HHS Public Access

Author manuscript

Trends Mol Med. Author manuscript; available in PMC 2023 March 01.

Published in final edited form as:

Trends Mol Med. 2022 March ; 28(3): 237–250. doi:10.1016/j.molmed.2022.01.002.

Adaptive immune cell responses as therapeutic targets in antibody-mediated organ rejection

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Abstract

Humoral alloimmunity of organ transplant recipient to donor can lead to antibody-mediated rejection (ABMR), causing thousands of organ transplants to fail each year worldwide. However, the mechanisms of adaptive immune cell responses at the basis of humoral alloimmunity have not been entirely understood. In this review, we discuss how recent investigations have allowed to uncover the key contributions of T follicular helper and B cells, and their coordinated actions in driving donor-specific antibody generation and the immune progression towards ABMR. We show how recognition of the role of T follicular helper-B cell interactions may allow to elaborate improved clinical strategies for immune monitoring and to identify novel therapeutic targets to tackle ABMR that will ultimately allow to improve organ transplant survival.

Keywords

Alloimmunity; humoral response; T follicular helper cells; B cells; organ transplantation

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Disclaimer Statement

The authors declare no conflicts of interest.

Resources

I - https://clinicaltrials.gov/ct2/show/NCT04066114
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Antibody-mediated rejection: an ongoing threat for organ transplant survival

Solid-organ **transplantation** (see Glossary) has emerged as the gold standard therapy for millions of individuals with end-stage organ failure worldwide from a clinical and an economic standpoint. However, thousands of transplanted organs fail each year, for which the first cause identified is represented by antibody-mediated **organ rejection** (ABMR) in kidney transplantation but also in heart, lung and liver [1]. ABMR is observed in organ recipients transplanted with an **allograft** from a genetically incompatible donors, and results from deleterious IgG donor-specific antibody (DSA) interactions with the allogeneic endothelium that triggers C1q-dependant complement activation, and recruitment of cytotoxic NK and monocytes cells that ultimately lead to severe inflammation and injury in both microvascular and macrovascular compartments of the organ allograft [2,3]. There are strong associations between the degree of **human leukocyte antigen** (HLA) mismatches within donor-recipient pairs and ABMR, and anti-HLA DSA are present and used for diagnosis, consistent with a pathogenic upstream involvement of adaptive cellular immune responses represented by alloantigen-specific T and B cells [4,5].

Yet, it is only recently that detailed mechanistic studies investigating the underlying contribution of T follicular helper (T_{FH}) and B cells to the generation of DSAs and development of ABMR have become available in animal models and in human [6–9]. Additionally, progresses have been made in understanding why patients mounting DSAs do progress to ABMR and some do not, and what cellular and molecular states of T_{FH} and B cells underlie the early onset of ABMR versus a late *de novo* occurrence of ABMR after organ transplantation. These recent discoveries have the potential to benefit in the future the transplant community for the diagnosis, risk stratification and therapeutic interventions of patients undergoing ABMR.

T_{FH} cell contribution to humoral alloimmunity in organ transplantation

T_{FH} cell and germinal center responses at the basis of DSA generation

T_{FH} cells are a defined subset of “B helper” CD4 T cells that reside in germinal centers (GC) and actively recirculate in blood, and are pivotal in orchestrating the elaboration of antibody responses against protein antigens including HLA molecules [10,11]. Although the role of T_{FH} cells has long sparked the interest of the transplant immunologists, clear recognition of their contribution to **humoral alloimmunity** has been documented only through the last five years [12–34] (Table 1). A growing number of studies in humans have shown that circulating T_{FH} (cT_{FH}), detected as $CD4^+$ $CXCR5^+$ cells, are expanded in frequencies or numbers in blood of patients mounting DSA post-transplant as compared to those who do not develop DSAs. These cT_{FH} cells can be either in central ($CCR7^+/CD62L^+$) or effector ($CCR7^-/CD62L^-$) **memory** differentiation states, display GC T_{FH}-like activation features ($ICOS^+PD\text{-}1^+$) and Th1 or Th17 polarized phenotypes ($CXCR3^+$ or $CCR6^+$). Importantly, cT_{FH} cells are enriched in $CD40L^+$ donor-specific cells and respond to donor-antigen by producing IL-21, which can be detected in pre-transplant (indicating pre-existing memory

to donor), or post-transplant at time of *de novo* generation DSA emergence [21] [24]. While overall functional potency or direct alloimmune responses of T_{FH} cells can be easily assessed *in vitro* (e.g (i) T_{FH} and B cells co-cultured with SEB [17], (ii) T_{FH} and B cells co-cultured with irradiated donor PBMCs [35] or (iii) recipient PBMCs co-cultured with irradiated donor PBMCs [36]), indirect alloimmune responses are more difficult to detect *in vitro* [37] (e.g recipient PBMCs pulsed with donor cell lysate [24,38]). Longitudinal studies have shown that expansion of alloreactive $ICOS^+PD\text{-}1^+$ cT_{FH} cells in blood tracks with GC reactivity in allograft-draining lymph nodes and precede DSA formation [19].

Distinct T_{FH} cell phenotypic and molecular states underlie DSA pathogenicity, ABMR severity and timing

One main indicator of DSA pathogenic potential is the evidence for histological antibody-related injury lesions in the allograft (and thus ABMR diagnosis), which is associated with DSAs of predominant IgG1/IgG3 isotypes with the most potential to activate complement and recruit cytotoxic $Fc\gamma R^+$ innate cells [39,40]. Although the abovementioned studies have linked T_{FH} response to DSA formation, their involvement in promoting DSA pathogenicity and ABMR was yet unclear. Recently, Louis et al. have designed a study to specifically compare patients mounting post-transplant DSA and undergoing ABMR (DSA+ABMR+) to those mounting DSA but with no evidence of ABMR throughout two years of clinical follow-up (DSA+ABMR−) [17]. Louis et al. found in DSA+ABMR+ patients increased frequencies of cT_{FH} cells with unique enrichment in cT_{FH} clusters comprising proliferating $Ki67^+ICOS^+$ and early memory precursor $CCR7^+CD127^+$ cells. The emergence of these $Ki67^+ICOS^+$ cT_{FH} clusters coincided with concomitant expansion of blood $Ki67^+$ activated $CD20^+CD38^{lo}$ B cells and $CD20^+CD38^{hi}$ plasma-blasts, correlated with plasma-released CXCL13 and levels of DSA generated, suggesting a more robust, GC reaction in DSA+ABMR+ compared to the DSA+ABMR− condition. In addition to their increased cell numbers in DSA+ABMR+ patients, these $Ki67^+ICOS^+$ cT_{FH} were specifically polarized with dominant Th1 and Th17 features, which paralleled the detection of IgG1 and IgG3-switched DSAs in patients' sera. This is consistent with the known roles of INF-g, IL-17 and IL-21 in isotype switching toward IgG3 [41]. Also, IgG1 and IgG3 are most likely to be the first IgG subclasses to be generated as dictated by their genomic order, and thus, detected during acute ABMR occurring post-transplant, whereas IgG4 DSAs are usually detected in late and chronic forms of ABMR [39,42]. The transcriptional analyses of these DSA+ABMR+ associated cT_{FH} clusters supported an enrichment in precursors (*LEF1, TCF1*), effectors (*CD28, MAF, IL-21R*) and Th1-polarized gene signatures. This distinct transcriptional programing of cT_{FH} cells during ABMR resulted consistently in their potent functional capacity *in vitro* to provide help to cognate B cells that differentiated into plasma cells in response to vigorous cT_{FH} cell help and generated DSAs enriched in IgG1 and IgG3 isotypes. In DSA+ABMR- patients, there was a lower amplitude of $Ki67^+ICOS^+$ cT_{FH} with differing transcriptional programming (*LEF1, SLAMF6, CD40LG*), lower CXCL13 and thus most likely extrafollicular responses resulting in less pathogenic DSAs generated [17] (Figure 1).

