Cytokine Profiles in Children After Pediatric Kidney Transplantation With Acute Cellular Compared to Chronic Antibody-mediated Rejection and Stable Patients: A Pilot Study

Nadja Borsum, MSc,1 Murielle Verboom, Ing.,2 Thurid Ahlenstiel-Grunow, MD,1 and Lars Pape, MD, PhD1

Background. Different patterns of plasma cytokines can be expected in the case of chronic active-antibody-mediated (cAMR) and acute cellular rejection (AR) after kidney transplantation (KTx). Methods. IL-2, 4, 6, 10, 17A, tumor necrosis factor alpha, and interferon gamma were measured in 51 pediatric KTx recipients at time of renal biopsy (17 AR, 14 cAMR, 20 normal). Patients were divided into a training (n = 30) and a validation (n = 21) set. Results. IL-6 was significantly higher in AR patients and significantly lower in the case of cAMR. In children with s-creatinine increase, IL-6 values were significantly different between AR and cAMR. IL-10 levels showed similar tendencies. For IL-2, 4, 17A, tumor necrosis factor alpha, and interferon gamma, no differences were found. In the independent validation cohort, the receiver operating characteristic area under the curve for IL-6 was 0.79 and 0.70 for AR and cAMR. In children with AR, an IL-6 <1141 fg/ml, and in those with cAMR, an IL-6 >721 fg/ml was associated with a specificity of 86%/76%, a sensitivity of 71%/80%, a positive predictive value of 56%/45%, and a negative predictive value of 92%/94%. Conclusions. In this pilot study, the plasma IL-6 level is a promising biomarker to identify pediatric kidney transplant recipients free from AR and cAMR and might help to distinguish between both entities, whereas there is only a nonsignificant trend toward the usability of IL-10. Validation in larger cohorts in combination with other biomarkers are warranted.

(Transplantation Direct 2019;5: e501; doi: 10.1097/TXD.0000000000000943. Published online 8 October, 2019.)
IFNy- ratio is associated with normal Th1 cytokines, suppressed Th2 cytokines and poor graft survival. Low levels of the proinflammatory cytokine IL-17 were associated with reduced expression of the Th1 cytokine IFNy and less graft damage and better survival in a murine model of KTx. In a pretransplant risk model, high soluble IL-17 levels were associated with a higher risk of future rejection; however, no measurements were taken at the time of rejection. In kidney biopsies following acute rejection, IL-17 could be found as a marker of rejection. In the case of inflammation, Treg can be converted into harmful Th17-producing cells. Treatment of inflammation can lead to TNFβ production and thereby a reswitch to Treg that protect the graft from immunological complications.

B cells also contribute to enhanced T-cell activation and differentiation, as well as formation of memory T cells by production of the cytokines IL-6 and TNFα. It has been shown in experimental models that the proinflammatory cytokine IL-6 is upregulated in the case of acute rejection. Of additional interest, plasma cells are supported by stromal cell-secreted IL-6 in their surviving niches.

Th1 cells mainly produce IFNy, IL-2, and TNFβ and evoke cell-mediated immunity and phagocyte-dependent inflammation, whereas Th2 cells secrete IL-4, IL-5, IL-6, IL-9, IL-10, and IL-13. Their activation leads to strong antibody responses and eosinophil accumulation but inhibits several functions of the phagocytic cells. The “classical” Th1/Th2 paradigm in allograft response states that Th1 response (IL-2 and IFNy) is associated with rejection, whereas the Th2 response is linked to the development of tolerance. In adults, an increase in the Th1 cytokines IL-6 and IL-10 has been shown in the case of chronic cellular rejection, whereas IL-10 and IFNy were increased in patients with acute rejection (as defined by Banff 2007 criteria). In those patients with stable graft function, IFNy and Th2 cytokines were downregulated. In pediatric liver transplantation, an association of increased IL-2 and decreased IFNy was found in the cases of acute rejection. Recently, it has been shown that in preactivation of endothelial cells with anti-HLA-DR antibody, allogenicity is redirected towards a pro-inflammatory response by decreasing amplification of functional Treg and by further increasing IL-6-dependent Th17 expansion.

According to these findings, it can be hypothesized that acute rejection with acute inflammation might be associated with higher levels of immune activating and proinflammatory cytokines, whereas cytokines that are expressed in protolerogenic states might be primarily found in stable patients. It was the intention of this trial to test this hypothesis in children after KTx.

