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Cross-talk between T and B cells in the Germinal Center following transplantation

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Abstract

Cross-talk between B and T cells in transplantation is increasingly recognized as being important in the alloimmune response. T cell activation of B cells occurs by a 3-stage pathway, culminating with costimulation signals. We review the distinct T cell subtypes required for B cell activation, and discuss the formation of the Germinal Center (GC) after transplantation, with particular reference to the repopulation of the GC following depletional induction, and the subsequent effect of immunosuppressive manipulation of T-B cell interactions. Additionally, ectopic GCs are seen in transplantation, but their role is not fully understood. Therapeutic options to target T-B cell interactions are of considerable interest, both as immunosuppressive tools, and to aid further understanding of these important alloimmune mechanisms.

1. General introduction

The two major cellular components that constitute the alloimmune response in transplantation, T and B cells, play major roles in graft rejection. In the absence of immunosuppression, organ transplantation elicits intense responses from T and B cells, resulting in cell- and antibody-mediated rejection, respectively. Unsurprisingly, ‘pure’ alloimmune responses, limited exclusively to either type of rejection are infrequent in clinical settings. Increasingly, it is recognised that the role of B cells in transplantation is not

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Author Contribution

J.K. participated in the concept, manuscript writing, figures drawing. M.M. participated in the concept, manuscript co-writing. E.P, C.B, and J.H. participated in manuscript co-writing. S.J.K participate in the concept, manuscript writing.

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restricted to their effector function, the humoral response, alone: antigen presentation of B cells also contributes to the optimal immune response[1]. Similarly, although graft rejection had been considered largely mediated by T cell effector function, there is growing evidence that B cells and their immunoglobulin products (alloantibody) may play a role in the process[2]. In this review, we wish to focus on crosstalk between T and B cells in antibody-mediated rejection (ABMR), following transplantation.

2. B cell in Transplantation

2.1. T- cell dependent B cell activation in Transplantation

Following transplantation, there are 3 signaling pathways required for T cell dependent activation of B cells. Initial B cell activation is driven by alloantigen (Figure 1). Alloantigen is delivered to the mature B cell (IgM^+IgD^+) rich area known as the B cell follicle (cortex) within the secondary lymphoid organ.

These naïve mature B cells are able to recognize both membrane bound and soluble alloantigens as a function of the B cell receptor (BCR). BCR recognition (signal 1) of the cognate alloantigen provides an activation signal via CD19 complex (which comprises CD19, CD21, CD81 and CD225)[3–5]. The BCR is also responsible for internalization (endocytosis) of alloantigens derived from the allogeneic cells, which are degraded and presented via MHC II molecules[6,7]. Primed B cells are translocated into the T cell rich area (T cell zone, paracortex) and only B cells which interact with cognate T helper cells (Tfh) receive further activation signals.

In addition to antigen recognition through the BCR, the second signal for B cell activation is costimulation (signal 2). A cognate interaction between helper T cells and B cells provides multiple costimulation signals for B cell activation. CD40L from T cells has been extensively studied; signaling via CD40 on B cells drives B cell proliferation, antibody affinity maturation and class switch recombination (via activation of NF-kB)[8]. In mice, deficiency of CD40 or CD40L results in the absence of IgG production, and Ig class switch defects[9,10]. Corroborating this, targeting CD40 or CD40L in organ transplantation results in a reduction of alloantibody production[11,12]. Following alloantigen recognition and costimulation, B cell activation requires cytokines (signal 3), produced by various helper T cells including Th1, Th2, and Th17. In support of this various cytokines are known to affect antibody production. Furthermore, numerous cytokines (including IL-6, IL-21, IL-12, IL-23, and IL-27) appear to be capable of inducing enhancing or sustaining Tfh cell-like phenotypes, which become important in the Germinal Center (GC) response. These cytokines act through phosphorylation of STAT1, STAT3 or STAT4 to regulate the Tfh cell – associated gene expression[13–15]. However, it is now known that BCL-6 is the required transcription factor for Tfh cell differentiation[16,17], which implies that the Tfh is a separate lineage of helper T cell. This is supported by the finding that the expression of BCL-6 is suppressed by IL-2 acting through STAT 5[18], and that BCL-6 expression represses of T-bet (Th1 differentiation) and RORγc (Th17 differentiation)[15]. More GC-associated cytokines will be discussed later.

2.2. T cell independent Signals for B cell activation

Although B cell activation is predominantly through T cell dependent mechanisms, there is interest in T cell independent mechanisms by which B cell activation might occur in organ transplantation.

Innate immunity—Complement split products, are bound by complement receptors CD21 (C3b, C3dg & C3d) and CD35 (C3b & C4b), which then inactivate them to iC3b and iC4b respectively[19]. CD21 expression is limited to B cells and follicular dendritic cells, while CD35 is expressed more widely. In cross talk between complement and the adaptive immune system, opsonized alloantigen activates the classical complement pathway and may engage the complement receptor, CD21/CD35, to form a co-receptor with the BCR, thereby lowering the threshold for B cell activation[20,21].

Cytokine stimulation—Part of the TNF family of cytokines, the BLyS (B Lymphocyte stimulator) family of ligands (BLyS, BAFF – B cell activating factor) and receptors (BAFFr - B cell activating factor receptor; present on each B cell, TACI - transmembrane activator and calcium modulator and cyclophilin ligand interactor; present on memory B cells & BCMA - B cell maturation antigen; present on plasma cells) are co-stimulators of B cells and necessary for the survival and maturation of B cells to plasma cells[22]. A proliferation inducing ligand (abbreviated as APRIL), like BAFF, is produced as a transmembrane protein, and then proteolytically cleaved to be released in soluble form[23].In mice, BAFF has been shown to independently promote TLR7/9 expression and autoantibody production, in the absence of T cells.[24].

Other mechanisms for T cell independent B cell activation include BCR recognition of polysaccharides, and costimulation with TLRs[25]. Although this is predominantly of importance in bacterial immunity, it may be of relevance in ABO-incompatible or xenotransplantation.