Analyzing frequencies and phenotypes of cT_{FH} clusters with histological and clinical profiles of patients, Louis et al. found that the magnitude of the expansion of the

Ki67$^+$ICOS$^+$ and CCR7$^+$CD127$^+$ cT$_{FH}$ cells was mostly associated with late onset and a more severe phenotype of ABMR, which was linked to significant decrease in kidney allograft survival [17]. These more severe forms of ABMR were characterized by more microvascular inflammation but also extensive arteritis and interstitial inflammation, that are both also features of T-cell–mediated rejection, suggesting an allograft-infiltrating T$_{FH}$ cell component which has been demonstrated by confocal microscopy by other groups [43,44].

These allograft-infiltrating T$_{FH}$ cells likely arise from cT$_{FH}$ cells that would have migrated from blood to target tissues as they display a CXCR5$^+$PD-1$^+$ phenotype. Also, intriguingly, these local T$_{FH}$ cells can cluster to form of ectopic/tertiary lymphoid structures defined by dense cell aggregates of T$_{FH}$-B cells reminiscent of B cell follicles/GC structures of secondary lymphoid organs [43,45]. Recent data have also found that T$_{FH}$ cells to be located within allografts of patients showing chronic ABMR [7]. Interestingly, concomitant with the presence of CXCR5$^+$ PD-1$^+$ cells, a substantial number of these allograft-infiltrating CD4 T cells in ABMR were CXCR5$^-$PD-1$^+$, indicative of a peripheral helper phenotype as previously described in autoimmunity [46,47].

These findings in patients are consistent with preclinical animal models demonstrating the crucial implication of T$_{FH}$ cells in promoting DSA responses towards ABMR, as deletion of T$_{FH}$ cells at the time of transplant resulted in significantly less severe allograft ABMR in mice [15]. Using several other animal models, including rats and non-human primates, as well as other models of transplantation such as heart and skin transplant, studies have consistently shown that T$_{FH}$ cells by their capacity to respond to donor antigens by producing IL-21, providing potent B cell help, and promoting GC hypertrophy and reactivity, are important in the generation of isotype-switched IgG DSAs that leads to ABMR and premature allograft failure (Table 1). Along with integration of routine clinical and virologic parameters, characterization of cT$_{FH}$ and B cell phenotypic and functional profiles have clinical potential as biomarkers for patient management to predict DSA generation before its onset and distinguish the less robust phenotype (DSA+ABMR−) from pathogenic (DSA+ABMR+) humoral responses after organ transplantation.

Effector B cell contribution to humoral alloimmunity in organ transplantation

Memory B cells as effectors for DSA generation

For obvious reasons, efforts in understanding of the immune pathogenesis of humoral alloimmunity and ABMR have focused on the characterization of the B cell response and its alloreactive potential [48–58] (Table 2). Most studies have identified important changes in the B cell compartment during an ongoing alloimmune humoral response; with increase in frequencies or number of antigen-experienced (i.e. memory) B cells in patients developing DSA in post-transplant. These memory B cells (MBCs) were generally isotype-switched (IgD$^-$) and expressing the key **costimulatory receptor** CD27, which is acquired through GC experience [59]. Importantly, MBCs are highly enriched in donor HLA-specific cells and strongly respond to polyclonal stimulation comprised of TLR9 agonists and **cytokine** cocktails leading to DSA production *in vitro* [54,60]. These

cells can also be detected pre-transplant (indicating pre-existing MBCs to donor-HLA antigens), or post-transplant by donor-HLA-specific ELISPOT at time of *de novo* DSA emergence [57]. Longitudinal studies have further shown that donor-HLA-specific MBC expansion in blood tracked with increased in GC size containing GL-7^{+}CD95^{+} GC B cells, was proportional to DSA titers, and not surprisingly preceded of several weeks DSA emergence in circulation [53,57]. The phenotype of these cells was described as Ki67^{+}CD19^{+}CD27^{+}IgD^{-} in both animal models as well as in transplant patients manifesting DSAs, reminiscent of the activated B cells previously reported after flu vaccination [31,61]. Based on these previous data, Louis et al. inquired whether these proliferating B cells can distinguish asymptomatic DSA+ABMR− patients from the DSA+ABMR+ status [17]. Louis et al. found that Ki67^{+}CD19^{+}CD27^{+}IgD^{-} cells could be further separated into CD20^{+}CD38^{lo} activated B cells and CD20^{-}CD38^{hi} antibody-secreting cells. While CD20^{-}CD38^{hi} cells were consistently elevated in all DSA+ situations, CD20^{+}CD38^{lo} were highest in DSA+ABMR+ patients [17]. The emergence of both B cell subsets in blood coincided with increase in Ki67^{+}ICOS^{+} cT$_{FH}$ cells, release of CXCL13 and the peak of DSA levels in circulation, overall indicative of GC-dependent T$_{FH}$-B cell interactions during DSA responses that are exacerbated during ABMR [17].

