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Tocilizumab in a patient affected by chronic active antibody-mediated rejection; histological improvement, reduction of proteinuria and renal function stabilization

Laura Panaro^{1*}, Matteo Trezzi¹, Michela Ardini¹, Michele Marchini¹, Marco Delsante², Davide Rolla¹

¹Divisione di Nefrologia, Dialisi e Trapianto, Ospedale Civile Sant'Andrea di La Spezia, La Spezia, Italy

²Divisione di Nefrologia, Azienda Ospedaliera Universitaria di Parma, Parma, Italy

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ABSTRACT

Introduction: Chronic active antibody-mediated rejection (cAMR) is a significant and rapid destructive form of allograft rejection and it is related to donor specific antibodies (DSA). Interleukin 6 (IL-6) plays an important role in mediating the allograft rejection by promoting CD4+ T cells differentiation to Th17 phenotype while inhibiting Treg. Tocilizumab is a humanized monoclonal antibody directed to IL-6 receptor (IL-6R). The aim of the study is to demonstrate the efficacy of tocilizumab as rescue therapy for cAMR.

Case Presentation: A 50-year-old man with Alport syndrome and with positive DSA against B7 e B55 underwent a second kidney transplant (HLA 2 mismatch). He received thymoglobulin and three plasma exchanges as induction therapy. Proteinuria (1-1.3 g/24 h) and decline in kidney function (serum creatinine; 1.5 mg/dL) appeared at 9 months. Kidney biopsy showed endocapillary proliferation, mononuclear cells infiltration, glomerular basal membrane duplication and tubulitis suggestive of cAMR. The patient has been treated with tocilizumab (6 mg/kg/mon) for six months. Reduction of proteinuria (0.6 g/24 h) and mild improvement of kidney function (serum creatinine; 1.3 mg/dL) were observed after tocilizumab treatment. A second biopsy revealed a significant decrease of glomerulitis and peritubular capillaritis. A significant reduction in DSA was detected.

Conclusion: Inhibition of the IL-6 receptor by tocilizumab may represent a novel and cheering approach to treat cAMR.

Implication for health policy/practice/research/medical education:

Chronic active antibody-mediated rejection (cAMR) is a significant and rapid destructive form of allograft rejection, where IL-6 plays an important role. We report a case of 50-year-old man with kidney transplant, who developed cAMR and has been treated with tocilizumab, a humanized monoclonal antibody directed to IL-6 receptor (IL-6R). We demonstrate its efficacy as new treatment strategy in patients who are resistant to current therapies.

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Introduction

Chronic active antibody-mediated rejection (cAMR) is a significant and rapid destructive form of allograft rejection. Five thousands allografts are lost every year in the United States, primarily due to cAMR (1). The current treatment consists in the reduction of antibody levels using rituximab, immunoglobulins and plasma exchange; however, cAMR is often unresponsive to such therapies.

Recently, there has been interest in targeting interleukin

6 (IL-6). IL-6 is a pleiotropic key cytokine that regulates inflammation and the development, maturation and activation of T cells, B cells, and plasma cells. Circulating IL-6 activates cells by binding to the non-signaling membrane receptor (IL-6R). The IL-6/IL-6R cassette can activate two major pathways: the signal transducer and activator transcription 3 (STAT3) and the mitogen activated protein kinase. Under extreme conditions of immune activation, as in acute humoral rejects,

*Corresponding author: Laura Panaro, Email: laura.panaro@asl5.liguria.it

serum levels can surge to more than 10 000 pg/mL (2). Interaction of IL-6 with IL-6 R induces acute phase reactants' synthesis, including C-reactive protein, serum amyloid A, hepcidin and fibrinogen, while it reduces levels of albumin, fibronectin and transferrin (3).

Tocilizumab is a humanized monoclonal antibody aimed at inhibiting IL-6 binding to its receptor. Tocilizumab binds to both soluble and membrane-bound form of the IL-6R. It has obtained FDA approval for some immune disorders and has been recently evaluated as rescue therapy for cAMR plus donor specific antibodies (DSA).