3 Germinal Centre Development

The Germinal center (GC) is a specialized structure containing proliferating antigen-specific B cells that promote antigen-dependent affinity maturation, which is located within B cell follicles of secondary lymphoid organs. The GC response arises from the interaction between follicular helper T cells (Tfh) and B cells. It is now known that Tfh cells and their interaction with B cells are required for GC formation, the generation of long-lasting antibody production[26–28], and high-affinity antibody generation.

3.1. Follicular Helper T cell (Tfh) and B cell interaction

Tfh cells upregulate the chemokine receptor 5 (CXCR5) whilst downregulating chemokine receptor 7 (CCR7). This facilitates migration towards B cell follicle[29,30]. Additional surface molecules including PD-1, ICOS, and SLAM family of receptors are highly expressed by Tfh cells (Figure 2). These molecules are now used for the identification of Tfh cells. It is now known that BCL-6 is the key transcription factor for Tfh cell differentiation[15–17].

CXCR5^{+}PD-1^{hi}ICOS^{+}BCL-6^{+} CD4 T cells also have been shown to localize to the GCs. Taken together, Tfh cells are a terminally differentiated effector T cell lineage[31].

As shown in figure 1, the T cells located at the T:B border are considered to be precursors of Tfh cells, which briefly express some of the Tfh cell-associated markers. It is believed that these cells initiate primed B cell proliferation and differentiation. Only once B cells receive the described activation signals, will they proliferate and differentiate along the both follicular and extrafollicular pathway[32]. While some fully differentiate into short-lived plasma cells and memory B cells (via the extrafollicular pathway) others move through the dark zone (centroblasts; CXCR4^{+}CD83^{+}CD86^{+}) into the light zone of the germinal center (GC) via chemokine receptor CXCR5 (binding its ligand CXCL13; Figure 1). These B cells (known as centrocytes; CXCR4^{-}CD83^{+}CD86^{+}) have already experienced somatic hypermutation (SHM) and interact with follicular dendritic cells (FDC) and Tfh cells. Selected B cell clones, via the cognate interaction with Tfh cells, differentiate into plasma cells (short-lived and long-lived) and memory B cells or, alternatively, some migrate back to the dark zone for further proliferation[8,33]. It is notable that memory B cells emerge earlier than plasmablast with less Tfh cell requirement[34,35]. Clonal expansion of these cells, to become the hyperplastic GC, occurs as result of their interaction with cognate-follicular helper T (Tfh: CXCR5^{+} PD-1^{hi}ICOS^{+} CD4^{+}) cells, via CD40 signaling and IL-21[36]. Further costimulation signaling, via ICOS and PD-1 interactions with ICOS-L and PD-L1/L2 on the B cell provides optimal signals for hyperplastic GC formation and antibody production[37–39]. Defects of these surface molecules in human have been shown to result in impaired GC formation and an improper antibody response[37,38,40,41].

CD28 and CTLA4 interactions to CD80 or CD86 on B cells are involved in both positive and negative regulation of B cells. Signaling of CD28 via CD86 promotes B cell proliferation and Ig class switching, while interaction of CD28 to CD80 inhibits this response[42,43]. CTLA4 counteracts the CD28 signal by binding to CD80/86 with higher affinity[44]. In transplantation, the efficacy of CTLA4-Ig (Belatacept) to suppress the anti-donor humoral response has been demonstrated[45–48]. However, like to ICOS and PD-1, it is still unclear if this costimulation signal is critical for T-B cell cooperation directly, or whether the mode of action is predominantly by limiting Tfh cell or B cell function/ proliferation.

The costimulation interactions described between T & B cells require additional binding to provide physical stabilisation. Initially this is by adhesion molecules, LFA-1 and ICAM, however subsequent maintenance of the T-B conjugation is dependent on SLAM-associated protein interactions, including CD84[49]. Targeting these interactions with an anti-LFA-1mAb also significantly reduces both alloantibody production as well as the GC formation (manuscript in preparation). Additionally CD84 deficiency has been shown to result in an impaired humoral response in mice[49].

Within the germinal center, cytokines (IL-6 and IL-21) remain crucial for further B cell differentiation[50]. Tfh cells are the major source of IL-21. Both Tfh cells and GC-B cells express IL-21 receptors. IL-21 signaling in GC-B cells promotes proliferation, plasma cell differentiation, and antibody production[51–53]. IL-21 signaling also stimulates Tfh

formation[54,55]. IL-6 provided by dendritic cells (DC, including Follicular DC) induces IL-21 production from Tfh cells[56]. Interestingly, IL-6 production from DC is enhanced after IFN-γ activation[57]. The role of IFN-γ may be very limited since the BCL-6 represses IFN-γ expression. It also has been shown that Tfh cells produce less IFN- γ[15,58].From the auto-immune literature, it would appear that IFN-γ promotes BAFF production from myeloid cells that may be indirectly involved in the GC response[59]. Cancro and colleagues elegantly showed that within the GC, a further role of BAFF produced by Tfh cells for GC B cells[60,61]. In transplantation, the role of BAFF is a developing field and its effect on Tfh within the GC remains to be fully elucidated.

3.2. Distinct T cell subtypes in the Germinal Centre

Tfh cells are known to be the key helper T cell phenotype however the mechanism of Tfh cells in GC homeostasis is incompletely understood. Here, we briefly outline the other known T cell phenotypes involved in the process:

T follicular regulatory cell (Tfr)—The selection of GC-B cells expressing high affinity variants of BCR via cognate Tfh cells prevents an irrelevant germinal center response. However this does not explain the control of possible cross-reactive GC-B cells which can recognize both target and self-antigen. More recently, CXCR5 expressing T reg ($FoxP3^+$) cells (T follicular regulatory cells; Tfr) have been identified in the germinal center[62–64]. Like Tfh cells, the high surface expression of CXCR5 is a useful marker to identify the subset of Tfr cells involved in regulation of germinal center in secondary lymphoid organ[65]. The Tfr cells share a similar genetic profile to Tfh cells and Treg cells including BCL-6, FoxP3, CXCR5, PD-1, ICOS and CTLA-4. An important distinguishing feature is that the Tfr does not express certain molecules necessary for B cell help such as CD40L, IL-4 and IL-21[63]. A lack of Tfr promotes increased Tfh, GC-B cells, and antibody secreting cells[62]. However, the relevance of these cells in transplantation is not fully elucidated, and it is still unclear how these Tfr affect the GC response.

