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Name of Journal: ***World Journal of Transplantation***

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Answering reviewers

We thank Editors and Reviewers for the careful review of our review entitled "B cells with regulatory properties in transplantation tolerance" 21206 in "World Journal of Transplantation".

Reviewer Comments:

In this excellent review authors reported an overview on how regulatory B cells may interfere and facilitate allograft acceptance. The review is well written although a little bit tok long and on the other side lacking of a crucial paragraph (The clinical side). I thus suggest the following before publication: 1. Reduce by 30% the length of the review, particularly for the section on complement which appear to long and the section on IL10 / IL35 which is way to long 2. Chapter on B cells and transplant rejection: again please cut the section on C3/C4d too much. Please include some relevant reference when discussing how to depleting or targeting B cells, the use of anti-CD22 should be mentioned [(i) Carvello M et al. Diabetes. 2012 Jan;61(1):155-65, (ii) Fiorina P, Sayegh MH. F1000 Biol Rep. 2009 May 28;1:39], furthermore the use of anti BLyss strategy should be mentioned as well (Zekavat G et al. J Immunol. 2008 Dec 1;181(11):8133-44). 3. In the section on Emerging role of regulatory B cells authors should mentioned and reinforce the appearance and reshape of B cell pool after the use of B cell deploying strategy. It is interesting to note that more immature B cells appear to be more regulatory (Fiorina P et al. Diabetes. 2008 Nov;57(11):3013-24) and how the targeting of B cells may be a double edge sword (also discussed in Kim JI1, Rothstein DM, Markmann JF. Curr Opin Organ

Transplant. 2015). Where is really ending the benefit and starting the harm in B cells depletion therapy? This is an open point. 4. Again the chapter on IL10/IL35 appeared to extensive , clearly well developed should clarify how sìstudy on extreme phenotype may help (Kleffel S et al Diabetes. 2015 Jan;64(1):158-71). Is it possible to see if patients with operative tolerance have different levels of Bregs? can be some of the study simply doomed by the study of Breg peripheral instead of more properly at Breg generation ability? Finally I really would like to see a chapter on clinical relevance or take home message for the clinician.

As you will see, we improved the manuscript by reducing some parts as requested by the reviewer and by adding what has been suggested in red in the revised version as follow :

"Different strategies have been developed to reduce the level of donor-specific antibodies in transplanted patients. One approach is to induce the depletion of B cells using depleting antibodies such as anti-CD20 (Rituximab) or anti-CD22. Rituximab is a glycosylated immunoglobulin G (IgG) chimeric mouse/human antibody that binds to the CD20 antigen present on the majority of circulating B cells from the pre-B-cell stage until terminal differentiation into plasma cells. However, CD20 is not found on pro–B cells or mature plasma cells (the latter produce 90% of circulating IgG), thus, rituximab eliminates peripheral B cells without preventing the regeneration of B cells from precursors, and does not directly affect immunoglobulin levels [15]. Rituximab is efficient to treat auto-immune diseases and lymphoma [16], however, in clinic, no convincing benefit was found so far as induction therapy in renal transplantation. However, in conjunction with other treatment it has been reported to have a beneficial effect on antibody production in chronic antibody-mediated rejection [17]. CD22 is a glycoprotein of the Ig superfamily in the sialoadhesin subclass and act as an inhibitory receptor. In mice, anti-CD22 conjugated to calicheamicin,

has been shown to deplete B cells in peripheral blood, spleen, bone marrow, and lymph nodes and given that CD22 also is expressed on CD138+ plasma cells, this compound has also a potential effect on autoantibody production [18]. Thus, this antibody promotes in mice the reduction of the anti-islet immune response in various models of islet transplantation [19]. In Human, Epratuzumab, a humanized anti-CD22 antibody, has been shown to induce some depletion of naive and transitional B cells, producing a 35% reduction in total B-cell numbers and can also inhibit B-cell activation and proliferation. It has shown beneficial effect for immunotherapy of systemic lupus erythematosus [20]. An other strategy is to modulate B cell response by targeting B-cell survival, proliferation and maturation. In this regard, to modulate the BAFF pathway is promising [21]. BAFF (B-cell-activating factor belonging to the tumour necrosis factor family, also known as BLys, TALL-1, and THANK) exists in both membrane-bound and soluble forms and is produced by monocytes, macrophages and dendritic cells. There are three BAFF receptors; BAFF-R (also known as BR3), TACI (transmembrane activator and calcium modulator and cyclophyllin ligand interactor) and BCMA (B-cell-maturation antigen) expressed on follicular, germinal centre and memory B cells, but BCMA is preferentially expressed on plasma [22]. In vivo BAFF neutralization has been shown to be efficient in experimental models of auto-immune diseases such as diabete [23]. In transplantation, BAFF-deficient recipients had extended transplant survival in a murine cardiac allograft model [24]. In addition, in an islet allograft model, BAFF blockade with a monoclonal antibody (in combination with rapamycin) resulted in long-term MHC-disparate allograft survival [25]. A pilot study to assess the use of belimumab (a humanized monoclonal antibody that inhibits BAFF) as monotherapy to reduce antibody levels in sensitized patients was terminated after recruiting eight patients because of the lack of efficacy (ClinicalTrials.gov Identifier: NCT01025193). BAFF-blockade, must now be tested in combination with other immunosuppressive agents, since these

