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Kinetic analysis of changes in T- and B-lymphocytes after anti-CD20 treatment in renal pathology

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ABSTRACT

Introduction: The main objective of this study is to describe qualitatively and quantitatively the different immune lymphocyte phenotypes of patients with renal disease after treatment with anti-CD20.
Material and methods: Two cohorts of transplanted and autoimmune kidney patients were compared: (1) Those who began treatment with Rituximab, matched (for sex, age and general clinical parameters) with (2) Non-treated control kidney patients. Different analyses were performed: (A) B-lymphocyte subpopulations; (B) T-cell subpopulations; (C) serum levels of BAFF, APRIL, Rituximab and anti-Rituximab; (D) rs396991 polymorphism of CD16a and at different time points for each type of analysis: (i) at baseline, (ii) day 15, (iii) at three and (iv) six months post-antiCD20.
Results: (A) A depletion of all B cell subsets analysed was observed preferentially decreasing the $CD40^{+}$memory B-cells, switched memory cells and plasmablasts. (B) A significant decreased percentage of $CD4^{+}$T-lymphocytes was observed. A significant decrease of the percentage of memory T-cells and an increase in naïve T-cells was also observed. (C) A significant increase for APRIL was observed, as well as a positive correlation between the APRIL levels, and the differential of B-cells. (D) The presence of CD16a Valine-variant induced greater changes in the variations of total T-cell and T-naïve subpopulations.
Conclusion: Our results highlight that the treatment of renal disease with Rituximab affects T-cells, particularly naïve/memory balance, while APRIL could be also a secondary marker of this treatment. The sequential analysis of phenotypic alterations of B- and T-cells could help patient management, although further studies to identify periods of remission or clinical relapse are warranted.

1. Introduction

Anti -CD20 monoclonal antibodies were first used in the treatment for non-Hodgkin lymphoma during the late nineties. Currently in Europe, their use is only approved for the treatment of four conditions: non-Hodgkin lymphoma, chronic lymphocytic leukaemia, rheumatoid arthritis and vasculitis (granulomatosis with polyangiitis and microscopic polyangiitis) (Perosa et al., 2010; Alduaij and Illidge, 2011; European Medicines Agency, 2016). In renal pathology, Rituximab has been proven to be useful in the treatment of some primary glomerulopathies, vasculitis with renal involvement, severe lupus with kidney disease, and especially in renal transplantation, where it is applied during the treatment of desensitization in hyperimmunized patients, patients receiving transplantation from an ABO incompatible living donor (Faguer et al., 2007), and patients with antibody-mediated rejection (Faguer et al., 2007; Salama and Pusey, 2006; Sugiura et al., 2011; Mosquera Reboredo and Vázquez Martul, 2011; Vo et al., 2008; Becker et al., 2004; Genberg et al., 2008; Kaposztas et al., 2016). The B-lymphocyte antigen CD20, is expressed at all stages of B cell maturation; from late pro-B cells and progressively increasing in concentration until maturity, but is absent on plasma

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blasts and plasma cells. Anti-CD20 agents produce a depletion of $CD20^+$lymphocytes reducing the potential of the humoral response and leaving only pre-existent antibody-producing plasma cells (Vieira et al., 2004). Three different mechanisms by which the drug leads to depletion of B-cells are known: (i) complement-mediated cytotoxicity, (ii) antibody-mediated cellular cytotoxicity (ADCC) and (iii) apoptosis induced by CD20 surface recognition (Pescovitz, 2006; Renaudineau et al., 2009; Taylor and Lindorfer, 2008). It was described (Egawa et al., 2007; Schmidt et al., 2009; Vital et al., 2011a) that the degree of B-cell depletion in treated patients is the factor that best correlates with clinical response to the drug (Thurlings et al., 2010). Other items that may also explain the different clinical and biological responses to the treatment are: (a) polymorphisms in Fc-gamma immunoglobulin receptors (Cañete et al., 2009) highlighting the V(valine) at position 158 (rs396991) variant of FCGR3A gene that leads to an increased receptor function; (b) changes in the complement system, such as those associated with CD59 that decreases the drug's effectiveness (Hu et al., 2011); (c) BAFF (B-cell Activating Factor) and APRIL (A Proliferation-Inducing Ligand) both promoting B-cell survival and proliferation [Rituximab induces increased BAFF serum levels in patients with systemic lupus erythematosus (SLE), rheumatoid arthritis or Sjögren's syndrome. For APRIL, results are less consistent]; both molecules are considered possible future targets for treatment (Pescovitz, 2006; Renaudineau et al., 2009).