CD8 T cells in humoral response—The other notable cell population involved in the GC response is CD8 T cells. It has been shown that a fraction of the CD8 T cell population suppresses B cell response[66]. More recently, $CD8^+$ regulatory T cells which are crucial for the maintenance of self tolerance in the GC were identified in autoimmune disease models.[67] These suppressive CD8 T cells do not share common developmental pathway with CD4 Treg cells. Unlike Tfr, CD8 regulatory cells in the GC express ICOS ligand (ICOS-L) but they do not express ICOS or PD-1[68]. However, they are both dependent on the transcription factor Helios to maintain suppressive phenotype[69]. In transplantation, Bumgardner and colleagues also observed the role of CD8 T cells in alloantibody reduction[70]. In their model, allo-primed CD8 T cells targeted allo-primed B cells via perforin and FasL in a antigen specific manner[71].

4. Kinetics of GC development after organ transplantation

The GC response after organ transplantation has not been fully described, this is mostly due to scarcity of post-transplant samples of secondary lymphoid samples in human patients.

The GC is, however, an important site of interaction between T & B cells, which has been investigated more thoroughly in animal models. In nonhuman primate model, we have traced the in vivo GC response with immunohistochemical analysis. The GC-associated Tfh cell has been also visualized via PD-1 and their functional cytokine, IL-21 in rhesus macque (Figure 3)[72,73]. Interestingly, we did not see an impressive development of GC after skin transplantation in spite of DSA elevation (Data not shown). It is partially because the base line level of GC response is high in monkeys since it is not germ-free animals. The robust Th1 immune reponse, especially IL-2, induced by skin transplantation (without immunosuppression) may also limit the Tfh cell differntiation[18]. IL-2 represses Tfh cell differentiation by upregulating BLIMP-1, a transcription factor that is mutually antagonistic to BCL-6, which is critical for Tfh cell differentitation..

We have developed a NHP AMR model which mimics clinical observations seen with alemtuzumab. Cai et al found that 42% of their alemtuzumab and sirolimus treated renal transplant recipients developed HLA antibodies, 60% of which were donor-specific[74]. Other studies have observed more frequent occurrences of acute antibody-mediated rejection after renal transplantation with alemtuzumab induction[75–78]. We treated our monkeys with CD3-Immunotoxin, Alefacept (LFA-3-Ig; anti-CD2 mAb), and tacrolimus[79]. Comparing this combination therapy to those monkeys treated with CD3-Immunotoxin and tacrolimus alone, we found that Alefacept treatment resulted in significant production of de novo antibody. Since Kirk and colleagues showed that higher CD2 expression on activated (or memory) CD8 T cells[80,81], our findings[45,79,82], support Bumgardner's work on the role of CD8 T cells in GC regulation, as previously described.

We have also assessed T cell depletion in different immune-compartments and GC of the secondary lymphoid organs following CD3-immunotoxin. We found that GC size and frequency are highly correlated to both DSA production and AMR score[45,82]. In the AMR-inducing model, T cell depletion allowed us to study the reconstruction of GC after eradication due to the depletional therapy. GC areas containing proliferating B cells (Ki67+) were measured using Hoechst staining of nuclei, demonstrating markedly less focal staining than the smaller marginal B cells[72,73]. In kidney transplantation, we found that T cell depletion results in elimination of GC in the first week after transplantation (Figure 4). There was also an absence of visible GC in the spleen and peyer's patches within the gut (Figure 5). In addition, systemic T cell depletion resulted in a reduction in the circulating number of B cells, despite the B cell follicle remaining intact. This suggests that, in the nondepleted steady state, B cells from the GC contribute to circulating B cell numbers[45,82].

Immunohistochemistry within the B cell follicles this early after transplantation with T cell depletion, showed no evidence of T-or proliferating B cells (Ki67). The GC-like structures without cell components may represent the effect of rapid T cell depletion on the GC (Figure 4 and 5). However at this stage, although very few T cells were identified in the B cell follicle, residual T cells in the medulla appear more evident.

By 2–4 weeks post transplantation, the GC shows evidence of rapid reconstruction. Within the medulla, there is clear evidence of T cell presence, with small numbers of T cells detectable in the B cell follicle and germinal center. It would appear that, following T cell

depletion, repopulation of the T cell occurs in the LN before the circulation. Supporting this finding, Kirk et al also showed that any remaining T cells after Alemtuzumab treatment are found in the lymph node, specifically the mantle zone[83]. Of note in the NHP model, T cells are not fully removed by T cell depletion[79].

In a mouse model of chronic AMR, using human CD52 transgenic mice[84], following alemtuzumab treatment, we also demonstrated eradication of the GC with residual T cells in the LN (MS in preparation). This mouse model also showed that T cells in the LN were more resistant to those in spleen (Data not shown). Like our NHP observations, it is notable that there was more remaining T cells and faster repopulation in the lymph node compartments compared to spleen. We currently don't know what the driving force of GC reconstruction is, under the current immunosuppressive regimen. We speculate that CNI based regimen does not efficiently suppress Tfh cell during homeostatic repopulation. It might be due to their great efficacy on suppressing Th1 response. It is also possible that Tfh cells are relatively spared by depletional induction due to their physical location (surrounded by densely populated B cells in GC). These remnant Tfh cells (or memory Tfh cells) could repopulate relatively faster under the lack of Th1 immune response. Currently we are further analyzing these T cells in the GC, in order to discover what type of T cell sub-population they represent.