**MATERIALS AND METHODS**

**Patients**

Fifty-one kidney transplant patients under the age of 18 years (21 females, mean age 13.0 ± 3.9 y) were classified into 3 groups according to graft function based on clinical symptoms, Banff classification of graft biopsy, and DSA analysis. Seventeen children were diagnosed with acute cellular rejection (AR), Banff 4, (8 females, mean age 13.0 ± 3.4 y) and 14 children with biopsy-proven chronic humoral rejection, Banff 2 (7 females; mean age 14.7 ± 2.9 y). The control group consisted of 20 children with baseline creatinine and normal protocol biopsy, Banff 1 or 6 (7 females, mean age 11.7 ± 4.5 y). The actual Banff classification based on definitions, Banff Lesion Scores, and Banff Diagnostc Categories during time of biopsy was used in each case. Patients from each group were divided randomly 3:2 into a training (n = 30) and a validation (n = 21) set.

Exclusion criteria were symptoms of infections and severe illnesses as well as mixed Banff classifications (combination of cellular and humoral rejection). Patient characteristics are given in Table 1.

**Plasma Samples**

Whole blood samples were collected at a timepoint of renal protocol biopsy 6 months or a later annual control after KTx.

| TABLE 1. Patient characteristics |
|----------------------------------|
| Control group (N = 20) | Acute rejection (N = 17) | Chronic antibody-mediated rejection (N = 14) |
| Mean Age (y) | 11.7 ± 4.5 | 13.0 ± 3.4 | 13.9 ± 3.7 |
| Age range | 2–18 | 6–18 | 5–17 |
| Sex (male/female) | 13/7 | 9/7 | 7/7 |
| Mean eGFR | 65.2 ± 21.8 | 59.6 ± 27.9 | 43.5 ± 26.8 |
| Range of eGFR | 28.4–95.9 | 26.0–133.0 | 16.7–104.0 |
| Serum creatinine (µmol/l) | 91.2 ± 52.9 | 101.9 ± 30.8 | 116.4 ± 43.9 |
| Mean Time (d) Transplantation – biopsy | 1015 | 418 | 2379 |
| BMI | 19.4 ± 3.9 | 21.7 ± 6.9 | 20.7 ± 5.5 |
| First transplantation | 16 | 12 | 10 |
| Second transplantation | 3 | 5 | 1 |
| Donor LD/DD | 4/15 | 3/14 | 5/9 |
| Mean level of IFTA on biopsy (ci/ct) | 0/1 | 1/1 | 1/1 |
| Presence of HLA DSA (timepoint of biopsy) | | | |
| Class I only (%) | 0 | 0 | 1 (7.1) |
| Class II only (%) | 1 (5.2) | 5 (31.3) | 8 (57.2) |
| Class I and II (%) | 0 | 0 | 1 (7.1) |
| Non-HLA-DSA (ATII Receptor A8) (%) | 0 | 0 | 4 (28.6) |

BMI, body mass index; DD, deceased donor; DSA, donor-specific antibodies; eGFR, estimated glomerular filtration rate; HLA, human leukocyte antigen; IFTA, interstitial fibrosis and tubular atrophy; LD, living donor.
or during episodes of kidney transplant rejection. Samples were drawn by venipuncture in S-Monovette 7.5 mL LH (Sarstedt AG & Co. KG, Nümbrecht, Germany) and centrifuged immediately at 315 g for 10 min at room temperature. Lithium heparin plasma aliquots were stored at −80°C until required for flow cytometric cytokine measurements.

Acute rejection episodes (AREs) were categorized as follows: (1) biopsy-proven acute rejection (BPAR) Banff score ≥IA on indication biopsy; (2) BPAR including borderline findings on indication biopsy, triggering antirejection therapy; (3) overall treated ARE (BPAR plus ARE, where a graft biopsy was either logistically not possible or medically contraindicated, but where antirejection therapy was initiated).