Memory B cell subsets derived from distinct inflammatory signals are involved during ABMR

Organ transplantation is characterized by a sustained antigenic exposure and an IL-21 and IFN-g biased chronic inflammatory environment [62], both of which are known to deeply affect B cell differentiation and function resulting in the generation of unusual MBCs lacking CD21 and expressing the transcription factor T-bet [63,64]. A recent study have identified the expansion of unusual MBC clusters lacking CD21 and expressing T-bet, which largely distinguished patients without DSA from all DSA+ patients [48]. According to CD27 expression, Louis et al. further delineated CD27^{+}CD21^{-} activated memory (AM) from CD27^{-}CD21^{-} tissue-like memory (TLM) cells, while their resting memory (RM) counterparts were identified as CD27^{+}CD21^{+} cells similar to other studies [65]. Although increased frequencies of both AM and TLM cells were an immune feature of all DSA+ patients, the AM cell expansion was much more pronounced in the DSA+ABMR+ group. T-bet was not expressed in RM cells, while expressed at intermediate levels in AM cells and most highly expressed in TLM cells, consistent with previously published data outside of the field of transplantation (Figure 2) [66].

RM cells, that do not express T-bet, represented the great majority of MBCs in healthy individuals and in patients who do not develop DSA [48]. Their transcriptional profile is consistent with a resting and central memory state with self-renewal capabilities. Conversely, AM and TLM cells shared the features of expression of T-bet and downregulation of CD21, and displayed several fundamental differences in their phenotype, differentiation, transcriptomic states and effector functions. AM cells comprised both proliferating isotype-switched (Ki67^{+}IgD^{-}) and unswitched (Ki67^{+}IgD^{+}) B cells which were predominantly found elevated in early ABMR (memory response) and late (*de novo* response) ABMR, respectively. Transcriptionally, AM cells appeared poised for plasma cell differentiation with high expression levels of the transcription factors IRF4 and Blimp1, as well as the

genes *MZB1* and *XBP1*, that are known to modulate plasma cell differentiation [67,68]. Specifically, unlike those from the DSA+ABMR− group, AM cells of DSA+ABMR+ patients were selectively enriched for *IGHV* germ line genes that have been previously documented to predominate during organ rejection, including *IGHV3–7*, *IGHV3–15*, *IGHV3–23* and *IGHV3–74* [69–71]. Consistently, AM cells could be detected within dense infiltrating B cell clusters from kidney allografts with diagnosis of acute ABMR. Their pathogenic *IGHV3–7*, *IGHV3–15*, *IGHV3–23* and *IGHV3–74* transcripts could also be detected within the global transcriptome of kidney allograft showing ABMR [48]. This restricted *IGHV* usage reflects clonal selection likely due to a common pool of antigens that may drive organ rejection, although direct evidence that the expansion of these clones is alloimmune requires additional *in vitro* and animal studies. Interestingly, Grover et al. demonstrated that antibodies encoded by *IGHV3–23* gene were recognizing components of bacteria [72]. Whether antibodies encoded by these dominant *IGHV* genes recognize HLA molecules or are crossreactive to HLA due to molecular mimicry remains to be investigated.

In Louis et al. study, AM cells could be generated *in vitro* after priming of naive B cells and their stimulation with IL-21. Because of their CD27 expression, AM cells likely represent GC emigrants and not surprisingly were more responsive to cognate T_{FH} cell help. They expressed high levels of IL-21R and CD40 at baseline and produced DSAs when co-cultured with T_{FH} cells in an IL-21 dependent manner [48]. Conversely, and consistent with previous findings [73,74], TLM cells lacked CD27 and CD40, phenotypically resembled exhausted cells, were enriched with for the inhibitory receptors CD72, CD32B and FcRL5, and therefore were hyporesponsive to T_{FH} cell help and did not produced DSA *in vitro*. Compared to DSA+ABMR−, TLM cells from DSA+ABMR+ patients displayed a more pronounced exhaustion-like transcriptional profile with increased in *TOX*, *DOCK3* and *FCRL4* (Figure 1). Their immune repertoire was different than that of AM cells and lacked enrichment in the pathogenic *IGHV* associated with ABMR. TLM cells could be generated in vitro from naive B cell priming and stimulation with IFN-g but not IL-21 [48]. Consistent with their T-bet^{hi} phenotype, they have been recently shown to localize outside of GCs, to be virtually absent from the lymphatic circulation [75]. Also, it has been shown that these cells were mainly generated through the extrafollicular pathway, had poor affinity maturation, generated short-lived plasma cells in response to TLR stimulation and IFN-g, signals mainly found outside GCs [76,77]. As both IL-21 and IFN-g coexist during ABMR, both T-bet^{int} AM cells and T-bet^{hi} TLM cells are generated and expanded in patients. It is like that TLM cells can counterbalance the hyperactivation state of AM cells in the context of chronic activation. Indeed, TLM cells from HIV infected patients were reported to be responsive to the binding of circulating IgG3 and C1q (also present in excess during ABMR) onto their surface IgM, conveying a strong B cell receptor-mediated inhibitory signal to these cells [78].

Coordinated T_{FH}/B cell responses determines the progression towards ABMR

Identification of novel T_{FH} and B cell subsets that shape humoral alloimmunity

The contribution of T_{FH} and B cells to the immune pathogenesis of DSA generation and progression to ABMR is now increasingly documented. However, only few studies have concomitantly profiled T_{FH} and B cell compartments and investigated the importance of T_{FH}-B cell interactions in response to common alloantigenic stimulations and proinflammatory instructions. Identification of coordinated adaptive T_{FH}/B cell immune signatures can be linked to specific trajectories of disease severity and have clinical implications for the design of more targeted and personalized therapeutics in ABMR. The emergence of novel high-dimensional and integrative technologies such as high-dimensional flow cytometry, next generation RNA-sequencing and multiplex immunoassays, can be used for deep profiling of patient clinical phenotypes. These methodologies have been applied in multiple discipline of medicine including autoimmunity, infectious disease and cancer, and are now ready for prime time in the field of transplantation.

Multidimensional approaches have been used to concomitantly profile several T_{FH} and B cell immune signatures in kidney transplant recipients undergoing active humoral alloimmune responses. Recent works have identified highly coordinated responses of T_{FH} and activated B cells at phenotypic, transcriptional and functional levels in the asymptomatic DSA+ABMR- versus the pathogenic DSA+ABMR+ clinical trajectory. Common cell subsets were identified in both DSA+ABMR− and DSA+ABMR+ patients when compared to no DSA situation, including $Ki67^+ICOS^+$ cT_{FH}, $Ki67^+CD27^+IgD^-$ $CD20^+CD38^{lo}$ activated B cells (ABCs), AM and TLM B cell subsets [17,48]. However, patients in the two cohorts, while well matched for clinical parameters (including the lack of ongoing viral infections) and analyzed at similar time points post-transplant and with a similar clinical follow-up of 2 years, differed in cT_{FH} and B cell subsets: (i) in absolute numbers and frequencies, (ii) phenotype, and (iii) transcriptional programming (Figure 1). More importantly, these quantitative and qualitative cellular differences correlated with the amount and quality (class-switching) of DSAs, as well as with levels of CXCL13 produced as an indicator of ongoing GC reaction in DSA+ABMR+ versus DSA+ABMR− patients. Specifically, cT_{FH} and B cells from DSA+ABMR+ patients are both activated and proliferating, more poised for cT_{FH}-B cell interaction and GC activation, for elevated DSA production and capacity to class-switch towards the pathogenic IgG1 and IgG3 as compared to cT_{FH} and B cells in DSA+ABMR- patients. Thus, differences between DSA+ABMR− and DSA+ABMR+ patients are most likely driven by distinct cT_{FH} and B cell profiles and may be used as surrogate markers for predictive, diagnostic or therapeutic purposes in the clinic. However, other immune triggers (e.g active infection, inflammation) may induce phenotypic and functional changes in cT_{FH} and B cells, which may participate to modulate their capacity to promote DSA formation [79], and these remain to be determined in future studies. In combination with histological and long-term clinical follow-up data, recent data further identified that DSA+ABMR+ patients with highest frequencies of both $Ki67^+ICOS^+$ cT_{FH} and ABCs displayed more allograft arteritis and interstitial inflammation, and progressed to premature kidney allograft loss, compared to those with lower frequencies