By 30 days after transplantation, recipients treated with CD3-IT with either tacrolimus or rapamycin showed GCs which are similar to the naïve animals. In the spleen and gut, GC reconstruction was negligible. This suggests that it is the residual T cells, following depletion, which contribute to GC reconstruction. In our AMR-model, with considerable immunosuppression, we found that in conjunction with elevated DSA, the rebound increase in GC size and frequency at later time points exceeded baseline. This finding suggests that T-B interactions in the germinal center can be completely eradicated by current cytolytic induction. However, repopulating Tfh cells within the germinal center provide B cell help, despite potent immunosuppression to control T cell mediated rejection.

5. Ectopic GC (Tertiary Structure) in Transplantation

In addition to the changes within hyperplastic Germinal Centre, it is known that germinal center-like structures develop in transplanted organs (kidney, lung tx). These ectopic intragraft GCs (also known as tertiary lymphoid organs) appear during chronic rejection, however, their role in graft rejection is debated. Originally, it was believed that they were related to rejection response by providing both a humoral response[85] and/or allo-T cell priming[86] directly at the site of alloantigens. Clinically, it is also been suggested that ectopic germinal centers correlate with poor survival[87]. However, these ectopic GC have also been found in transplanted organs with no evidence of dysfunction[88].

In nonhuman primate kidney transplantation model, we have made similar demonstrations of ectopic GCs in kidney transplant recipients in whom immunosuppressive drug therapy has been discontinued after initial long-term treatment. We observed that the tertiary GC structure developed rapidly in conjunction with graft rejection. However, even with well-

developed T and B cell zones, there was no evidence of a B cell clonal expansion (Data not shown).

We also found evidence of ectopic GC within a well-functioning graft after drug discontinuation. These ectopic GCs are composed of T and B cells with often hyperplastic GC (showing B cell clonal expansion), although there was no evidence of circulating DSA. As found in other lymphoid organs including lymph nodes, spleen and payer's patch (figure 4 and 5), GCs in the tertiary lymphoid aggregates are clearly identifiable based on their typical architecture. Staining of ectopic GC within the graft showed Ki67+B cells (Figure 6). We also observed an inverse BAFF staining pattern which suggests a consumption of BAFF in the GC-B cells in the ectopic GC – a finding which was also demonstrated in the hyperplastic GCs (data not shown). It remains unclear which factors mediate the differential GC morphologies in the graft after discontinuation of immunosuppression. Further analysis of T and B cells in the ectopic and hyperplastic GCs in well functioning (tolerant) nonhuman primate kidney recipient is likely to provide an insight on how these structures regulate the alloimmune response in recipients with long-term graft function and survival.

6. Possible drug interventions on T:B cross talk in transplantation

There are a number of drug therapies which have the ability to influence this crosstalk between T and B cells.

6. 1. Costimulation blockade

Co-stimulation pathway blockade has been in use in preclinical and clinical transplantation for some time[89]. Belatacept is a second generation fusion protein (CTLA4-Ig) which blocks the CD28/CTL4:CD80/86 costimulation axis, and has been licensed for use in transplantation as maintenance therapy since 2011. Clinically, a Cochrane review has found that there is no difference in effectiveness of belatacept, compared to CNI based maintenance, with respect to graft rejection or survival, although it is less nephrotoxic than CNI[90]. This review however acknowledges that the evidence so far has not fully evaluated the role of belatacept in transplantation, particularly with respect to costimulation blockade resistant rejection. Of pertinent interest, in a recent paper based on an alemtuzumab, belatacept, rapamycin based regimen in human kidney transplantation, it has been shown that, following depletional induction, the rapidly repopulating B cell phenotype is predominantly naïve and regulatory in nature[47].

CD40 ligand pathway inhibitors are also currently of interest in preclinical models of transplantation[91]. In a case report of liver transplantation in a patient with CD40L deficiency, it was been noted that although B cell immunity is severely compromised due to CD40L being required for B cell activation, germinal center formation and class switching, the lack of CD40L on T- and NK-cells did not result in any differences in T cell and NK-cell responses early after transplantation[92]. In NHP models of transplantation, using CD40L blockade (Chi220) resulted in prolonged graft function, and peripheral B cell depletion, although this was associated with severe systemic CMV infection[93]. We have investigated CD40L (2C10) blockade, in conjunction with belatacept and, as described above in our

AMR inducing model, and noted decreased DSA production, and suppression of the GC response[45]. To date, no human studies of CD40 inhibition in transplantation are available.

Although anti-LFA1 blockade is appealing, Efalizumab (Anti LFA-1 blocking monoclonal antibody) has been used in a Phase I/II trial human kidney transplantation, where although the graft rejection rates were low, and mild, higher dose treatment was associated with PTLD[94]. Anti-LFA-1 has been also used in combination with belatacept in an NHP model of renal transplantation where it failed to significantly prolong graft survival[95]. Efalizumab is also no longer commercially available.

6. 2. Modulation of B cell development via B-lymphocyte stimulator/BAFF or APRIL pathways

Belimumab (Benlysta), is a fully human IgG1 monoclonal antibody directed against circulating B-lymphocyte stimulation factor (BLyS) or B cell activating factor (BAFF) which binds soluble human BLyS is currently in clinical use in autoimmune disease.

Atacicept is a fusion protein which targets not only BLyS, but also APRIL pathways. Again, clinical use to date has been reserved to autoimmune indications, and outcomes have been somewhat controversial[96]. However, as discussed in our NHP models, in a T cell depletion model, using Taci-Ig, successfully reduced the DSA response[82].

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Summary

The role of crosstalk between B & T cells is increasingly recognized as being an important in the activation of the B cell, and development of the humoral response in transplantation. In particular, this cross talk is vital for the development of the Germinal Center. There are multiple pharmaceutical possibilities for intervention, although further studies in preclinical transplant models are necessary to fully evaluate the mechanisms described.

