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Review article

Follicular helper T cells and humoral response in organ transplantation

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ABSTRACT

Antibody mediated rejection has been recognized as an important contributor to long-term graft loss in most solid organ transplants. Current immunosuppressive regimes are not capable of preventing anti-HLA antibody formation and eventual damage to the graft, and there is a need to develop drugs directed against novel targets to avoid graft allorecognition. In this review we introduce follicular helper T cells (Tfh), a subtype of lymphocyte specialized in helping B cells to differentiate into plasmablasts and produce class-switched antibodies. We focus on the role of Tfh in solid organ transplantation, what is known about Tfh and the production of alloantibodies, how current immunosuppressive therapies affect Tfh and what new molecules could be used to target Tfh in transplantation, with the goal of improving graft survival.

Contents

1. Introduction

Solid organ transplantation is a highly successful therapeutic approach for the treatment of different organ failures. Short- and medium-term survival of grafts has improved greatly due to technological advances, improvements in transplant programmes and better medications. Especially relevant was the arrival of the calcineurin inhibitors in the 1980's which increased the survival of allografts by significantly decreasing acute rejection. However, the allorecognition of the graft by the patient's immune system in the long term cannot be prevented. T cell mediated rejection is the most common reason for graft failure during the first year post-transplantation; however, after the first year, antibody mediated rejection (AMR) becomes the most common cause for transplant failure [1,2]. In particular, AMR is responsible for two thirds of the kidney graft losses [1], and a well-recognized cause of injury and rejection for heart, lung, pancreatic and liver allografts [3].

Anti-HLA antibodies have been shown to correlate with worse graft outcome, especially if they are donor specific antibodies (DSA) [4–6]. Tfh are specialized in providing help to B cells to differentiate into plasma cells and are required for the efficient production of antibodies [7,8], suggesting that Tfh could be a therapeutic target to prevent or

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minimize the consequences of AMR. This review will cover the current knowledge about the biology of Tfh and their role in health and disease, focusing on transplantation and how Tfh could be targeted to prevent humoral immune responses against the graft.

2. Follicular helper T cells: development and subsets

The first studies establishing the relevance of cognate interaction between T and B cells for antigen-specific B cell immunity date back to the 1960's [9], although it was not until much later that this T cell subset in the lymph nodes, characterized by CXCR5 surface expression, was termed follicular helper T cells [10,11].

Upon encountering their antigen, mature naive CD4+ T cells can differentiate into different helper subsets, namely Th1, Th2, Th17, Tfh and regulatory T cells, which show distinctive transcriptional profiles [12]. The commitment to these cell subsets is determined by the type of antigen encountered, cytokines present in the environment and costimulatory signals received at the time. Tfh are normally located in secondary lymphoid organs (SLO), mostly in lymph nodes, although they have also been found in non-lymphoid tissues with chronic inflammation forming tertiary lymphoid follicles [13,14].

The differentiation towards Tfh is regulated by the transcription factor B-cell lymphoma 6 (Bcl-6) which is critical for Tfh cell identity [15–17]. Coinciding with the STAT3-dependent upregulation of Bcl-6 there is repression of Blimp-1 [18]. These changes in transcription factors lead to the upregulation of the surface chemokine receptor CXCR5 and downregulation of CCR7, which allows pre-Tfh to migrate towards B cell areas in the lymph node [19]. Pre-Tfh interact with already activated B cells presenting cognate antigen at the T-B cell border, which drives their complete differentiation into Tfh. The full development of Tfh requires TCR/MHCII, CD28/CD86, ICOS/ICOSL, CD40/CD40L interactions with the cognate B cell, as well as the presence of cytokines such as interleukin-6 (IL-6), IL-12, IL-17, IL-21, IL-23 and IL-27 [20–22]. Especially important for the generation of Tfh appear to be IL-6 and IL-21, as knockout mice of these two cytokines exhibit greatly reduced numbers of Tfh [23,24]. IL-21 produced by Tfh can act in an autocrine manner to maintain Tfh differentiation, and in a paracrine way on GC B cells, promoting B cell growth, survival, and isotype switching [25,26]. Activation through the IL-21 receptor leads to phosphorylation of STAT3, another important transcription factor for the function of Tfh.

Mature Tfh are characterized by the expression of surface molecules such as CD40L, PD-1, and ICOS, and the secretion of IL-21 [27]. ICOS and PD-1 are considered to be activation markers of Tfh and the cells with higher surface expression of these two proteins are the most efficient at providing B cell help [28,29]. The interaction inside the follicles between Tfh, follicular B cells and other cells, such as follicular dendritic cells, is called the GC reaction, and it is necessary for the differentiation of B cells into memory B cells and into long-lived plasma cells able to secrete class-switched antibodies. The involvement of Tfh is also necessary for B cells to undergo somatic hypermutation at the antigen binding site, which results in the generation, and selection, of higher affinity antibodies [30–32].

