected to interfere with the lymphocytotoxicity crossmatch (LCT-XM) test when they are present in the tested sera because of their potential ability to activate or inhibit the complement.
Methods: The most frequent therapeutic antibodies (Abs) used in desensitization protocols (intravenous immunoglobulins, rituximab, basiliximab, eculizumab, antithymocyte globulin) were added to a negative- or a positive-control serum at various concentrations, and tested in vitro in a LCT-XM test.
Results: Rituximab turned the LCT-XM positive on B cells at 0.2 μg/mL and antithymocyte globulin turned the LCT-XM positive with T and B cells at 20 μg/mL and 200 μg/mL, respectively. Treatment with dithiothreitol sera, supplemented with rituximab (0.2 and 2 μg/mL) and antithymocyte globulins (20 and 200 μg/mL), partially or totally reduced this positive interference. Intravenous immunoglobulin, eculizumab, and basiliximab did not trigger any interference with the negative control serum. In a positive LCT-XM, eculizumab did not annihilate activation of the rabbit complement. Intravenous immunoglobulins (25 g/L) could partially or totally reduced lysis score of positive crossmatch with weak lysis scores.
Conclusion: If eculizumab within the serum did not annihilate rabbit complement activation and basiliximab did not interfere with the crossmatch reaction, treatments based on rituximab, antithymocyte globulin and intravenous immunoglobulins need to be taken into account when interpreting a positive or negative crossmatch test.

1. Introduction

Prior to transplantation, the crossmatch (XM) test is crucial to confirm compatibility between the donor and recipient, especially for a patient receiving a graft from an ABO-mismatched and/or HLA-mismatched donor, to prevent acute antibody-mediated rejection [1]. A complement-dependent lymphocytotoxicity crossmatch (LCT-XM) still remains the gold standard technique [2].

This test is based on the incubation of sorted B- and T-lymphocytes from the donor, with the sera of the recipient, and collected during pre-graft monitoring. If anti-HLA antibodies (Abs) that target the donor HLA antigens are present in the tested sera, the rabbit complement is activated, and donor cells are lysed by complement-dependent cytotoxicity. In this test, sera are tested before and after treatment by dithiothreitol (DTT): a reducing agent used in the XM assay at a concentration able to inactivate IgM but not IgG [3]. A positive LCT-XM on T-cells caused by IgG constitutes a contraindication for transplantation [4,5]. In cases where the donor's transplant is HLA and/or ABO mismatched, the recipients can be pre-medicated using various desensitization protocols,

Abbreviations: Abs, antibodies; ATG, antithymocyte globulin; BSX, basiliximab; DSA, donor-specific antibody; DTT, dithiothreitol; ECZ, eculizumab; FC, flow cytometry; HLA, human leukocyte antigen; IVIg, intravenous immunoglobulin; LCT, lymphocytotoxicity; MoAbs, monoclonal antibodies; RTX, rituximab; XM, crossmatch.

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designed to reduce the amount of preexisting antibodies to a level that allows successful transplantation [6,7].

The major treatments combine intravenous immunoglobulins (IVIgs), polyclonal rabbit antithymocyte immunoglobulins (ATGs), chimeric IgG1 monoclonal antibodies (MoAbs) that target the IL-2 receptor/CD25 (basiliximab: BSX) or CD20 (rituximab: RTX), and a humanized IgG2/IgG4 MoAb that targets the C5 fraction of human complement (eculizumab: ECZ). These treatments can be used in combination to assess the recipient's immunological risk, and have demonstrated their long-term efficiency in kidney transplantation [8–17]. Because of their ability to activate the complement system (RTX and BSX human IgG1 isotype or polyclonal rabbit ATG and IVIgs), or their ability to target complement components (ECZ) or HLA antibodies (IVIgs), they may also interfere with the LCT-XM reactions and, thus, provide false results.

This study investigated if the therapies used as desensitization drugs could interfere with the LCT-XM results. We added RTX, ECZ, IVIgs, BSX, and ATG to a positive control serum and a negative control serum to assess the LCT-XM reaction, at concentrations classically found in the blood of treated patients.

2. Materials and methods

2.1. Isolation of B- and T-lymphocytes

Cells were isolated, using the standard Ficoll method (Eurobio), from a triturated spleen taken from a human deceased organ-donor. Cells were washed and re-suspended in McCoy's buffer at 5×10 cells/mL [7]. B- and T-lymphocytes were sorted using an EasySep® Kit (STEMCELL Technologies) according to the manufacturer's recommendations.

2.2. Sera and reagents

A heat-inactivated AB Human Serum (Life technologies) was used as negative control. A serum containing anti-HLA Abs (One Lambda) was used as a positive control. Dilutions of positive control serum were performed in PBS-albumin buffer (Vialebex®, LFB, 60 g/L) at 1/10, 1/100, 1/1000, 1/10,000 and 1/100,000. The drugs tested were IVIgs (Tegeline®, LFB-BIOMEDICAMENTS and Privigen®, Csl Behring Gmbh), RTX (Mabthera®, Roche Registration Ltd), BSX (Simulect®, Novartis Europharm Ltd), ECZ (Soliris®, Alexion Europe), and ATG (Thymoglobulin®, Genzyme). The drug dilutions were prepared extemporaneously (Tegeline® and Privigen ® concentrations from 0.0025 to 25 g/L; RTX concentrations from 0.02 to 2000 μg/mL; BSX concentrations from 0.2 to 2000 μg/mL; ECZ concentrations from 0.2 to 2000 μg/mL; ATG concentrations from 0.2 to 2000 μg/mL) in PBS-albumin buffer (Vialebex®, LFB) at 60 g/L. Serum supplemented with RTX and ATG were tested before and after DTT treatment (Sigma-Aldrich) or PBS-albumin treatment for the control (2 μL at 37 °C for 15 min).