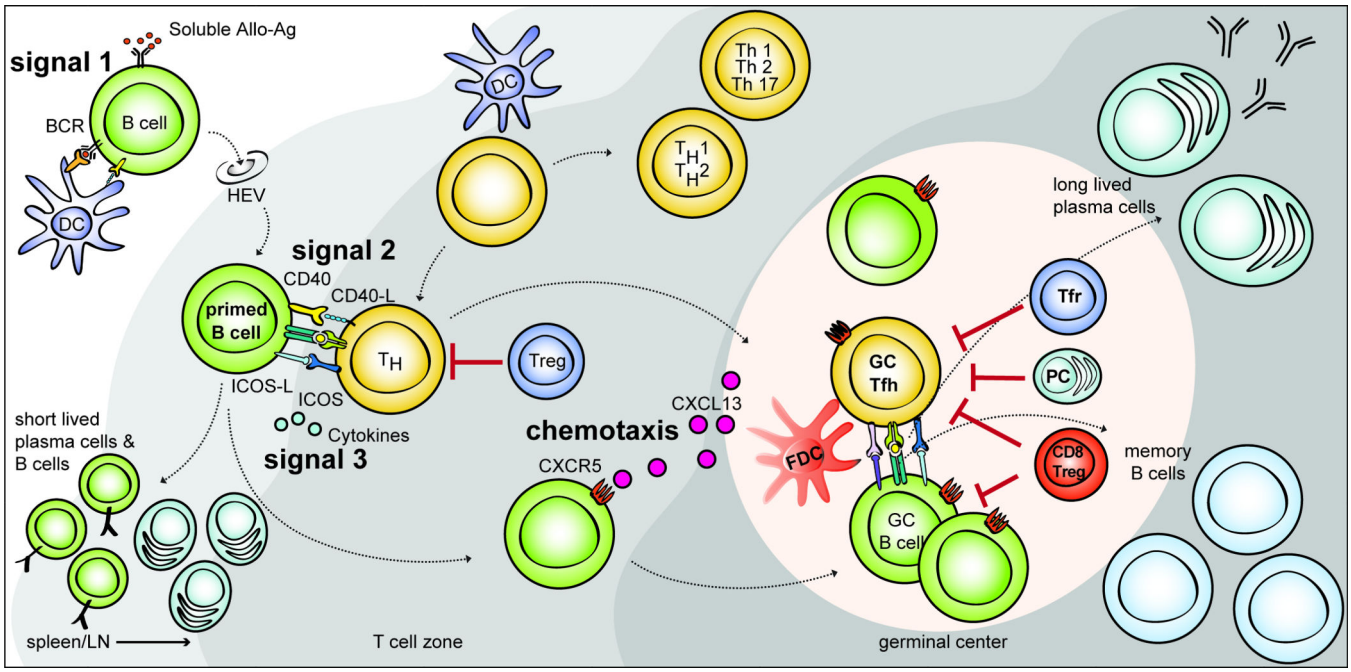

FIGURE 1. T cell dependent B cell activation via Mutiple T-B interations after allostimulation

Naïve mature B cells are activated through BCR recognition (signal 1) and migrate to the T-B border via HEVs (High Endothelial Venules). Primed B cells receive further signals from costimulation (signal 2), and cytokines (signal 3) in the T-B border. Some activated CD4 T cells can acquire charateristics of Tfh cell lineage and migrate into the B cell follicle via CXCR5. These Tfh cells provide IL-21 and costimulation and induce the proliferation of cognate B cells, isotype switching, and somatic mutation. This massive B cell expansion and differentiation leads to the formation of hyperplastic germinal center in the B cell follicle. Tfr cells and CD8 Treg cells are thought to suppress this germinal center response either directly, by depleting B cells or indirectly by modulating Tfh cells. The germinal center response induces the differentiation of isotype-switched affinity mature B cells into memory B cells or into long-lived plasma cells.