Since 2011, peripheral blood CD4+CXCR5+ T cells have been considered as circulating Tfh (cTfh) and described as counterparts of the Tfh found in the GC of SLO [33,34]. cTfh not only display similar phenotype and signature cytokines to GC Tfh, but are also capable of driving B cell differentiation into IgG-producing plasma cells in vitro. It is commonly agreed that cTfh are memory CD45RO+ Tfh, which constitutively express CXCR5, CD28 and CD40L on their surface, and upon activation upregulate ICOS and PD-1 [20]. The more activated Tfh are, the lower their CCR7 expression and the higher their helper capacity. Unlike Tfh from SLO, cTfh do not actively express Bcl-6 [35,36].

Tfh can also be divided into subsets: Tfh1, Tfh2, Tfh17 [20,33]. Tfh1 express Tbet together with Bcl6, they are CXCR3+CCR6-, but they generally have no helper capacity. Conversely, Tfh2 and Tfh17 have helper capacity supporting plasmablast differentiation and antibody production. Tfh2 express Gata3 and Bcl-6, are CXCR3-CCR6- and secrete IL-4, 5 and 13, while Tfh17 express RORγt together with Bcl6, are CXCR3-CCR6+ and produce IL-17 and 22.

3. Follicular helper T cells: function and regulation

The pivotal role of Tfh in the production of high affinity, class switched antibodies, as well as their discovery as circulating cells, has made Tfh a very active field of research in different clinical settings. Tfh are responsible for protective humoral responses against pathogens, either during natural infections or in response to vaccination. Tfh promote humoral responses in healthy volunteers and HIV+ patients receiving influenza vaccination [34,37,38], with correlation between the numbers of cTfh and titres of specific influenza antibodies [36] and between activation of cTfh and development of specific memory B cells [39]. cTfh levels were also increased in HIV+ patients who produced high titres of anti-HIV neutralizing antibodies [37]. These researchers described a highly functional, cTfh polarized population, represented by CD4+PD1+CXCR3-CXCR5+ cells which drove the development of highly neutralizing antibodies. This cTfh subset had a transcriptomic profile very similar to that of GC and was the most efficient at antibody production, promoting B cell survival and plasmablast differentiation. In a cohort of paediatric patients with recurrent streptococcal tonsillitis, antigen-specific Tfh were decreased in number and dysfunctional, and antibody responses were reduced compared to children without recurrent tonsillitis [40].

On the other hand excessive activation of Tfh can lead to autoimmunity and, therefore, their differentiation and function must be tightly regulated. Control of the GC reaction is provided by T follicular regulatory cells (Tfr), a subset of regulatory T cells which express Foxp3 together with Bcl-6 [41–44]. Tfr derive from thymic precursors and differentiate in SLO, where they limit the function of Tfh as well as the development of GCs [43]. They regulate the process of somatic hypermutation, which is needed to limit autoimmunity [45]. Tfr have been, in fact, found to be impaired in multiple sclerosis and Sjögren's disease among other autoimmune disorders [46,47]. Data on Tfr and their likely role in limiting antibody formation in transplantation is still limited. Indirect data comes from a study in mice showing that increased Tfr attenuated antibody dependent graft-versus-host disease (GVHD) after hematopoietic stem cell transplantation [48], and a study in humans showing low levels of circulating Tfr in renal transplant recipients with chronic AMR [49].

4. Follicular helper T cells in autoimmunity

Tfh can facilitate autoimmunity by supporting the emergence of autoreactive B cell clones in the GC [45,50]. In a mouse model of autoimmune hepatitis, the liver had severe T-cell infiltration and mice produced large amounts of antinuclear antibodies [51]. Tfh cells were found in enlarged GCs, and blocking of ICOS or IL-21 suppressed Tfh generation and the development of autoimmune hepatitis. Similarly, different mouse models of systemic lupus erythematosus (SLE) have shown that Tfh are involved in the pathophysiology of this disease. Lupus ameliorates when Bcl6 is knocked out [52], and mice deficient in IL-21 receptor lack the abnormalities characteristic of SLE [53].