of the two cell subsets [17]. Similar conclusions were reached for high frequencies of AM B cells in the same cohort of deeply phenotyped kidney transplant patients. Comparable blood cT_{FH} and activated B cells subsets were independently identified by several other groups, which were also predictive of poor prognosis in patients mounting DSA with or without ABMR [14,53] [80–82]. These findings highlight the importance of simultaneous immune monitoring of cT_{FH} and activated B cells for improving patient's risk stratification and clinical management (see Clinician's Corner).

T_{FH} and B cells as concomitant therapeutic targets in ABMR

There is an urgent need for innovative treatments to assist clinicians in the management of ABMR. Although patients with acute ABMR have an acceptable response to standard-of-care treatment (i.e. corticosteroids, plasmapheresis and intravenous immunoglobulins), the risk of relapse upon treatment withdrawal is high (>40%) [83]. In addition, the use of the B cell- and plasma cell-depleting agents Rituximab and Bortezomib was not associated with significant improvement of long-term outcome in ABMR [84,85]. For chronic forms of ABMR, no efficient immunosuppressive treatment exists and the poor response to the standard-of-care treatment is linked to allograft loss [86]. Moreover, it is difficult to predict clinical outcome at diagnosis, upon ABMR treatment and before complete treatment withdrawal. For these reasons, more specific targeted therapies aiming T_{FH}-B cell interactions may represent a timely option as the abovementioned ABMR therapy depleting DSAs and plasma cells are insufficient. One successful use of biotherapy in organ transplantation has been illustrated by the use of the costimulatory blockade molecule belatacept (CTLA4-Ig) that received FDA approval for prophylaxis of humoral organ rejection in kidney transplantation, which significantly diminished the incidence of *de novo* DSA and improved long-term allograft function, when compared to cyclosporine [87]. However, the use of belatacept has not been tested so far for ABMR treatment.

Targeting costimulatory molecules during ABMR

Because several costimulatory receptor pairs (CD28/CD80-CD86, CD40/CD40L, ICOS/ICOSL and CD70/CD27 axes) are at the basis of optimal control of T_{FH} cell help to B cells, other costimulatory molecules antagonists are currently evaluated in transplantation (Figure 3, **Key Figure**). Donor-specific GC T_{FH} cells, which upregulate CTLA4, and $CD95^+$ $GL7^+$ GC B cells can be significantly inhibited by anti-CD28 domain antibody at a greater extent than by CTLA4-Ig [26]. Of note, the superior inhibition of GC T_{FH}-B cell interactions with selective CD28 blockade was CTLA-4 dependent and T_{FH} cell specific [16]. Additionally, the new anti-CD28 molecule (lulizumab), because it preserves the regulatory CTLA4-mediated signals and controls the proliferative response of pre-existing T_{FH} cells better than belatacept, has raised high interest [88,89] and is currently tested in clinical trials. An open-label single-arm Phase I clinical trial (NCT04066114 [I]) is currently recruiting patients to evaluate the safety of lulizumab in the context of combined immunosuppressive regimen (steroids, belatacept, tocilizumab and everolimus) in kidney transplantation. Another open-label randomized Phase II clinical trial (NCT04903054 [II]) was launched to evaluate the safety and efficacy of lulizumab compared to tacrolimus as the primary immunosuppressant in first-time renal transplant recipients. While interventional clinical trials using anti-CD40L agents were halted because of severe thromboembolic complications [90], anti-CD40 is

now developed as a safer and promising alternative. Anti-CD40 effectively disrupt GCs by reducing GC T_{FH}-cell numbers and their IL-21 production resulting in attenuated DSA production in animal models. Preliminary trials using bleselumab have shown its acceptable efficacy and safety for preventing acute rejection in kidney transplantation [91] and further clinical trials are ongoing. Although efficacy of ICOS-Ig was unsuccessful at preventing allograft rejection [92], anti-ICOS efficiently inhibited DSA generation in islet xenografts recipients, and when combined with anti-CD40 led to decrease in DSA titers and improved histology as compared with anti-CD40 alone in a cardiac model of chronic ABMR [93]. A double-blind randomized Phase I clinical trial (NCT01127321 [III]) is evaluating the safety and tolerability of the anti-ICOS MEDI-570 in patients with lupus and another open-label single-arm Phase I clinical trial (NCT02520791 [IV]) was launched to determine the safety and maximum tolerated dose of MEDI-570 in patients with T-cell Lymphoma. Thus, future clinical trials evaluating the potential use of anti-ICOS in transplantation are warranted. CD70/CD27 are mutually expressed on T_{FH} and B cells; CD70 stimulates cytokine production in T_{FH} cells [94] while it synergizes with the CD40 pathway to activate B cells and antibody production, and CD27, the ligand of CD70, is expressed on activated/memory T_{FH} and B cells as well as plasma cells [95]. In transplant animals, anti-CD70 administration diminished prolonged corneal or heart allograft survival [96,97]. While there are ongoing clinical trials with the anti-CD70 cusatuzumab in acute myeloid leukemia [98], studies in transplant recipients are lacking and warranted.