Case Presentation

A 51-year-old obese male underwent a second kidney transplant in 2018. The patient's history shows micro-hematuria and hearing loss from childhood secondary to Alport syndrome. He had been on hemodialysis from the age of 17 years old. His first kidney transplant was performed in 1990, but because of an early failure, he started again the hemodialysis treatment three years later, in 1993.

In the period following the transplant, the patient contracted viral hepatitis B, for which he is still being treated with Lamivudine. In 2012, adenocarcinoma of the colon was diagnosed during an ordinary screening, reason why he was suspended from the waiting list for 5 years.

In May 2018, the patient underwent a second kidney transplant. The donor was a deceased 51-year-old female;

cold ischemia lasted 13 hours and the transplant was complicated by delayed graft function. Induction therapy was made by basiliximab, thymoglobulin (3 mg/kg), three plasmapheresis/plasma exchange (PLEX) and pulse steroid. The maintenance therapy was composed of tacrolimus (TL between 6-8 µg/dL for the first year), mycophenolate mofetil (MMF) and steroid. Renal function was mildly depressed at discharge (serum creatinine; 1.6 mg/dL).

At the time of the second transplantation, immunology tests showed two mismatches for HLA and the presence of anti-HLA antibodies directed against DR 53 (fluorescence intensity (MFI) pre-kidney transplant 15000).

DR 53 is considered a "public superantigen", because it shares some epitopes with other HLA class II antigens (DR4, DR7, DR9), but donor-specific anti-HLA-DR53 antibodies are not considered an exclusion criteria in the algorithm for the transplant candidate selection. The donor presented a DR 7 antigen.

After 9 months from kidney transplant, we noted a slight increase in proteinuria (from 0.5 to 1.3 g/24 h), and a mild deterioration of renal function (serum creatinine from 1.3 to 1.5 mg/dL). The research for DSA confirmed only a high rate of anti-HLA-D53 [fluorescence intensity (MFI) 15000-16000]. Thus, we decided to perform a renal biopsy.

The histological report showed a cAMR. Light microscopy examination revealed severe chronic transplant glomerulopathy, glomerulitis and focal severe peritubular capillaritis. The biopsy also showed T-cell infiltrate underneath thickened sub-endothelial space of an interlobular artery, consistent with chronic transplant arteriopathy, and focal interstitial T-cell dominant inflammation with moderate tubulitis (Figure 1A and 1B). C4d deposition in peritubular capillaries was negative.

The usual therapy of cAMR consists of reducing the antibody levels by rituximab + immunoglobulins + plasma exchanges, but it is often resistant to such therapies. Hence, we decided to start the treatment with tocilizumab (RoActemra at a dose of 6 mg/kg) after premedication with steroids [methylprednisolone 60 mg (intravenous; i.v.)], paracetamol, and chlorphenamine in our "Day hospital", with constant monitoring of vital signs during the sixty-minute infusion time.

Before the initiation of tocilizumab, the patient was tested for HBV-DNA (neg.), HCV and HIV serology, and Quantiferon-TB. Prophylactic antiviral therapy with acyclovir (200 mg/d) and Pneumocystis Carinii prophylaxis with trimethoprim+sulfamethoxazole (160 plus 800 mg; half a tablet three times a week) was established. During the treatment period (one year) we observed two adverse events; a transient increase of HBV-DNA (1190 IU/mL), treated with entecavir (tablet 0.5 mg/d), and an episode of pneumococcal pneumonia,

Table 1. Demographic characteristics

| Recipient characteristics | |
|--|--------------------------------|
| Age at transplant (y) | 51 |
| Gender | Male |
| Previous kidney transplantation | Yes |
| Time between dialysis and transplant (y) | 15 |
| Donor characteristics | |
| Age (y) | 51 |
| Gender | Female |
| Cold ischemia time (h) | 13 |
| Delayed graft function | Yes |
| Immunology at the time of transplantation | |
| HLA mismatches (n) | 2 |
| Anti-HLA DSA positive | B7 (MFI 2450) - B55 (MFI 1100) |
| Functional data at discharge | |
| s-Creatinine (mg/dL) | 1.26 |
| eGFR (mL/min) | 65.6 |
| Proteinuria (g/d) | 0.5 |
| Time from transplantation to treatment (mon) | 14 |
| Functional data at the time of diagnosis of cAMR | |
| s-Creatinine (mg/dL) | 1.64 |
| eGFR (mL/min) | 47.4 |
| Proteinuria (g/d) | 1.3 |
| Anti-HLA DSA positive | DR53 – MFI 15000 |
| Rejection episodes before cAMR | No |