In humans, cTfh levels have been found increased in SLE [34,54–56], particularly in those patients with greater disease severity and higher titres of ANA and anti-dsDNA autoantibodies. Similarly, increased cTfh together with enhanced expression of IL-21 have been detected in patients with type 1 diabetes mellitus [57]. In addition, cTfh from these patients correlated with serum autoantibodies and C-peptide level. In another study, two cohorts of patients were compared, one suffering juvenile dermatomyositis producing large amounts of antibodies and another suffering arthritic psoriasis and making no antibodies [33]. cTfh1 were decreased while cTfh2 and cTfh17 were increased in dermatomyositis patients, the disorder with large amounts of autoantibodies.

Altogether these studies support the involvement of Tfh in the production of pathogenic antibodies which are an important element in the pathophysiology of most autoimmune diseases.

There is increasing evidence that antibodies against non-HLA autoantigens can produce transplant associated autoimmunity, favouring rejection and poorer graft survival [58–61]. In a mouse model of cardiac allograft vasculopathy (CAV), Qureshi et al. have demonstrated that although the key players initiating humoral autoimmunity are donor CD4 T cells, the maintenance of this autoimmunity is dependent on help provided by recipient Tfh [62]. Therefore, targeting Tfh in transplantation could be relevant to improve graft function and survival by preventing or treating not only allorecognition of the graft, but also transplant associated autoimmunity.

5. Follicular helper T cells in renal transplantation

The study of Tfh in transplantation is timely because AMR is the main cause of kidney transplant failure nowadays [1]. Pre-existent as well as de novo anti-HLA DSA (dnDSA) are associated with worse renal graft outcome [5,6], and AMR is more frequent, and more rapid, in patients with DSA pre-transplantation [63]. Patients with DSA also have increased rates of chronic graft dysfunction, graft loss and death [4]. Anti-HLA antibodies are predominantly class switched and persist in the circulation for many years both pre and post-transplantation, suggesting that Tfh have been involved in the differentiation of long-lived plasma cells in GCs [64–66]. The antibody subclasses IgG1 and IgG3 especially associate with poorer graft survival, due probably to their complement-binding capacity [67].

The first publication showing the relationship between Tfh and allograft reactivity comes from the group of Carla Baan in Rotterdam [68]. They found no difference in cTfh number before and after transplantation in a cohort of kidney transplant patients, and showed that the capacity of cTfh to derive plasmablasts in vitro was reduced post-transplantation, although only partially. In fact, the capacity of cTfh to promote IgG production in vitro was similar pre and post-transplantation. Patients with preformed DSA had higher numbers of cTfh post-transplantation than patients without pre-existent DSA. In kidney biopsies diagnosed as acute rejection, they identified structures similar to tertiary follicles with CD3+ Bcl6+ cells that co-localized with B cells, suggesting that Tfh were interacting with B cells in the rejected graft. This group has also shown in an in vitro alloantigen setting, that plasmablast differentiation and immunoglobulin production were both dependent on IL-21 producing cTfh [69].

Our group has analysed the relationship among cTfh and anti-HLA sensitization in a prospective cohort of 206 kidney transplant patients [70]. Patients with previous transplants or blood transfusions had increased cTfh pre-transplantation, which suggested that cTfh were expanded in patients with previous exposure to alloantigens. According with the capacity of alloantigens to stimulate the production of alloantibodies, we found that patients with anti-HLA antibodies pre-transplantation had more pre-transplantation cTfh than non-sensitized patients, and anti-HLA antibody titres positively correlated with cTfh. cTfh increased during the first six months post-transplantation, and this expansion was more noticeable in patients who developed de novo anti-HLA antibodies. There was also a correlation between the anti-HLA antibody titre post-transplantation and cTfh. In addition, cTfh 6-months after transplantation retained the capacity to derive plasmablasts and promoted IgG production, in spite of the presence of immunosuppressive therapy. We did not observe an association between cTfh and dnDSA as the number of patients who developed dnDSA was very low. A recent CyTOF analysis of PBMC in 26 recipients failed to observe the post-transplant expansion of cTfh recorded by us [71].

Chenouard et al. found that cTfh are defective in operational tolerant patients, showing impaired B cell help [72]. cTfh cells from tolerant patients exhibited a remarkably different transcriptomic signature compared to stable patients under immunosuppressive therapy, failed to produce IL-21 and did not support IgG production in vitro. Of note, post-transplantation cTfh were higher in stable renal transplant patients subsequently developing dnDSA. The functional impairment of cTfh, together with the low incidence of dnDSA in this cohort of tolerant patients, suggests that targeting Tfh could reduce the risk of developing DSA. Consistent with the role of Tfh in DSA formation, Macedo et al. have recently found in a kidney transplant cohort that patients who developed DSA post-transplantation had higher percentage of cTfh and lower percentage of regulatory T cells (CD4 + CD25hiCD127-FOXP3 +) post-transplantation, resulting in increased cTfh:Treg ratio at DSA occurrence [21]. In addition, half of the patients who developed dnDSA had pre-transplantation donor-reactive memory IL21+ cTfh.