**Donor-specific Antibodies**

Human leukocyte antigen (HLA) antibodies were measured before engraftment and at least annually posttransplant by the LABScreen single-antigen beads Luminex Kit (One Lambda, Canoga Park, CA) which uses single HLA-coated beads and enables identification of IgG alloantibody specificities against HLA-A, -B, -C, -DRB1/3/4/5, -DQA1, -DQB1, -DPA1, and -DPB1 antigens. Because no clinically validated cutoff for the Luminex assay is recommended by the provider company, a mean fluorescence intensity of ≥1 000 was used to define the cutoff for antibody positivity. For high-resolution typing, CTS-Sequence Kits (Heidelberg, Germany) and Olerup-SSP Kits (Olerup-SSP AB, Stockholm, Sweden) were used.

Estimated glomerular filtration rate was calculated using the complete 2009 Schwartz formula.26

**Cytometric Bead Array Immunoassay**

Because of IL-6 and IL-10 levels around the detection limit of the standard set, cytokine plasma levels, including IL-2, IL-4, IL-6, IL-10, IL-17A, TNFα, and IFNγ, were simultaneously quantified with the Human Th1/Th2/Th17 Cytokine Kit and additional Enhanced Sensitivity Flex Set IL6/IL10 (all BD Biosciences Pharmingen, San Diego, CA), according to the instruction manual. Data acquisition and analysis was performed with a FACSVerse flow cytometer, using FACSuite and FCAP Array software (BD Biosciences Pharmingen).

**Statistical Analysis**

Data were expressed as median and range for each group. The difference between groups was analyzed by the Wilcoxon two-sample test. All analysis, including the receiver operating characteristic (ROC) analysis were performed with GraphPad Prism 6. A P ≤0.05 was considered statistically significant. In the ROC analysis, the patients were grouped as AR versus combined rejection (cAMR + controls) and cAMR versus combined rejection (AR + controls).

This study was approved by the ethics committee of Hannover Medical School (Number 2336-2014) and all families and patients gave informed consent.

**RESULTS**

**Interleukin 6**

In patients with AR Banff ≥IA (training set), IL-6 concentration (3 751 SD 3 214 versus 840 SD 645 [fg/ml], P = 0.0016) was significantly higher than in the other patients. In children with cAMR, IL-6 (579 SD 692 versus 2 258 SD 2 559 [fg/ml], P = 0.0071) was significantly lower. Among patients with increase in s-creatinine, IL-6 values (3 751 SD 3 214 versus 579 SD 692 [fg/ml], P = 0.0031) were significantly different between patients with AR and cAMR (Figure 1).

In the training cohort, AR showed an area under the ROC curve (AUC) for IL-6 of 0.84 (95% confidence interval [CI], 0.66-1.03, P = 0.002); for cAMR, the AUC for IL-6 was 0.81 (95% CI, 0.64-1.00, P = 0.16) (Figure 2); AR IL-6 <1631.0 fg/ml and cAMR IL-6 >901.7 fg/ml were associated with a specificity of 80%/88%, a sensitivity of 85%/64%, a positive predictive value of 52%/57%, and a negative predictive value of 96%/91% at 20% prevalence.

In the independent validation cohort, AR showed an AUC for IL-6 of 0.79 (95% CI, 0.55-1.02, P = 0.04); for cAMR, the AUC for IL-6 was 0.81 (95% CI, 0.64-1.00, P = 0.16) (Figure 3); AR IL-6 <1141.0 fg/ml and cAMR IL-6 >721.0 fg/ml were associated with a specificity of 86%/76%, a sensitivity of 85%/64%, a positive predictive value of 52%/45%, and a negative predictive value of 92%/94% at 20% prevalence.

**Interleukin 10**

Measurements of IL-10 concentration showed the same tendency as for the IL-6 results but no significant differences. Patients with AR Banff ≥IA (training set) showed higher IL-10 concentration (2 686 SD 5 598 versus 519 SD 965 [fg/ml], P = 0.05) than the other patients but not significantly. There
FIGURE 2. In the training cohort, (A) acute cellular rejection (AR) showed an area under the receiver operating characteristic (ROC) curve (AUC) for IL-6 of 0.84%. In cAMR (B), the ROC analysis indicates an AUC for IL-6 of 0.81%. The diagonal lines indicate random guessings associated with an AUC of 50%. cAMR, chronic antibody-mediated rejection; IL, interleukin.