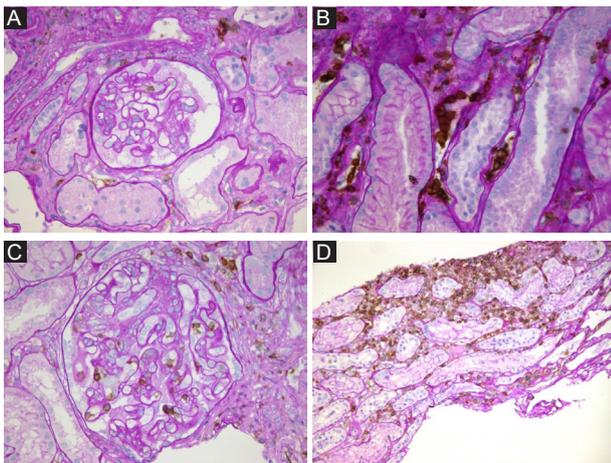


Figure 1. Microvascular inflammation before (upper panels) and after (lower panels) tocilizumab administration, with leukocyte common antigen (LCA) immunohistochemistry. The first biopsy showed severe chronic transplant glomerulopathy and glomerulitis (A, LCA+PAS, 400x) and focal severe peritubular capillaritis (B, LCA+PAS, 400x). After tocilizumab therapy, glomeruli showed thickening and reduplication (chronic transplant glomerulopathy) of basement membranes but no significant glomerulitis (C, LCA+PAS, 400x); inflammatory infiltrate was mostly interstitial with only mild and focal peritubular capillaritis. Mild tubulitis was observed (D, LCA+PAS, 200x).

responsive to antibiotic therapy.

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On follow-up, renal function and 24-hour proteinuria were monitored. Figure 2 shows renal function and 24-hour proteinuria, before and after tocilizumab.

After 6 months of tocilizumab treatment, we repeated a renal biopsy: the glomeruli showed a thickening and a reduplication (chronic transplant glomerulopathy) of basement membranes but no significant glomerulitis. Inflammatory infiltrate was mostly interstitial with only mild and focal peritubular capillaritis (Figure 1C-D). After 12 months, in addition to a clear histological improvement, we observed a functional renal stabilization and a persistent reduction of proteinuria (Figure 2), and we assisted to a reduction in DSA (MFI 15000 pre-tocilizumab, MFI 7500 post-tocilizumab (Figure 3).

Discussion

IL-6 consists of 184 amino acids and was originally identified by Narazaki and Kishimoto (4) as B-cell stimulatory factor 2. In healthy subjects, this cytokine is not expressed, but it is rapidly synthesized by tissue injury, as observed in chronic immune disorders (rheumatoid arthritis, systemic juvenile idiopathic arthritis, and polyarticular juvenile rheumatoid arthritis), transplant rejection and graft versus host disease (5). In the kidney, IL-6R is present on mesangial cells and IL-6/IL-6R