Targeting cytokines to treat ABMR

Biotherapeutic targeting of cytokines axes mediating T_{FH}-B cell interactions may result in complex immune modulation beyond T_{FH} and B cell responses. However, recent data encourage further cytokine inhibition-based biotherapeutic development in ABMR. IL-6 has known direct effects on B cell maturation and differentiation towards plasma cells, and also controls plasma cell homeostasis [99]. Additionally, IL-6 plays a major role in T_{FH}-cell differentiation and function, and B cells and plasmablasts produce large amounts of IL-6, which in turn, stimulate T_{FH} cells via IL-6R [100]. The IL-6R antagonist tocilizumab administration resulted in reduction in T_{FH} cells in experimental models [101], and leaded to stabilization of kidney allograft function and significant decreased in DSA MFI levels in patients with chronic ABMR [102]. A recent double-blind randomized phase II clinical trial (NCT03444103 [V]) has evaluated the safety, tolerability and efficacy of clazakizumab in kidney transplant recipients with late ABMR. Ten patients received placebo and 10 patients received clazakizumab for 12 weeks, and in the second part, all 20 patients were treated for 40 weeks with clazakizumab. 5/20 patients under active treatment developed serious infectious events and 2/20 patients developed diverticular disease complications, leading to trial withdrawal. The mean renal function decline during the first part of the trial was slower with clazakizumab compared to placebo. Importantly, clazakizumab was associated with significantly decreased DSA levels, resolution of ABMR activity, and diminished ABMR-related gene-expression patterns [103]. While IL-21 is a master regulator of optimal T_{FH}–B cell cross talk [104], its therapeutic blockade was found to have double-edge sword effects in clinical settings and should be used with caution. Donor-reactive cT_{FH} cells produce IL-21 [17], which is detected at high levels in sera in experimental models [25]. Anti-IL-21R administration has been tested *in vitro* resulting in diminished plasma cells differentiation

and IgG production in a coculture model of cT_{FH}-B cells stimulated with donor antigen [35], and prolonged allograft survival *in vivo* in mice models [105]. Safety and efficacy data are accumulating in clinical trials in lupus and rheumatoid arthritis for anti-IL-21 [106] and in healthy subjects for anti-IL-21R [107]. Thus, blocking IL-21/IL-21R axis should be tested in future clinical trials in transplantation.

Recent *in vitro* data exposing cT_{FH} cells to IL-2 induced their reprogramming with increased T-bet expression and IFN-g production leading to reduced IgG production by cognate B cells [108]. On the B cell side, an excessive expression of T-bet was also associated with diminished proliferation capacity, response to T_{FH} cell signals and programming towards a B cell inhibitory state [109]. Overall, data support that a biased Th1 polarization on both T_{FH} and B cells may diminish their effector function and lead, if sustained exposure, to cell state resembling exhaustion. Signals that may diminished effector function while favoring hyporesponsiveness of T_{FH} and B cells, like blocking IL-21 and administration of low-dose IL-2 and IFN-g warrant further investigations. However, while cytokine manipulation appears promising, this should also be done with caution, and account for the complexity of interplay between the cognate signals involved in T_{FH}-B cell cross talk and for the pleiotropic effects of cytokines beyond T_{FH} and B cells.

Concluding remarks

Understanding of the pivotal role of adaptive immune cell responses in directing DSA generation leading to ABMR has been substantially improved by the profiling of cellular and molecular cues concomitantly occurring in T_{FH} and B cells. The coordinated T_{FH}-B cells responses in ABMR are now recognized to be underlied by common allo-antigeneic and dominant inflammatory triggers, and to involve several key cognate costimulatory receptors and cytokine signaling pathways. As depleting plasma cells and DSAs is certainly not efficient enough to tackle ABMR, targeting the upstream T_{FH}-B cell interaction represents a novel attractive option for future biotherapeutic strategies in ABMR. Future investigations need to address whether therapeutic manipulation of T_{FH}/B cell fate for promoting exhausted/tolerogenic profiles versus effector function is safe, and whether combined targeting of T_{FH}-B cell interaction, their plasma cell progeny and DSAs will result in prolonged allograft survival in ABMR (see Outstanding Questions).

Clinician’s corner

Immune monitoring of organ transplant recipients in the clinic is largely based on the measurement of anti-HLA DSAs. Implementation of additional non-invasive immune monitoring with assessment of concomitant blood cT_{FH} and B cells can help clinician to improve precision diagnosis of ABMR, to identify specific immunophenotypes of patients, to identify ongoing DSA generation and predict future ABMR occurrence for improved patient management.

Unified assays for measuring cT_{FH} and B cells and their donor-reactive potential, as preformed memory or *de novo* responses in both pre-transplant and post-transplant settings,

are currently under thorough investigation by several independent laboratories in order to render them readily usable in clinical practice.

Because patients with the highest frequencies of activated cT_{FH} and B cells at ABMR diagnosis have increased risk of allograft loss long-term, measuring these cells at diagnosis may help risk-stratify patients at high risk based their T_{FH} and B cell immunophenotypes. In addition, measuring activated cT_{FH} and B cells may help predict non-responder patients to treatment of ABMR and predict relapse after treatment discontinuation.

Development of novel biotherapeutic tools is growing exponentially, with the already successful use of belatacept which remarkable efficacy in preventing *de novo* DSA responses is largely due its intrinsic effect on optimal control of T_{FH}-B cell interactions. Rituximab is part of the armamentarium of biotherapy used in ABMR, in addition to standard-of-care treatment (corticosteroids, plasmapheresis and intravenous immunoglobulins). However, its immunological impact in terms of successful GC B cell and regulatory B cell depletion (without targeting T_{FH} cells), DSA reduction, and its clinical benefits in terms of allograft survival are yet to be demonstrated in larger clinical studies. As for Bortezomib, while depleting the direct source of DSAs, it certainly triggers paradoxical effect upstream plasma cells such as T_{FH} and B cell proliferation compensating the plasma cell loss, resulting in the absence of significant decreased in DSA and lack of clinical effects in ABMR, when used alone.

Other promising biotherapeutic agents including tocilizumab (which targets T_{FH}-B cell interaction) have yielded promising results on diminishing T_{FH} cells and DSA levels, and stabilizing allograft function during ABMR with low rate of adverse events. This paves the way for further testing of the clinical efficacy and safety of cytokine manipulation to temper T_{FH} and B cell responses.

Acknowledgments

This work was supported by the following grants: R21-AI116746 (DM), R01-AI130010 (DM) and the Human Immunology Program at the Starzl Transplantation Institute.