FIGURE 3. In the independent validation cohort, (A) acute cellular rejection (AR) showed an area under the receiver operating characteristic (ROC) curve (AUC) for IL-6 of 0.79%. In cAMR (B), the ROC analysis indicates an AUC for IL-6 of 0.70%. The diagonal lines indicate random guessings associated with an AUC of 50%. cAMR, chronic antibody-mediated rejection; IL, interleukin.

FIGURE 4. Plasma concentration of IL-10 of patients with long-term stable graft function (control), patients with acute rejection (acute), and patients with chronic antibody-mediated rejection (cAMR). Results are represented as median, minimum, and maximum concentration. AR, acute cellular rejection; IL, interleukin.
levels. In AMR, it can be speculated that no acute systemic end-products were increased but, interestingly, not IL-6. Serum levels of IL-17, IL-33 and receptor for advanced glycaation is increased in patients with chronic graft dysfunction. This is especially interesting as the IL-6 antibody tocilizumab has proven to be an effective treatment for AMR.27 The IL-6 pathway is active and high IL-6 production is associated with activation of Th17 cells and inhibition of Treg with attendant inflammation.28 IL-6 drives B-cell activation and differentiation of B-cells to antibody-producing plasma cells. In AR, there is inflammation which leads to the upregulation of IL-6 production, with high levels measured as shown previously.29 Chung et al30 have shown that the Th17-L phenotype is increased in patients with chronic graft dysfunction. Serum levels of IL-17, IL-33 and receptor for advanced glycation end-products were increased but, interestingly, not IL-6 levels.30 In AMR, it can be speculated that no acute systemic inflammation occurs but that both IL-6 and IL-17 primarily bind in the graft and are thereby reduced in the serum.

IL-6 is a pleiotropic cytokine with proinflammatory and anti-inflammatory properties and acts according to 2 different receptor pathways. In classic signaling, target cells are stimulated via IL-6, interacting with the membrane-bound IL-6 receptor (mIL-6R). The resulting IL-6/mIL-6R complex associates with the signaling receptor protein gp130 and activates an intracellular signaling cascade. Only a few cell types express mIL-6R, mainly hepatocytes, neutrophils, monocytes, and some leukocyte subpopulations, as well as some T- and B-cells. IL-6-trans-signaling acts via the soluble IL-6 receptor (sIL-6R). The soluble IL-6/sIL-6R complex can bind to gp130 on cells that lack the membrane-bound IL-6R. Membrane-bound gp130 is omnipresent and thus the spectrum of IL-6 target cells will be enlarged.31

In several experiments with mouse models of human disease, it could be shown that IL-6 classical signaling, activating STAT3, represents the anti-inflammatory or regenerative axis of IL-6, whereas IL-6 trans-signaling typifies the proinflammatory part of the IL-6 axis.32-35 The IL-6/sIL-6R complex seems to promote the shift from acute to chronic inflammation via transition from neutrophil to mononuclear cell infiltrate and activation of the immune system.36-38 Selective blocking of sIL-6R via sgp130Fc protein inhibits the proinflammatory but not the anti-inflammatory mIL-6R pathway. This could lead to new therapeutic options in chronic rejection after KTx despite tocilizumab therapy. The sgp130Fc was efficient in several preclinical models of inflammation (intestinal inflammation, rheumatoid arthritis, asthma, and inflammation-associated cancer) and initiated phase II clinical trials in patients with active ulcerative colitis.39

IL-6 plasma levels of healthy men varied between 0.9 and 30.6 pg/ml (mean 3.1 pg/ml) in line with their circadian rhythms.40 This data support the finding of low IL-6 plasma in pediatric patients. In healthy individuals, approximately 30% of circulating IL-6 is free-floating in the blood and able to bind to mIL-6R. The larger part (~70%) is bound in IL-6/sIL-6R complexes. A mathematical model demonstrated that a 2-fold increase of sIL-6R results in a 43% decrease of free IL-6 concentration.41 The finding of low plasma IL-6 in AMR does not implicate the absence of IL-6 production. In fact, circulating IL-6 could be bound to sIL-6R promoting the proinflammatory trans-signal pathway. The circulating IL-6/sIL-6R complex possibly conceals the effective amount of free circulating IL-6. To substantiate our data, future measurements of sIL-6R protein could further clarify the difference between acute and chronic renal rejection. Analyses of IL-8 could verify this additional approach, because IL-8 induces IL-6R shedding from neutrophils.36,37

On the one hand, as the measurement of IL-6 (and highly sensitive IL-6) is routine in many laboratories for diagnosis of acute inflammation, this test could easily be introduced in transplanted patients and might therefore be used as a routine surrogate marker for AMR or AR. On the other hand, it has to be taken into account that in the case of elevated values of IL-6 the clinician will have to differentiate between an inflammation because of AR and an impairment of graft function that has been caused by a viral or bacterial infection, eventually in combination with dehydration.