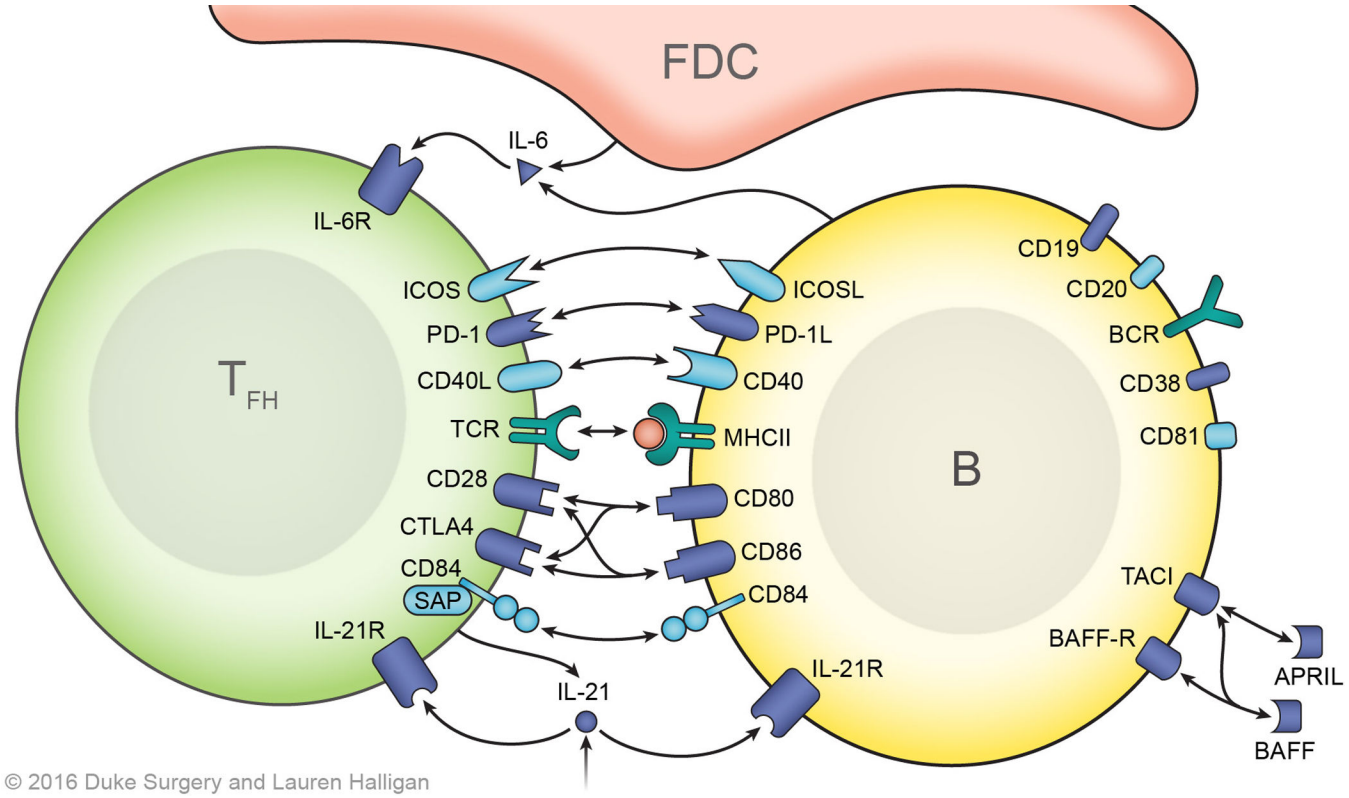

FIGURE 2. T-B cell interactions and signals

The initial B cell activation signal is provided by cognate BCR-alloantigen interaction and requires the CD19, CD21, CD81 complex for an optimal signaling. Congnate follicular helper T cells provide costimulation following presentation of antigenic peptides in MHC class II by the B cells in the germinal center. T:B interactions, especially ICOS-ICOSL, CD40-CD40L, and CD28-B7 interactions, are central to this process. Members of the SLAM (Signaling lymphocytic activation molecule) family of receptors, particularly CD84, are also important for stable T:B cell interactions, which also contribute to Tfh cell differentiation through SAP (SLAM associated protein)-mediated signaling. Cytokines, IL-6 and IL-21 contribute to Tfh cell differentiation via upregulation of BCL-6. The cross-talk between T and B cells in the GC is bi-directional mediated by ligand-receptor interactions, soluble mediators (cytokines). Biologics targeting these signals can modulate post-transplant humoral response.

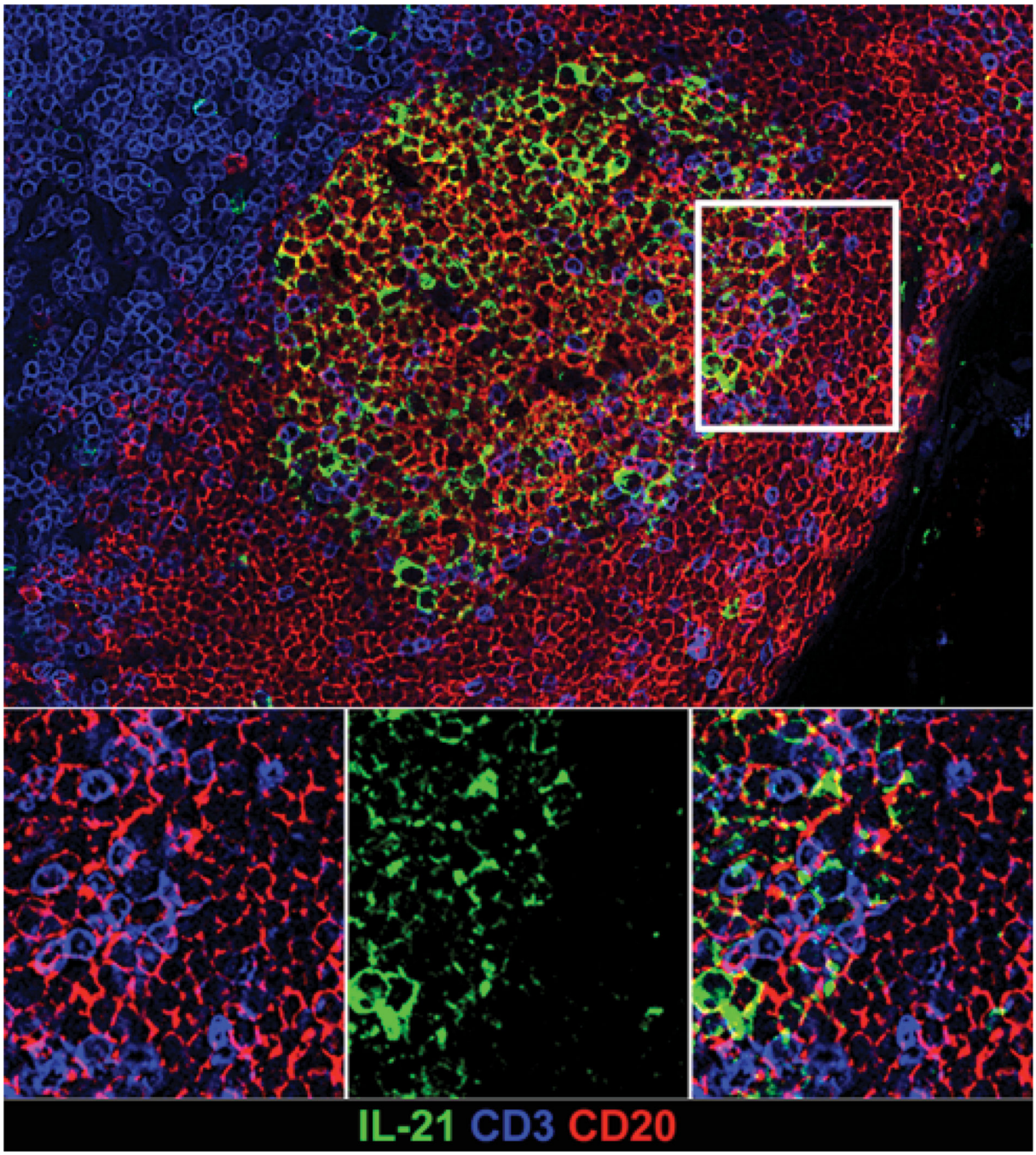

FIGURE 3. In situ IL-21 staining in the GC

IL-21 expression is increased in hyperplastic follicles during antibody-mediated rejection. Representative immunofluorescence image of GC staining with CD3 (Blue), CD20 (Red), IL-21 (green) in lymph node of rhesus macaque.