Glossary

Allograft
a tissue or an organ removed from an individual and implanted to another individual from the same species

Alloimmunity
an immune response that attacks cells or tissues from another individual of the same species

Costimulatory receptor
a class of receptors expressed by immune cells that regulates the activation and generation of effector or regulatory cell responses

Cytokine

substances such as interferons, interleukins or growth factors, that are secreted by certain cells of the immune system and have effects on other cells

Humoral
relating to the body fluids especially with regard to immune responses involving antibodies

Human leukocyte antigen (HLA)
complex of genes in humans which encodes cell surface proteins responsible for the regulation of the immune system including immunity against allograft

Memory
immunity mediated by certain immune cell types that have previously encountered a given antigen and that on reexposure to the same antigen strongly and rapidly initiates a recall immune response

Organ rejection
process in which the immune system of an organ transplant recipient attacks the transplanted organ or tissue

Transplantation
the process of taking a living organ or tissue and implanting it in another part of the body or in another body

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Outstanding questions

- Are both T_{FH} and B cell clonally expanded during ABMR? Are there dominant T_{FH} and B cell clones that drive DSA generation and ABMR? Are these exclusively allospecific, or also comprise of autoreactive or crossreactive expanded clones?
- What is the role of regulatory T and B cells in the control of humoral alloimmunity? Are regulatory T and B cell responses also coordinated?
- Can effector T_{FH}-B cell be reprogrammed into regulatory or exhausted T_{FH}-B cells and vice-versa?
- Do T_{FH} and B cell responses resolve after standard-of-care treatment of ABMR? Can monitoring T_{FH} and B cell identify patients in need for second-line therapy in ABMR?
- Can the use of IL-21/IL-21R blockers or low-dose IL-2 promote anti-inflammatory/tolerogenic environment to counteract pro-inflammatory signals in ABMR?
- Is personalized targeting of cytokines and T_{FH}-B cell interactions enough to tackle ABMR or should it be implemented to conventional antibody removal and plasma cell depletion?

Highlights

The effector mechanisms by which donor-specific antibodies injure organ transplants leading to antibody-mediated rejection are now well recognized, but the pivotal role of the adaptive immune cell responses upstream of such humoral alloimmunity has been much less appreciated.

Knowledge of the contributions of both T_{FH} and B cells to pathogenic humoral alloimmunity may improve outcomes for patients. Of particular interest is how the coordinated T_{FH} and B cell responses can shape DSA pathogenicity and lead to ABMR.

This allows to understand how patients would benefit from concomitant monitoring both T_{FH} and B cell responses to improve patient management and why targeting their interactions is key to tackle ABMR therapeutically.

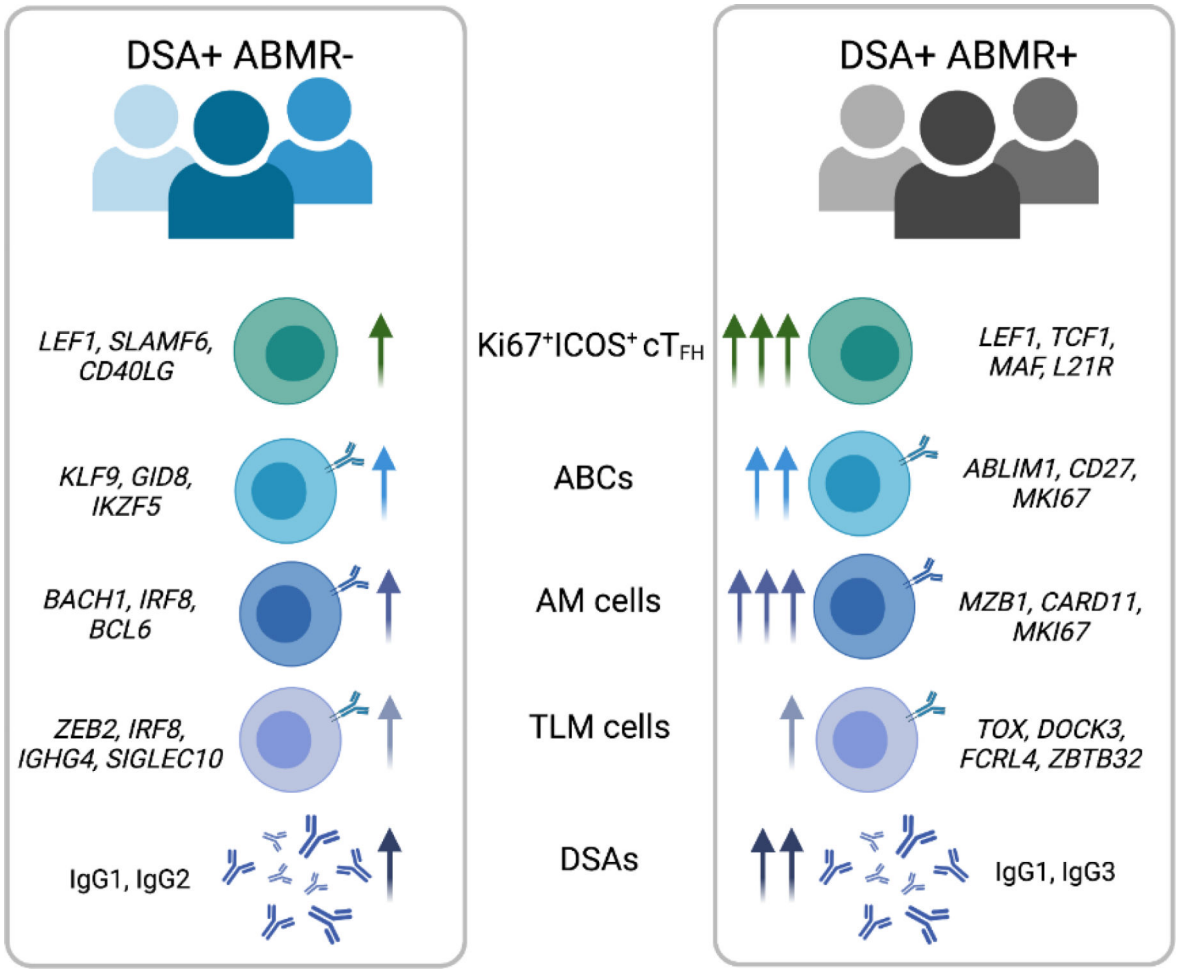

Figure 1. Differing cellular and molecular states of T_{FH} and B cells underlying progression to ABMR.

Common circulating T_{FH} (cT_{FH}) and B cell subsets can be found elevated in blood of patients developing DSA but who do not progress to ABMR (DSA+ABMR−) and patients developing DSA who progress to ABMR (DSA+ABMR+). These proliferating donor-specific cT_{FH}, activated B cells (ABCs), activated memory (AM) and tissue-like memory (TLM) cell subsets were elevated at different magnitudes in blood, indicated by the arrows, and displayed distinct transcriptional programming, indicated by gene names in italics. These changes in cT_{FH} and B cell subsets are linked to distinct magnitude and IgG subclass composition of donor-specific antibodies (DSAs) generated in the two types of patients. This figure was created with BioRender.