IL-10 is a cytokine with anti-inflammatory and immunomodulation properties. It influences the release of immune mediators, both antigen presentation and the phagocytosis of macrophages. This inhibits the release of proinflammatory mediators such as TNFα, IL-1β, IL-6, and others.42 IL-10 increase can be induced by elevated IL-6 plasma levels.43 This confirms our findings of high IL-6 plasma levels in correlation with high IL-10 plasma levels during acute rejection in our study cohort. In fact, IL-10 inhibits the release of proinflammatory mediators from monocytes and macrophages; it acts as a negative feedback on IL-6 secretion.44 In IL-10 deficient mice,45 as well as in diseases with a relative or absolute IL-10 deficiency (eg, rheumatoid arthritis46 or after organ transplantation47), there is ongoing immune activation. It has been shown that the production of IL-10 is part of an autocrine pathway to reduce uncontrolled activation of IFNγ in Th1 cells. The inability to produce enough IL-10 is associated with an unregulated antiodor response and can be linked to a higher proportion of graft loss over time.47 It could also be shown that IFNγ/IL-10 ratios were higher in patients with AMR as compared with other patients after renal transplantation.48 These findings correspond to our experience of decreased IL-10 levels in children with AMR.

Our study is limited by the relatively small number of pediatric patients included. As measurements were not performed at regular timepoints before biopsy, no prediction values for the markers for development of cellular or humoral rejection after early detection of changes in IL-6 or IL-10 before renal biopsy could be calculated.

Therefore, future longitudinal studies should evaluate whether IL-6 and IL-10 levels can differentiate earlier between patients with acute rejection and cAMR before renal function...
decreases and indication biopsies are perfumed. Consequently, an increase in immunosuppressive therapy based on these biomarkers could be a possible intervention to prevent the full picture of clinical rejection. Second, further studies should determine if IL-6 decrease and IL-10 decrease in patients with cAMR can be detected before cAMR is diagnosed clinically. Renal biopsy could then be performed earlier, and interventions could lead to more timely intensification and change of immunosuppression or IL-6 antibody therapy and thus preserve the graft.

In conclusion, serum IL-6 and possibly IL-10 are promising biomarkers that might help the clinician to identify kidney graft recipients free from acute rejection and cAMR and to distinguish between both entities in the case of creatinine increase. However, this study only represents pilot data; therefore, future longitudinal studies in larger populations must confirm the potential of these 2 cytokines as diagnostic and possibly predictive markers of cellular and humoral rejections of kidney grafts to incorporate these markers in prediction models of rejection.