2.3. LCT crossmatch technique

Sorted B and T cells were incubated with a negative or a positive control serum, and rabbit complement (Servibio), in Terasaki plates (Ingen) for 90 min at 22 °C. Separation between alive and dead cells was achieved by fluorescent microscopy, using the FluoroQuench® staining/quenching reagent (Ingen); results were then codified (from 8 = total lysis, to 1 = no lysis).

3. Results

3.1. Interferences from drugs on a negative LCT-XM

A negative LCT-XM test was performed using sorted B and T cells incubated with the negative control serum supplemented or not with IVIgs, ATG, BSX, ECZ, and RTX at the concentrations, and corresponding to blood concentrations of patients reported in the literature (Fig. 1A) [18–21]. The LCT-XM was negative (cell lysis score = 1) on B and T cells with and without adding IVIgs, BSX, and ECZ to the negative serum, and at whatever concentrations tested (from 0.2 to 2000 μg/mL for BSX and ECZ, and from 0.0025 to 50 g/L for IVIgs). When negative sera were supplemented with 2 to 2000 μg/mL of RTX, we observed a positive LCT-XM on B-cells and a lysis score of 8, decreasing to 6 at 0.2 μg/mL, and a negative XM at 0.02 μg/mL.

A positive LCT-XM was obtained for both B and T cells when the negative control serum was supplemented with ATG concentrations from 0.2 to 2000 μg/mL, with a threshold of positivity at 20 μg/mL for T cells and 200 μg/mL for B cells (Fig. 1A). Interference with ATG and RTX on negative XM was tested for after treatment by DTT (Fig. 1A, left column). The lysis score was lower after DTT treatment in both T and B cells. Indeed, T-cell XM was positive at 20 μg/mL of ATG without DTT, and at 200 μg/mL of ATG with DTT. We noted B-cell XM positivity at a 200-μg/mL ATG concentration but a lower cell lysis score after DTT treatment (a lysis score of 8 vs. 2, respectively). With RTX, B-cell XM was positive at 20 μg/mL and at 0.2 μg/mL after treatment with and without DTT, respectively (Fig. 1A).

3.2. Interference of drugs on a positive LCT-XM

A positive LCT-XM test was simulated as described in Materials and methods, using a positive control serum that contained cytotoxic anti-HLA Abs (Fig. 1B). Whatever the concentrations of IVIgs and ECZ used, LCT-XM remained positive (cell lysis score = 8) on B and T cells (Tegeline® and Privigen® concentrations from 0.0025 to 25 g/L; ECZ concentration from 0.2 to 2000 μg/mL). No reduction in T- or B-cell lysis score was observed with BSX, ATG, or RTX at concentrations of 0.02 to 2000 μg/mL (data not shown).

Positive control serum dilutions were then incubated with IVIg [25 g/L] or ECZ [200 μg/mL] (Fig. 2). A negative LCT-XM was obtained cells since positive control serum was diluted at 1/100,000 and 1/10,000 for both B and T cells, respectively. No effect on B- and T-cell lysis scores was observed after DTT treatment. The adjunction of IVIg has triggered a decrease of the B-cell lysis score from 6 to 4, and from 4 to 1 with the positive control diluted at 1/100 and 1/1000, respectively. T-cell lysis scores were decreased from 4 to 2 and from 2 to 1 with the positive control diluted at 1/100 and 1/1000, respectively. ECZ addition did not modify B- and T-cell lysis score whatever the positive control dilution used (Fig. 2).

4. Discussion

LCT-XM is the sole XM technique that reveals the functional potential of anti-HLA Abs to activate complement. Its result constitutes an important datum in the decision to perform transplantation or not, especially when the recipient can have donor-specific antibodies (DSAs): thus driving the decision to establish prior desensitization protocols. It has been previously described that a false positive LCT-XM can be obtained in different situations, notably in cases where kidney recipients are suffering from an autoimmune disease [22].

In our study, we demonstrate the ability of RTX and ATG to provide a false positive LCT-XM result. As expected, for RTX, we found that LCT-XM positivity was restricted to B cells [23]. Philippe et al. have previously described a false positive LCT-XM in the presence of a RTX residual concentration of 0.6 μg/mL, which was observed at 78 days after the last infusion [24]. Our results are in accordance with these results. Moreover, we found that a false positive LCT-XM could be detected for lower RTX concentrations (i.e., 0.02 μg/mL), which corresponds to blood concentrations classically found several months after the last infusion. In vivo, ATG is responsible for depletion of T- and NK-cell populations, but not B cells and monocytes. However, we found a false positive LCT-XM for T and B cells at 20 and 200 μg/mL, respectively. These results suggest that ATG binds to B cells and can activate complement in vitro. This interaction has already been evoked in previous studies demonstrating that ATG can trigger B-cell apoptosis [25,26]. Because IVIgs are prepared from thousands of human plasma donors, it may contain anti-HLA Abs. Only a few studies have performed HLA antibody screening in IVIg preparations [27,28]. Recently, Ravindranath et al. showed, using Luminex, that IVIgs react with a wide array of HLA alleles and can recognize the HLA types of the corresponding donors [29]. In our study we didn't find any interference of IVIgs with a negative LCT-XM. This suggests an absence of HLA antibodies in the therapeutic preparation or at least their inability to activate rabbit complement due to their low levels, protein purification, or the pathogen-reduction steps, which could interfere with the functional abilities of IgG to activate complement. BSX, because of its IgG1 isotype, could theoretically activate the complement system, depending on the level of expression of the targeted antigen: CD25. We did not observe any false positive

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