agents have already been used in the treatment of autoimmune disease [26] such as systemic lupus erythematous (SLE) [27]."

And

"In long-term normoglycemic NOD mice, which are naturally protected from diabetes, islet-infiltrating B cells were identified as more antigen-experienced IL-10+ cells with more diverse B-cell receptor repertoires compared to those of hyperglycemic mice. In addition, healthy individuals showed increased frequencies of both CD40+ and IL-10+ B cells compared to type 1 diabetic patients [88]"

And

"Clinical relevance
Although largely described as involved in the prevention of auto-immune diseases, the importance of CD19+ CD24high CD38high immature B cells in kidney transplantation in a clinical setting, has been highlighted by their increased frequencies in operationally tolerant patients after immunosuppressive treatment cessation [55][56]. The proof as to their direct role in this phenomenon is still lacking but these studies suggest the relevance of these cells as biomarkers of tolerance. In this sense, a recent longitudinal prospective study aiming to track the temporal relationship between these regulatory, transitional B cells and clinical events demonstrates that transitional B cell frequencies (but not total B cells or "regulatory" T cells) were associated with protection from acute rejection [137]. Another study demonstrate that patients with chronic antibody-mediated rejection display a unique B-cell phenotype with a reduced ratio of activated to memory B cells associated with an impaired immunosuppressive activity that depends on their maturation status [138]. Therefore, these clinical studies highlighted the

potential utility of these cells as biomarkers of predictive graft outcome, to adapt immunosuppressive treatment.

According to immuno-suppression protocols, there constitution of the B cell compartment in the presence of alloantigens could create a favorable environment for the development and maintenance of tolerance towards antigens of the graft. Indeed, Parsons and his colleagues demonstrated in mice that depletion of the B cell compartment at the time of transplantation induces tolerance by depleting allo-reactiveB cell clones and reshaping the B cell repertoire [139].Following some immunosuppressive treatments, the B cell compartment is recolonized by B cell populations exhibiting a phenotype of regulatory B cells. For example, following an induction treatment with Alemtuzumab (anti-CD52), the authors observed a temporary increase in the proportion of transitional B cells, described as regulatory B cells [140]. It has also been sown that that Alemtuzumab rather than Basiliximab (anti-CD25) induction was associated with the appearance of a novel peripheral lymphocyte phenotype, although clinical outcomes were broadly similar. This phenotype was notable for the prominence of naïve, transitional and regulatory B-cell subtypes and was associated with more stable graft function. These transitional cells as immature cells are due to homeostatic repopulation following lymphocyte depletion [141]. Furthermore, similar results were obtained in non-human primates following depletion of B cells with Rituximab (anti-CD20). Indeed, the reconstitution of the compartment by immature and transitional B cells was associated with long-term graft survival of pancreatic islets [142]. In a model of diabetes in mice, anti-CD22 treatment also demonstrated the generation of a pool of immature reemerging B220+ CD93+ CD23+ IgMlow B-cells, functionally impaired in their ability to present antigen, and that can regulate at long-term the autoimmune response in vivo by establishing tolerance toward autoantigens [18].

Moreover, a novel role of CD24high CD27+ and IL-10+ plasmablast B cells has been suggested in the regulation of human chronic graft-versus-host

disease [143].

Therefore, we should question the paradigm of depleting B cells as the central strategy for controlling rejection. Depleting strategy at the induction phase may help to reshape the immune B cell repertoire and the reemergence of regulatory immature B cells but at a latter phase or for the treatment of antibody-mediated rejection, although prevent the donor-specific antibody formation, may be deleterious for the pre-existing regulatory B cell population. The exact therapeutical narrow of depletion and the beneficial effect of combined immunosuppressive regimens are now urgent to evaluate in the setting of transplantation. Another aspect to consider is the potential adverse side effects of B-cell modulation in the development of infections. Indeed, as such for immunosuppressive regimens, B cell depletion and emergence of regulatory B cells could lead to infectious complications and reactivation of some virus notably following B cell transfer [144]."