REFERENCES

1. Solez K, Colvin RB, Racusen LC, et al. Banff 07 classification of renal allograft pathology: updates and future directions. Am J Transplant. 2008;8:753–760.
2. Tavakoli-Ardakani M, Mehrpooya M, Mehdizadeh M, et al. Association between interleukin-6 (IL-6), interleukin-10 (IL-10) and depression in patients undergoing hematopoietic stem cell transplantation. Int J Hematol Oncol Stem Cell Res. 2015;9:80–87.
3. Kanzelmeyer NK, Ahlenstiel T, Drube J, et al. Protocol biopsy-driven interventions after pediatric renal transplantation. Pediatr Transplant. 2010;14:1012–1018.
4. Cosio FG, El Ters M, Cornell LD, et al. Changing kidney allograft histology early posttransplant: prognostic implications of 1-year protocol biopsies. Am J Transplant. 2016;16:194–203.
5. Chai H, Yang L, Gao L, et al. Decreased percentages of regulatory T cells are necessary to activate Th1-Th17-Th22 responses during acute rejection of the peripheral nerve xenotransplantation in mice. Transplantation. 2014;98:729–737.
6. Yu X, Jiang Y, Lu L, et al. A crucial role of IL-17 and IFN-γ during acute rejection of peripheral nerve xenotransplantation in mice. Plos One. 2012;7:e4419.
7. Karakhanov O, Oweira H, Steinmeyer B, et al. Interferon-γ, interleukin-10 and interferon-inducible protein 10 (CXCL10) as serum biomarkers for the early allograft dysfunction after liver transplantation. Transpl Immunol. 2016;34:14–16.
8. Limaye AP, La Rosa C, Longmate J, et al. Plasma IL-10 levels to guide antiviral prophylaxis prevention of late-onset cytomegalovirus disease, in high risk solid kidney and liver transplant recipients. Transplantation. 2010;100:210–216.
9. Pape L, Becker JU, Immenschuh S, et al. Acute and chronic anti-body-mediated rejection in pediatric kidney transplantation. Pediatr Nephrol. 2015;30:417–424.
10. Takatsuki K. Cytokines involved in B-cell differentiation and their site of action. Proc Soc Exp Biol Med. 1997;215:121–133.
11. Hoffmann W, Laikakis FG, Chalasani G. B cells, antibodies, and more. Curr J Am Soc Nephrol. 2016;11:137–154.
12. van der Vugt LE, Zinsou JF, Ozir-Fazalalikhan A, et al. Interleukin 10 (IL-10)-producing CD19+ regulatory B cells from schistosoma haematobium-infected individuals induce IL-10-positive T cells and suppress effector T-cell cytokines. J Infect Dis. 2014;210:1207–1216.
13. Yang M, Sun L, Wang S, et al. Novel function of B cell-activating factor in the induction of IL-10-producing regulatory B cells. J Immunol. 2010;184:3321–3325.
14. Cherukuri A, Rothstein DM, Clark B, et al. Immunologic human renal allograft injury associates with an altered IL-10/IFN-α expression ratio in regulatory B cells. J Am Soc Nephrol. 2014;25:1575–1585.
15. Kwan T, Chadban SJ, Ma J, et al. IL-17 deficiency attenuates allograft injury and prolongs survival in a murine model of fully MHC-mismatched renal allograft transplantation. Am J Transplant. 2015;15:1555–1567.