The available immunosuppressants in renal pathology of immune origin are based on modulating T-cells (Mesquita Júnior et al., 2016; Ingulli, 2010; Masri, 2003) therefore B-cell targeting by Rituximab could be an "alternative" selective immunomodulating treatment (Looney et al., 2010). Thus, in Rituximab treatment of ABO-mismatched kidney transplant recipients, a sustained decrease of anti-HLA-antibody levels was described as well as a reduction in the number of chronic humoral rejections (Kohei et al., 2012). Similarly, after anti-CD20 treatment of patients with SLE, published works describe clear and significant changes in the phenotype of circulating lymphocytes, in both B- and T-cells: (a) next to the expected depletion of B-cells by Rituximab, an unexpected decrease of naïve and memory T-lymphocytes have been described, particularly between three and six months after treatment, when a gradual recovery of naïve B- and T-cells occurs. These changes extend for two years post-treatment, in relation to disease remission, while the return of memory cells correlates with relapse (Iwata et al., 2011; Vallerskog et al., 2007). (b) An increase in regulatory T-cells in patients with clinical response to treatment has also been described (Sfikakis et al., 2007). (c) Finally, a reduction of co-stimulatory molecules (CD40 and CD80) suggests less T-cell activation has been also involved (Stroopinsky et al., 2012; Tokunaga et al., 2005). In a recent longitudinal study it has been described that a single dose of rituximab in the induction therapy in renal transplant recipients leads (in addition to a depletion of B-cells) to a relative increase of transitional and memory-like B cells, without affecting T-cell phenotype and function (Kamburova et al., 2014).

Despite obvious differences (antibodies against autoantigens versus anti-HLA antibodies, autoreactive T-cells vs alloreactive T-cells), there are certain similarities between the SLE immunopathology and humoral kidney rejection, involving both humoral (IgG and hypermutated antibodies in both humoral responses, involving T and B cooperation) and cellular immunity (Th1 and Th17 responses). Thus, as with the SLE studies, it seems logical to develop evaluations of lymphocyte dynamics in renal pathological processes (Iwata et al., 2011).

Based on these previous data, the results of this work have been systematically divided into four study groups in relation to treatment with anti-CD20: (A) phenotypic analysis of B-cell subpopulations, (B) T-cell phenotyping, (C) BAFF, APRIL, anti-Rituximab and Rituximab serum levels, (D) role of FCGR3A polymorphism (CD16a). Although broad statistical analyses of the studied results were undertaken, in this study we have only mentioned the significant results. The aim of this study was to define cell changes in B and T subpopulations so as to better understand the kinetics of immune response in renal pathology after anti-CD20 treatment.

2. Patients, samples, materials and methods

2.1. Patients and samples

Patients that were treated with anti-CD20 between June 2012 and November 2012 and attended at the Nephrology Service were included in this study. Rituximab was administered for: (i) desensitization of hyperimmunized patients prior to transplantation, and patients receiving a kidney transplant from an ABO-mismatched living donor; (ii) humoral rejection treatment in kidney transplant; (iii) glomerular pathology or systemic autoimmune disease treatments.

A total of 37 patients (18 female patients and 19 male patients) were included in the study, divided into two cohorts (groups), the Treatment Group (TG) and the untreated Control Group (CG). Patients, recruited in groups paired by sex, age and general clinical parameters, include 19 cases (TG) and 18 untreated controls (CG): (A) Six cases (TG) treated with Rituximab for desensitization prior to transplantation, matched with five controls (CG) living donor transplant; (B) five cases (TG) treated with Rituximab for acute humoral rejection paired with seven transplanted controls (CG) clinically stable; (C) eight cases (TG) treated with Rituximab for autoimmune or glomerular diseases, paired with six controls (CG) with autoimmune or renal glomerular disease clinically stable. We recorded the principal diagnosis and the Rituximab concomitant treatment for the TG and the treatment for the CG within each group and subgroup of patients (Table 1). Six patients in the TG were previously treated with Rituximab; following differential expression analysis no statistically significant difference was found between TG and CG when those retreated patients with Rituximab are discounted. The differences between first treatment and retreatment patients at baseline period are shown in Table 2.

Rituximab doses were prescribed and administered following the protocol of the Nephrology Service: for pre-transplant desensitization, the protocol recommends two doses of 200 mg for ABO mismatched living donor transplant (400 mg in total) and two doses of 400 mg for transplantation in hyperimmunized patients (800 mg in total), for acute rejection two doses of 400 mg (800 mg in total), and for renal autoimmune diseases two doses of 1 g (2 g total). Four patients received extra doses (more than 1 gr in total) during the study, due to clinical requirements, two of them 1200 and 1600 mg with extradoses during the same year of the study. The other two patients received 2400 and 1400 mg in total, four and five years before the study respectively.

Samples were obtained from all these patients, (5 mL EDTA blood to flow cytometry and 5 mL coagulated blood serum) before treatment (baseline), at 15 days, three months and six months. All patients provided signed informed consent under current regulations defined and approved by the Ethics Committee of Hospital Clinic, Barcelona.

2.2. Analytical studies

2.2.1. Cytometric studies: B-cell subpopulations (section A) and T-cell subpopulations (section B)

Multicolor cytometry was performed from whole blood samples using pooled monoclonal antibodies in panels selected to define the appropriate lymphocyte subpopulations (Supplemen-