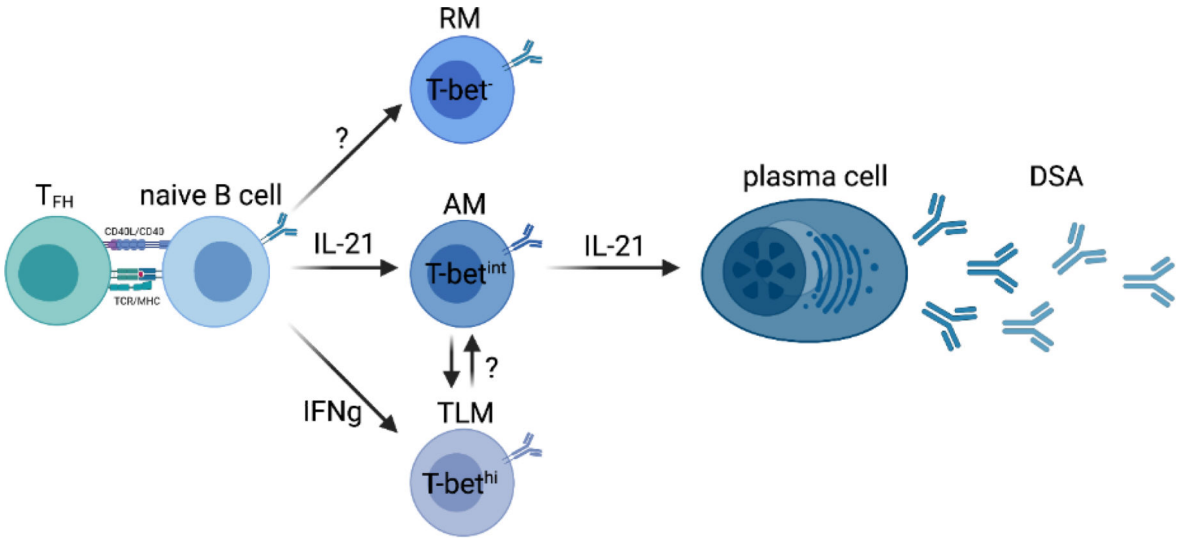

Figure 2. Alloreactive B cell differentiation according to cytokine instructions.

Fate of naive B cells dictated by IFN-g and T_{FH} cell-derived IL-21 and according to T-bet expression: resting memory (RM) cells represent the self-renewal reservoir of B cell memory, activated memory (AM) cells are poised for differentiation into donor-specific antibody-producing plasma cells driven by IL-21, and tissue-like memory (TLM) cells in an inhibitory state driven by IFN-g signals. Plasticity between AM and TLM cell fate may exist. This figure was created with BioRender. Abbreviations: CD, cluster of differentiation; MHC, major histocompatibility complex; TCR, T cell receptor.

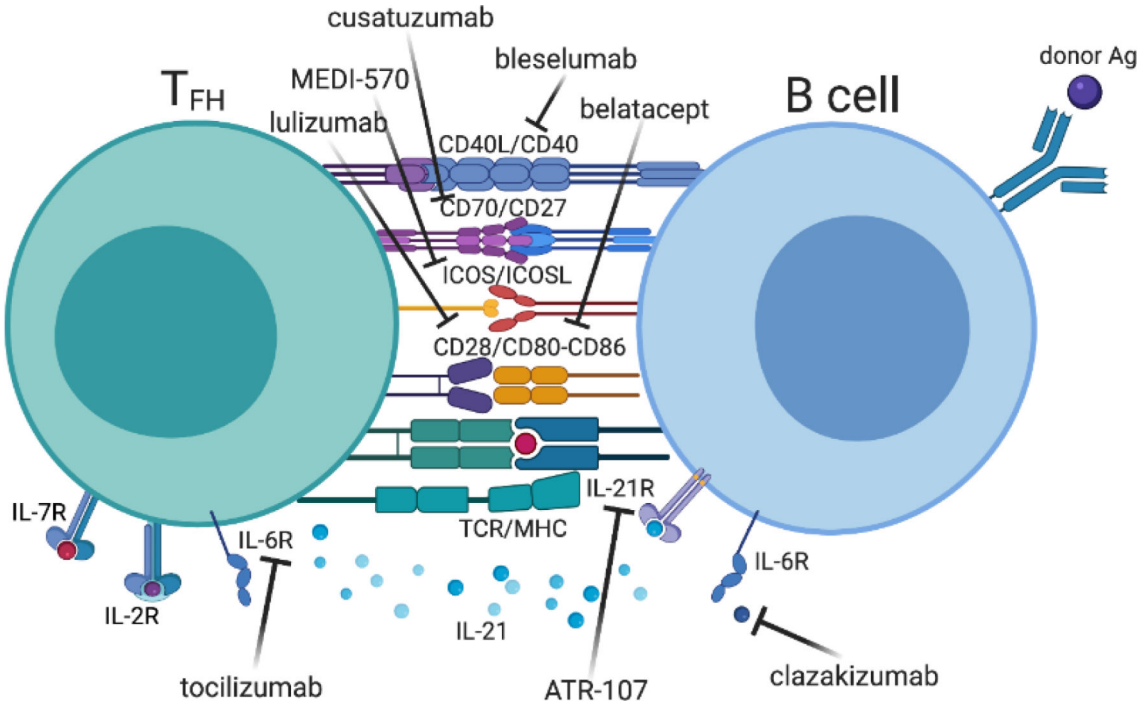

Figure 3. Key therapeutic targets in T_{FH} cell crosstalk with cognate B cell (key figure)

The major costimulatory and cytokine receptors involved in cognate donor-antigen specific T_{FH}-B cell interaction are depicted. Biotherapies currently evaluated in clinical trials and their specific targets are represented. This figure was created with BioRender. Abbreviations: CD, cluster of differentiation; ICOS, inducible T cell costimulator; IL, interleukin; MHC, major histocompatibility complex; TCR, T cell receptor.

Table 1.