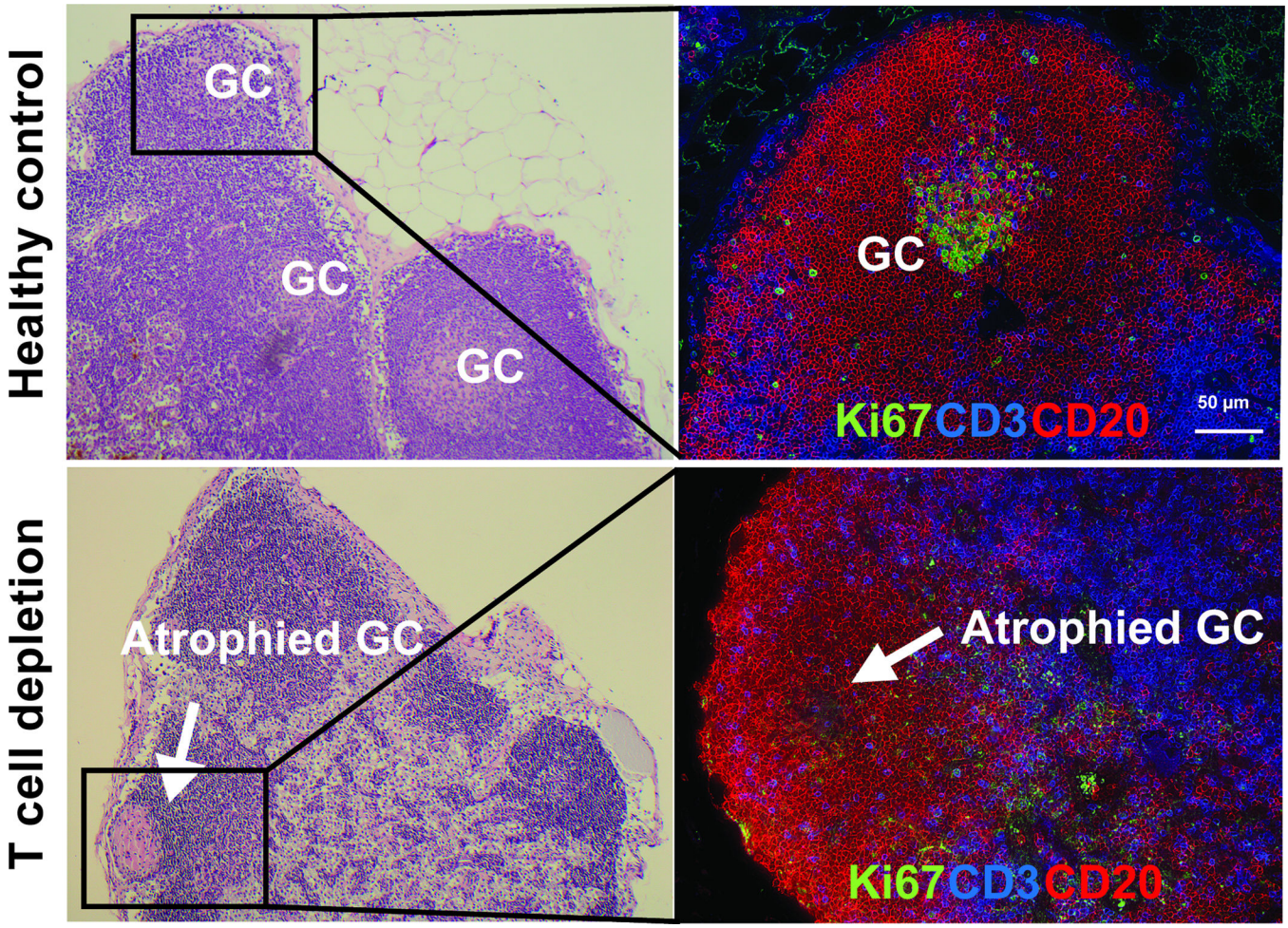

FIGURE 4. Eradication of GC in the lymph node after T cell depletion

Representative H&E (left) and immunofluorescence image (right) of GC staining with CD3 (Blue), CD20 (Red), Ki67 (green) in lymph node of rhesus macaque. Upper panels show sections of LN from healthy control with GCs and lower panels show atrophied GC (white arrow) after T cell depletion.