There is emerging data associating Tfh with acute graft rejection. Our group has found that higher pre-transplantation cTfh numbers are a risk factor for acute rejection, with a significant hazard ratio of 1.14 [70]. Moreover, the Rotterdam group has shown the presence of Tfh in acute kidney rejection biopsies [68]. They studied biopsies diagnosed as acute AMR, acute T cell mediated rejection grade I and acute T cell mediated rejection grade II. T cells could be detected in all biopsies but they found only in the acute T cell mediated rejection grade I biopsies that B cells formed aggregates surrounded by T cells, containing follicular dendritic cells, IgD and Ki67 staining and in which IL-21 co-localized with Bcl-6, showing the presence of Tfh in these ectopic lymphoid structures [13]. The presence of Tfh and ectopic lymphoid structures in these biopsies suggest a mechanism for B cell alloactivation at the graft itself during T cell mediated rejection. They also point to a role of Tfh in acute T cell rejection, not only antibody mediated rejection. These authors speculated that IL-21 secreted by Tfh could upregulate the production of granzyme B in CD8 T cells thereby enhancing their cytotoxic capacity and favouring T cell mediated rejection [73].

There is also data showing an association between Tfh and chronic graft rejection. In a recent study, IL21-producing cTfh cells (specifically cTfh2 and cTfh17) were increased, while Tfr were decreased in blood and kidney biopsies of patients with chronic AMR [49]. Still, these Tfr were functionally similar in patients with or without chronic AMR, exerting their inhibitory effect over Tfh partly through CTLA-4 and secretion of Il-10 and TGF-β. Similar results were found in another smaller cohort of kidney transplant patients, in which cTfh were increased in patients with chronic AMR compared to stable patients [74].

6. Follicular helper T cells in other solid organ transplants

The relevance of humoral allorecognition in liver transplantation outcome is not completely delineated. The liver is considered an immunoprivileged organ, which generally requires less immunosuppression following transplantation and is subject to less rejection. However, pre-formed and post-transplant anti-HLA antibodies have been shown to decrease liver allograft survival [75,76]. In a cohort of liver recipients cTfh remained stable post-transplantation, although Tfh1 and Tfh2 frequencies were reduced, and also stable remained the capacity of cTfh to help B cells to produce antibodies in vitro [77], suggesting the possibility that cTfh may be involved in alloreactive responses following liver transplantation. In a subsequent study cTfh2 were associated with acute graft rejection after liver transplantation. The proportion of B cells correlated with cTfh2 and serum IL-21 levels were higher in the patients with than without rejection [78].

The development of dnDSA in heart transplant patients is clearly associated with heart allograft failure and poor patient survival [79], especially if they are persistent DSA but transient DSA also associate to worse transplant outcome. In a prospective cohort of heart recipients, de novo anti-HLA antibodies associated with graft rejection, and those directed against class II HLA associated as well with allograft vasculopathy and patient death [80]. There is no published data on the role of Tfh in clinical heart transplantation, however, much work has been done in mouse models by the group of Gavin Pettigrew. Using mice reconstituted with

different TCR-transgenic CD4 T cells they found that indirect presentation of allopeptides to CD4 T cells was required for development of long-lasting alloantibody responses and generation of GCs with differentiated Tfh within them [81]. Later they performed MHC-mismatched heart transplants in mice reconstituted with "TCR75" CD4 T cells which differentiated into Tfh, and these mice developed long-lived class-switched alloantibodies and their heart grafts developed progressive CAV and were rejected chronically. When mice were reconstituted with "*Sh2d1a*$^{-/-}$ TCR75" CD4 T cells genetically incapable of providing Tfh function, alloantibody responses were abolished and heart grafts survived indefinitely, showing that Tfh were at the core of AMR [82]. In a different mouse model of allogeneic heart transplantation, DSA responses could not be observed in AβKO mice genetically deficient for CD4+ T cells [83]. All this work supports the idea that Tfh help is mandatory for DSA responses against heart allografts, and targeting Tfh may have therapeutic potential.

AMR has been recently recognized as a contributor to poor long-term survival in lung transplantation. Anti-HLA antibodies, and particularly DSA, have been associated with bronchiolitis obliterans syndrome and graft rejection [84,85], and anti-HLA antibody presence has been included as a diagnostic criterion in the latest guidelines for diagnosis of AMR of the lung [86]. Tfh and antibody deposition in the lungs has been associated with bronchiolitis obliterans syndrome in a murine model, suggesting that Tfh targeting could also be useful to improve lung transplantation outcome.