T_{FH} cell responses in humoral alloimmunity in transplantation

Model	location	T_{FH} cell immune state and function	References
rat kidney transplant	GC T_{FH}	proliferating Ki67+ GC T_{FH}, increased IL-21+ T_{FH} in splenic follicles and in serum from rats with ABMR (model using low dose cyclosporine)	[12]
mouse skin transplant	GC T_{FH}	increased splenic ICOS+ PD-1+ T_{FH} in skin rejection, which can be inhibited by anti-IL-21R Ab	[13]
human kidney transplant recipients	cT_{FH}	increased IL-21+ and CFSElow donor-reactive blood TH (CD3+ CD8-) cells in patients with de novo DSA	[14]
mouse kidney transplant	GC T_{FH}	increased CXCR5+ ICOS+ GC T_{FH} from the draining lymph nodes of the transplanted kidney in mice with ABMR	[15]
mouse skin transplant	GC T_{FH}	expansion of CXCR5+ PD-1hi GC T_{FH} in response to a skin graft, which could be diminished by selective anti-CD28 treatment. The selective CD28 blockade inhibition of T_{FH}-B cell interactions was CTLA-4-dependent and T_{FH}-specific	[16]
human kidney transplant recipients	cT_{FH}	increased of proliferating Ki67+ ICOS+ T_{FH}, CCR7+CD127+ T_{FH} and IL-21+ donor-specific T_{FH} in DSA+ABMR+ versus DSA+ABMR-patients	[17]
human kidney transplant recipients	cT_{FH}	ICOS+PD-1+CD38+CXCR5+ T_{FH} detected in highly sensitized patients, which were decreased after desensitization by belatacept and proteasome inhibitor	[18]
mouse skin transplant	cT_{FH}	ICOS+ PD-1+ T_{FH} are enriched for donor-specific cells, ICOS+ PD-1 T_{FH} expansion precedes DSA formation	[19]
human kidney transplant recipients	cT_{FH}	decreased CXCR5+ICOS+PD-1+ T_{FH} precedes de novo DSA formation	[20]
human kidney transplant recipients	cT_{FH}	increased IL-21+ donor-specific T cells pre-transplant and post-tranplant predict allograft rejection	[21]
human kidney transplant recipients	cT_{FH}	increased CD25+ CCR6+ T_{FH} after in vitro restimulation correlates with DSA generation	[22]
mouse heart transplant	GC T_{FH}	increased number of secondary follicles and T_{FH} cells in spleen at day 50 post-transplant from mice showing heart ABMR	[23]
human kidney transplant recipients	cT_{FH}	increased CD62L-CXCR3+ T_{FH} and IL-21+ donor-specific T_{FH} are associated with early DSA generation post-transplant	[24]
mouse heart	GC T_{FH}	increased T_{FH} numbers, GC size, high serum IL-21 in alemtuzumab-induced	[25]
transplant		chronic ABMR model, which could be reduced by anti-LFA-1 treatment	
mouse skin transplant	GC T_{FH}	expansion of CXCR5+ PD-1hi Bcl6+ donor-specific T_{FH} that upregulated CTLA4 in response to a skin graft. Anti-CD28 treatment led to superior inhibition of donor-specific T_{FH} and DSA formation compared to CTLA4-Ig.	[26]
human kidney transplant recipients	cT_{FH}	CXCR5+ CCR7lo PD-1hi T_{FH} correlated with de novo HLA sensitization post-transplant	[27]
human kidney transplant recipients	cT_{FH}	increased CD40L+PD-1+ T_{FH} in patients with de novo DSA at 1-year post-transplant	[28]
human kidney transplant recipients	cT_{FH}	increased CXCR5+ CCR7+ T_{FH} in ABMR patients with class II DSA compared to those with class I DSA	[29]
human kidney transplant recipients	cT_{FH}	increased of both CXCR3-CCR6-T_{FH} and CXCR3-CCR6+ T_{FH} in patients with ABMR	[30]
non human primate kidney transplant	GC T_{FH}	increased ICOS+PD-1hi GC T_{FH} and size of GC in sensitized primates with ABMR after T cell–depleting induction	[31]
human kidney transplant recipients	cT_{FH}	increased numbers of T_{FH} cells in patients with pre-existent DSA	[32]
non human primate kidney transplant	GC T_{FH}	Increased PD-1hi GC T_{FH} and number of GCs in ABMR which could be decreased by anti-CD40 and belatacept treatment	[33]

Model	location	T_{FH} cell immune state and function	References
mouse heart transplant	GC T_{FH}	differentiation of transferred TCR-transgenic CD4 T cells (mimicking indirect pathway) into T_{FH} phenotype that allow GC formation after heart transplantation of T cell-deficient mice	[34]

Table 2.

Effector B cell responses in humoral alloimmunity in transplantation

Model	location	Effector B cell immune state and function	References
human kidney transplant recipients	circulating B cells	increased T-bet+CD27+CD21+ memory B cells transcriptionally and functionally poised for plasma cell differentiation in ABMR	[48]
human kidney transplant recipients	intragraft B cells	intragraft B cells have transcriptional resemblance with mouse innate B cells with capacity to differentiate into plasma cells expressing self-reactive antibodies, driven by local intragraft antigens	[49]
rat kidney transplant	GC B cells	CD45R+CD27+ memory B cells in splenic follicles from rats with ABMR, which can be diminished by high cyclosporine treatment	[12]
mouse heart transplant	GC B cells	Alloreactive B cells contribute to transplantation tolerance by foregoing germinal center responses while retaining their ability to function as antigen-presenting cells and by actively suppressing de novo alloreactive B cell responses	[50]
mouse skin transplant	GC B cells	increased GL-7+CD95+ B cells correlate with ICOS+ PD-1 cTfh expansion and precedes DSA formation, which can be diminished by belatacept and anti-CD28 treatment	[19]
rat kidney transplant	GC B cells	presence of B cell follicles and AID mRNA in spleen of rats in ABMR model (low cyclosporine-induced) which can be diminished by anti-BAFF treatment	[51]
human kidney transplant recipients	circulating B cells	expansion of Ki67+CD20hiCD38loCD27+IgD- activated B cells in DSA+ABMR+ versus DSA+ABMR- patients	[17]
human kidney transplant recipients	circulating B cells	CD27-IgD+ anive and CD27+IgD− memory B cells detected in highly sensitized patients, which were decreased after desensitization by belatacept and proteasome inhibitor	[52]
human kidney transplant recipients	circulating B cells	increased IgD-CD27+CD38- memory B cells at time of DSA detection before onset of ABMR	[53]
human kidney transplant recipients	circulating B cells	high frequencies of donor-specific memory B cells in patients with acute and chronic ABMR	[54]
human kidney transplant recipients	circulating B cells	donor-specific memory B cells are detected by ELISPOT before transplantation in sensitized patients	[55]
non human primate kidney transplant	circulating B cells	increased blood Ki67+CD20+CD27+IgD− memory B cells in sensitized primated with ABMR after T cell–depleting induction	[31]
human kidney transplant recipients	circulating B cells	expansion of CD19+CD27-IgD- and IL-21R+ B cells in patients developping anti-HLA antibodies	[56]
human kidney transplant recipients	circulating B cells	high frequencies of donor-specific memory B cells are found at ABMR diagnosis and before transplantation, regardless of circulating DSA	[57]
non human primate kidney transplant	circulating B cells	presence of CD20+CD27+ B cells in ABMR which could be decreased by anti-CD40 and belatacept treatment	[33]
human kidney transplant recipients	circulating B cells	increased IgD-CD27+ switched memory and decreased IgD+ CD27- naive B cells during the first month post-transplant predicted de novo DSA development	[58]