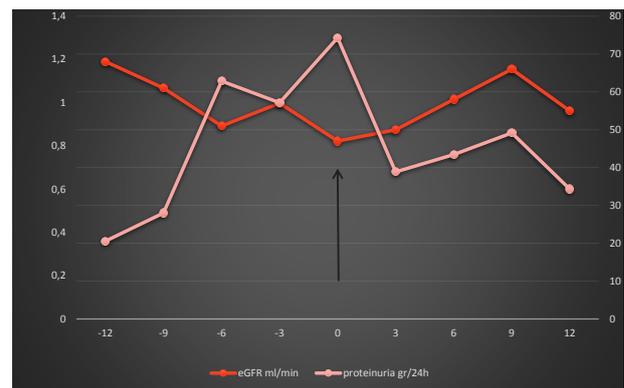


Figure 2. Renal function and 24-h proteinuria, before and after tocilizumab

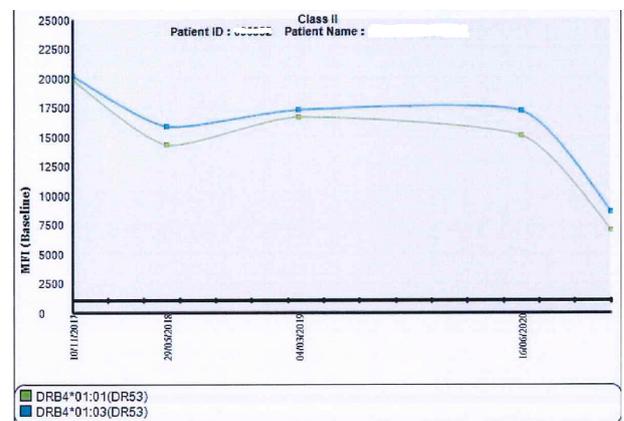


Figure 3. Reduction of DSA class II after tocilizumab treatment.

cassette is associated with mesangial cell proliferation, which seems to play a pathological role in the progression of experimental crescentic glomerulonephritis. In abnormal renal tissue, IL-6R is expressed on the surface of podocytes, tubular epithelial cells and arterial smooth muscle cells.

Clinical observations and animal models have demonstrated that IL-6 plays an important role in mediating the allograft rejection. IL-6 production is responsible for allogenic T-cells infiltration (6), while its inhibition with anti-IL-6R induces graft acceptance, in combination with costimulatory pathway blockage by CTLA-4 (cytotoxic T-lymphocytes associated protein 4) (7). B-cells that infiltrate the allograft produce massive pro-inflammatory cytokines, including IL-6, that can induce interstitial fibrosis and tubular atrophy (IF/TA). Circulating IL-6 activates cells by binding to the non-signaling membrane receptor (IL-6R). The IL-6/IL-6R cassette then activates cellular gp 130, which triggers the signaling transduction pathway that ultimately results in JAK/STAT activation and gene transcription events (8).

Other data from animal models have demonstrated an important role in the mediation of allograft vasculopathy

(9). Using a murine aortic interposition model of vascular rejection, donor-derived IL-6 amplifies allogenic T cell response that causes vascular rejection. Moreover, it is known that endothelial cells produce IL-6 as a major factor responsible for intimal proliferation. In summary, cytokines are essential in host defense and maintenance of tissue homeostasis, but abnormal or excessive IL-6 overexpression results in inflammation and tissue injury. Disruption of signaling pathways limits inflammation by inhibiting cell activation and expansion of effector cells, thus reducing chronic immune activity.

Long-term outcomes of patients with cAMR is very poor with a median graft survival of 1.9 years. The conventional “standard care therapies” (PLEX, IV-Ig and rituximab) are often disappointing and associated with higher risk of infectious complications which are particularly insidious in immunosuppressed patients.

Choi et al (10) in 2017 treated with tocilizumab 36 pts with cAMR plus DSAs and transplant glomerulopathy who failed standard care treatment. They demonstrated graft survival rates of 80% at 6 years with a stabilization of renal function at two years. Moreover, Lavacca et al (11) in 2020 adopted tocilizumab as a first-line approach in cAMR, showing early serological and histological improvement as demonstrated by protocol biopsies.

In our case, we decided to maintain the same chronic immunosuppression therapy and to associate tocilizumab, without prior standard therapy for cAMR. The control biopsy, after six months of tocilizumab, demonstrated a clear amelioration of glomerulitis and capillaritis. It is possible that the beneficial effects seen on glomerular compartment were due to the blockage of IL-6 local production by mesangial cells, which also express IL-6 receptor. Moreover, Lavacca et al (11) observed an increased expression of three genes (TJP-1, AKR1C3 and CASK) that are upregulated by tocilizumab treatment and that stimulate the regeneration of podocytes, mesangial and tubular cells. Because a rebound of IL-6 activity was demonstrated after stopping tocilizumab in desensitization protocol (12), we decided to continue tocilizumab, achieving a constant stabilization of renal function and a persistent reduction of proteinuria and DSA.

Conclusion

This case presentation showed that further studies are needed with rigorous clinical trials setting, but tocilizumab may represent a valid alternative to the management of cAMR.