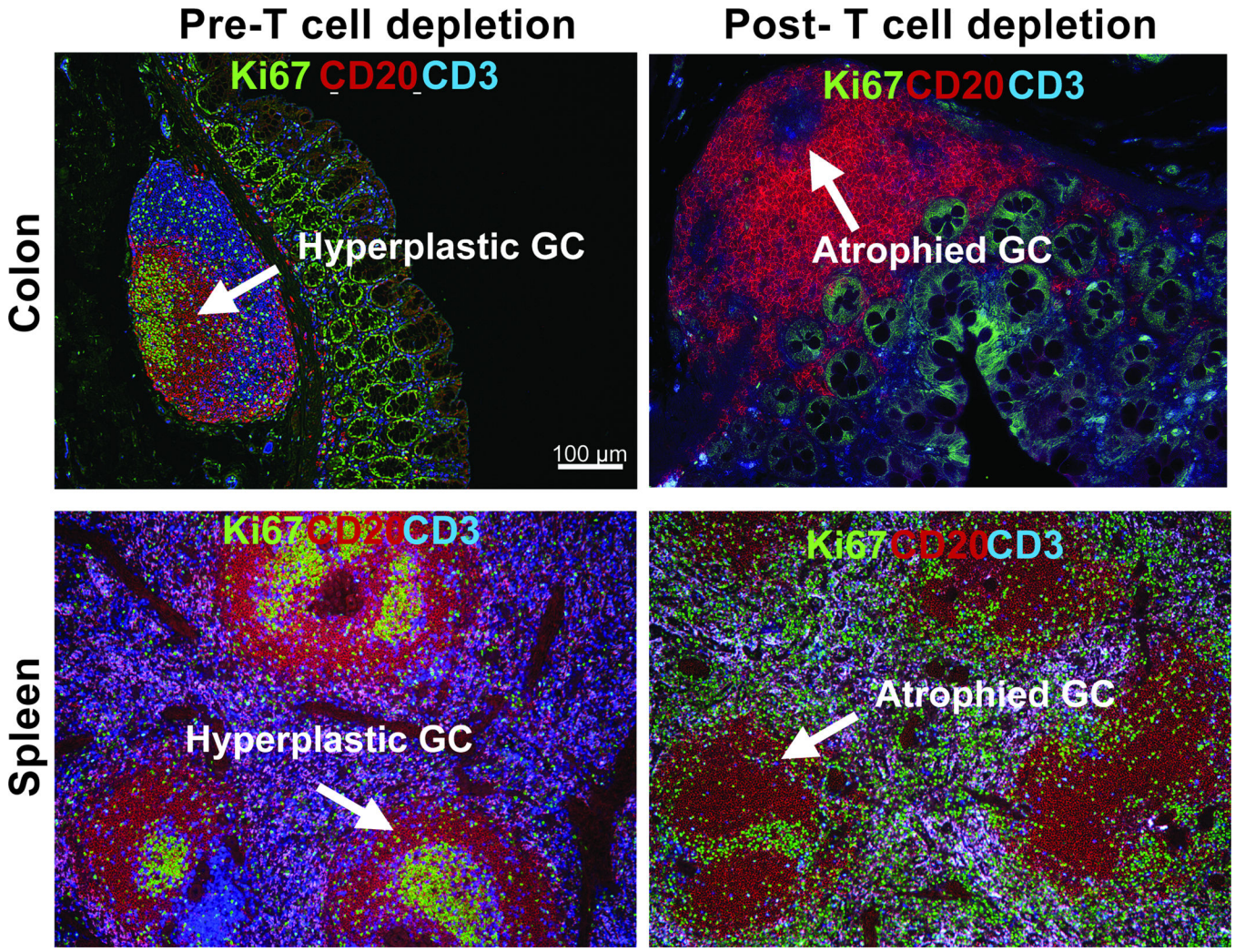

FIGURE 5. Disrupted GC after T cell depletion in spleen and colon

Representative IHC panels show sections of colon (Upper panel) and Spleen (lower panel) from before and After T cell depletion with large lymphoid aggregation (Peyer's patch) stained with Ki67, CD20, and CD3 antibodies. (A) Similar to the lymph node, Peyer's patch and spleen comprises T cell area and B cell follicle with germinal center containing large proliferating B cells. (B) GC in payer's patch in the descending colon and spleen of rhesus macaques were disrupted after T cell depletion.

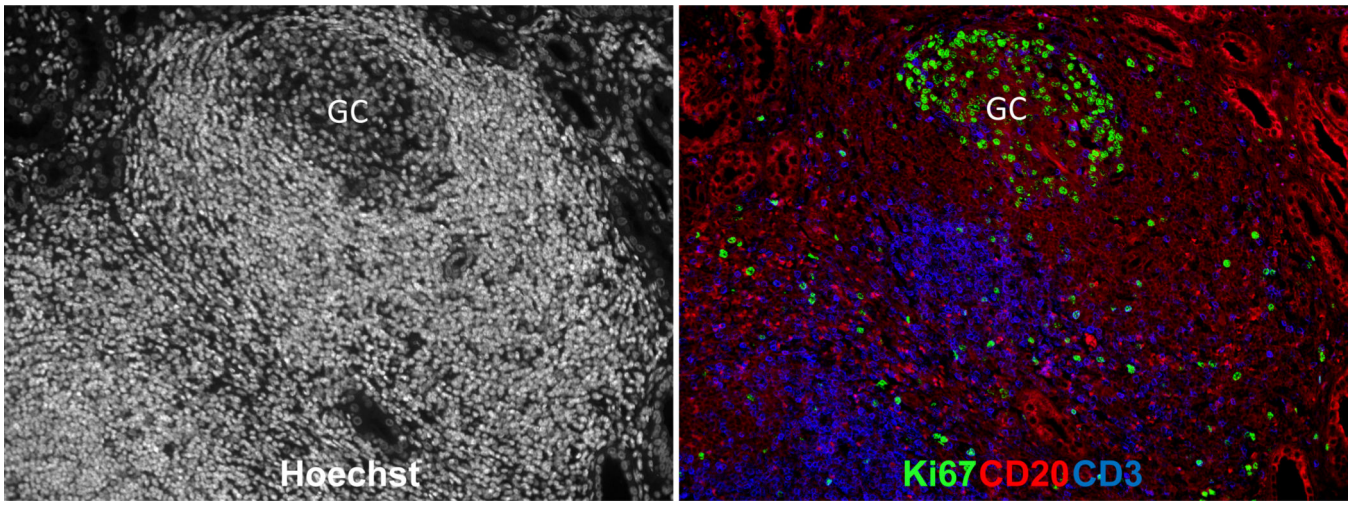

FIGURE 6. Ectopic Germinal Center in well-functioning graft

Representative GC response in the well-functioning kidney graft. Immunological profiles of Hoechst (left), CD20/CD3/Ki67 positive cells (right) within kidney graft section from renal transplant rhesus recipients. The sections were stained with hoechst dye (white), CD3 (Blue), CD20 (Red), and Ki67 (green) antibodies.