16. Millán O, Rafael-Valdivia L, San Segundo D, et al. Should IFN-γ, IL-17 and IL-2 be considered predictive biomarkers of acute rejection in liver and kidney transplantation? Results of a multicentric study. Clin Immunol. 2014;154:141–154.
17. de Menezes Neves PD, Machado JR, dos Reis MA, et al. Distinct expression of interleukin 17, tumor necrosis factor α, transforming growth factor β, and forkhead box P3 in acute rejection after kidney transplantation. Ann Diagn Pathol. 2013;17:75–79.
18. Hanidiziar D, Koulmanda M. Inflammation and the balance of treg and th17 cells in transplant rejection and tolerance. Curr Opin Organ Transplant. 2010;15:411–415.
19. Raad IV, Yang J, Chock S, et al. Jagged2-signalizing promotes IL-6-dependent transplant rejection. Eur J Immunol. 2013;43:1449–1458.
20. Romagnani S, T-cell subsets (Th1 versus Th2). Ann Allergy Asthma Immunol. 2000;85:9–18. Quiz 18, 21.
21. Strom TB, Roy-Chaudhury P, Manrho R, et al. The th1/th2 paradigm and the allograft response. Curr Opin Immunol. 1996;8:688–693.
22. Zhai Y, Gholibar R, Busuttil RW, et al. Th1 and th2 cytokines in organ transplantation: paradigm lost? Crit Rev Immunol. 1999;19:155–172.
23. Karczewski M, Karczewski J, Poniedziałek B, et al. Distinct cytokine patterns in different states of kidney allograft function. Transplant Proc. 2009;41:4147–4149.
24. Briem-Richter A, Leuschner A, Krieger T, et al. Peripheral blood biomarkers for the characterization of allogeneic reactivity after pediatric liver transplantation. Pediatr Transplant. 2013;17:757–764.
25. Rousso F, Simmonds N, Claasuen-van Groringen M, et al. A 2014 reference guide to the banff classification of renal allograft pathology. Transplantation. 2016;99:1795–1814.
26. Schwartz GJ, Muñoz A, Schneider MF, et al. New equations to estimate GFR in children with CKD. J Am Soc Nephrol. 2009;20:629–637.
27. Choi J, Aubert O, Vo A, et al. Assessment of tocolizumab (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. 2014;13:2381–2389.
28. Jordan SC, Choi J, Kim I, et al. Interleukin-6, A cytokine critical to mediation of inflammation, autoimmunity and allograft rejection: therapeutic implications of IL-6 receptor blockade. Transplantation. 2017;101:32–44.
29. Van Oers MH, Van der Heyden AA, Aarden LA. Interleukin 6 (IL-6) in serum and urine of renal transplant recipients. Clin Exp Immunol. 1989;71:314–319.
30. Chung BH, Kim KW, Kim BM, et al. Increase of th17 cell phenotype in kidney transplant recipients with chronic allograft dysfunction. Plos One. 2015;10:e0145258.
31. Scheller J, Chalaris A, Schmidt-Arras D, et al. The pro- and anti-inflammatory properties of the cytokine interleukin-6. Biochem Biophys Acta. 2011;1813:878–888.
32. Grivennikov S, Karin E, Karin N, et al. IL-6 and stat3 are required for survival of intestinal epithelial cells and development of colitis-associated cancer. Cancer Cell. 2009;15:103–113.
33. Barkhausen T, Tschernig T, Rosenstiel P, et al. Selective blockade of interleukin-6 trans-signaling improves survival in a murine polymicrobial sepsis model. Crit Care Med. 2011;39:1407–1413.
34. Atrey R, Mudder J, Finotto S, et al. Blockade of interleukin 6 trans signaling suppresses T-cell resistance against apoptosis in chronic intestinal inflammation: evidence in crohn disease and experimental colitis in vivo. Nat Med. 2000;6:583–588.
35. Rabe B, Chalaris A, May U, et al. Transgenic blockade of interleukin 6 transsignaling abrogates inflammation. Blood. 2008;111:1021–1028.
36. Marin V, Montero-Julian FA, Gries S, et al. The IL-6-soluble IL-6rα autocrine loop of endothelial activation as an intermediate between acute and chronic inflammation: an experimental model involving de Menezes NE. J Immunol. 2001;167:3435–3442.
37. Kaplanski G, Marin V, Montero-Júlían F, et al. IL-6; a regulator of the cytokine interleukin-6 receptor mediated transplant rejection. Am J Transplant. 2014;15:1555–1567.
38. Agarastos A, Hauger RL, Barakauskas DA, et al. Circadian rhythmicity, variability and correlation of interleukin-6 levels in plasma
and cerebrospinal fluid of healthy men. Psychoneuroendocrinology. 2014;44:71–82.
41. Gaillard J, Pugnière M, Tresca J, et al. Interleukin-6 receptor signaling. II. Bio-availability of interleukin-6 in serum. Eur Cytokine Netw. 1999;10:337–344.
42. Sabat R, Grütz G, Warszawski K, et al. Biology of interleukin-10. Cytokine Growth Factor Rev. 2010;21:331–344.
43. Steensberg A, Fischer CR, Keller C, et al. IL-6 enhances plasma IL-1ra, IL-10, and cortisol in humans. Am J Physiol Endocrinol Metab. 2003;285:E433–E437.
44. Köhn R, Löhler J, Rennick D, et al. Interleukin-10-deficient mice develop chronic enterocolitis. Cell. 1993;75:263–274.
45. Katsikis PD, Chu CQ, Brennan FM, et al. Immunoregulatory role of interleukin 10 in rheumatoid arthritis. J Exp Med. 1994;179:1517–1527.
46. DeBruyne LA, Li, Chan SY, et al. Lipid-mediated gene transfer of viral IL-10 prolongs vascularized cardiac allograft survival by inhibiting donor-specific cellular and humoral immune responses. Gene Ther. 1998;5:1079–1087.
47. Shiu KY, McLaughlin L, Rebollo-Mesa I, et al. Graft dysfunction in chronic antibody-mediated rejection correlates with B-cell-dependent indirect antidonor alloresponses and autocrine regulation of interferon-γ production by th1 cells. Kidney Int. 2017;91:477–492.
48. Shiu KY, McLaughlin L, Rebollo-Mesa I, et al. B-lymphocytes support and regulate indirect T-cell alloreactivity in individual patients with chronic antibody-mediated rejection. Kidney Int. 2015;88:560–568.