Authors' contribution

LP, DR and MA were the principal investigators of the study. LP, DR, MA, MT, MD and MM were included in preparing the concept and design. LP and DR rechecked

the manuscript and critically evaluated the intellectual contents. All authors participated in preparing the final draft of the manuscript, revised the manuscript and critically evaluated the intellectual contents. All authors have read and approved the content of the manuscript and confirmed the accuracy or integrity of any part of the work.

Conflicts of interest

Authors declare no conflict of interests

Ethical considerations

The use of tocilizumab was conducted in consideration of ethical issues in accordance to the Helsinki Declaration and was approved by both internal Ethical and Pharmaceutical Committees. The patient gave written informed consent after description of the potential risks and benefits of tocilizumab.

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References

1. Loupy A, Hill GS, Jordan SC. The impact of donor-specific anti-HLA antibodies on late kidney allograft failure. *Nat Rev Nephrol.* 2012;8(6):348-57. doi: 10.1038/nrneph.2012.81
2. Garbers C, Aparicio-Siegmund S, Rose-John S. The IL-6/gp130/STAT3 signaling axis: recent advances towards specific inhibition. *Curr Opin Immunol* 2015;34:75-82. doi: 10.1016/j.coi.2015.02.008
3. Tanaka T, Narazaki M, Masuda K, Kishimoto T. Regulation of IL-6 in Immunity and Diseases. *Adv Exp Med Biol.* 2016;941:79-88. doi: 10.1007/978-94-024-0921-5_4
4. Narazaki M, Kishimoto T. The Two-Faced Cytokine IL-6 in Host Defense and Diseases. *Int J Mol Sci.* 2018;19(11):3528. doi: 10.3390/ijms19113528
5. Hunter CA, Jones SA. IL-6 as a keystone cytokine in health and disease. *Nat Immunol.* 2015;16(5):448-57. doi: 10.1038/ni.3153
6. Jordan SC, Choi J, Kim I, Wu G, Toyoda M, Shin B, et al. Interleukin-6, A Cytokine Critical to Mediation of Inflammation, Autoimmunity and Allograft Rejection: Therapeutic Implications of IL-6 Receptor Blockade. *Transplantation.* 2017;101(1):32-44. doi: 10.1097/TP.0000000000001452.
7. Sebba A. Tocilizumab: the first interleukin-6-receptor inhibitor. *Am J Health Syst Pharm.* 2008 Aug 1;65(15):1413-8. doi: 10.2146/ajhp070449
8. Leonard WJ, O'Shea JJ. Jaks and STATs: biological implications. *Annu Rev Immunol* 1998; 16:293-322. doi: 10.1146/annurev.immunol.16.1.293.
9. Von Rossum A, Rey K, Enns W, Manku S, Cheema R, MacEwan GE et al. Graft-Derived IL-6 Amplifies Proliferation and Survival of Effector T Cells That Drive Alloimmune-Mediated Vascular Rejection. *Transplantation*

- 2016; 100(11):2332-41. doi: 10.1097/TP.0000000000001227
10. Choi J, Aubert O, Vo A, Loupy A, Haas M, Puliyaanda D, et al. Assessment of Tocilizumab (Anti-Interleukin-6 Receptor Monoclonal) as a Potential Treatment for Chronic Antibody-Mediated Rejection and Transplant Glomerulopathy in HLA-Sensitized Renal Allograft Recipients. *Am J Transplant*. 2017;17(9):2381-9. doi: 10.1111/ajt.14228.
 11. Lavacca A, Presta R, Gai C, Mella A, Gallo E, Camussi G et al. Early effects of first-line treatment with anti-interleukin-6 receptor antibody tocilizumab for chronic active antibody-mediated rejection in kidney transplantation. *Clin Transplant*. 2020; 34(8):e13908. doi: 10.1111/ctr.13908
 12. Vo A, Choi J, Kim I, Louie S, Cisneros K, Kahwaji J et al. A Phase I/II Trial of the Interleukin-6 Receptor-Specific Humanized Monoclonal (Tocilizumab) + Intravenous Immunoglobulin in Difficult to Desensitize Patients. *Transplantation*. 2015;99(11):2356-63. doi: 10.1097/TP.0000000000000741

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