7. Effect of current IS on Tfh

Current immunosuppressive therapies mostly prevent activation, proliferation and function of effector T lymphocytes. The fact that chronic graft damage eventually occurs, mainly through humoral allorecognition, indicates that current immunosuppressive drugs may not be completely efficient at limiting Tfh function.

Thymoglobulin is a polyclonal antibody commonly used as induction therapy to immunological high-risk patients. In a prospective cohort of kidney transplant patients, we found that thymoglobulin drastically reduced cTfh early post-transplantation and cTfh took six months to recover to pre-transplantation level [70]. In a different cohort, cTfh reconstituted after thymoglobulin-induced depletion displayed upregulation of PD1 expression, suggesting cTfh may become activated after this treatment [21]. We have also detected an increase in cTfh frequency between six- and twelve-months post-transplantation in thymoglobulin-induced patients (unpublished data). In our cohort, cTfh levels remained similar in patients with basiliximab (anti-IL2-R nondepleting antibody) and in patients who received no induction [70]. In fact, cTfh increased early post-transplantation despite basiliximab and maintenance immunosuppressive therapy. It has been shown that IL-2 signalling can shift CD4 T cell differentiation away from Tfh, by activating STAT5 which on the one hand suppresses STAT3 mediated upregulation of Bcl6 and on the other promotes expression of Blimp-1 [87,88]. Given that blocking the IL-2 receptor may promote Tfh differentiation, the utility of basiliximab in avoiding humoral immune seems questionable.

Alemtuzumab is an anti-CD52 T-cell depleting antibody. A higher incidence of DSA formation and humoral rejection following lymphocyte depletion with alemtuzumab has been found in certain human immunosuppressive protocols [89,90]. This finding is supported by a mouse model of heart transplantation, in which alemtuzumab promoted DSA, allo-specific B cells and CAV [91]. A possible explanation to this finding could be that after alemtuzumab treatment, during the reconstitution of the T cell compartment, there is an expansion of regulatory T cells [92] which could restrict IL-2 availability [93], favouring again Tfh differentiation.

Taking all of this data into account, despite the good efficacy of current induction therapies in controlling T cell mediated acute rejection, their efficiency in controlling the humoral immune response may be more limited.

Regarding immunosuppressive maintenance therapies, tacrolimus could in theory be effective at inhibiting Tfh because it inhibits the transcription factor NFAT and Tfh are dependent on NFAT signalling. However, despite tacrolimus being used worldwide, DSA formation and graft rejection remains a common problem. After transplantation, cTfh remain relatively stable in transplant patients under tacrolimus-based regimes [68,70,77]. Isolated cTfh from these patients have their function partially impaired but are still able to differentiate B cells into plasmablasts and promote antibody production. In vitro addition of tacrolimus to cell cultures did not inhibit Tfh generation and only partially prevented Tfh activation and plasmablast differentiation [94]. Conversely, a recent study has shown that a week of tacrolimus treatment pre-transplantation significantly reduces cTfh and lymph node Tfh while it has no effect on B cells, TFR and Treg [95], suggesting that calcineurin inhibitors may have a more important role in the prevention of antibody formation than previously understood.

Some patients convert from using calcineurin inhibitors to MTOR inhibitors. In a study with 26 kidney transplant recipients treated with tacrolimus and 13 with sirolimus, sirolimus supressed the quantity of cTfh, and the co-expression of PD1, more efficiently than tacrolimus [96]. However, in another study in which patients were treated with alemtuzumab followed by sirolimus, 25% of patients developed dnDSA at two years post-transplantation [89]. This latter result is supported by a study in a mouse model of heart transplant, in which adding rapamycin to alemtuzumab increased Tfh in lymph nodes, serum DSA levels and the incidence of vasculopathy [97].

The capacity of the CTLA4-Ig fusion protein belatacept to prevent dnDSA formation has been tested in a nonhuman primate kidney transplant model [98]. Belatacept was very efficient at reducing the early production of dnDSA, reducing the number of Tfh in GCs and the production of IL-21, as well as reducing B cell clonal expansion and isotype switching. Similarly, CTLA4-Ig has shown to be effective at reversing alloantibody responses and rescuing allografts from acute rejection in a mouse model of heart transplant [99]. CTLA-Ig was administered 6 days post-transplantation, when there was already significant C4d deposition, and three weeks later the presence of C4d was reduced. In a human study, belatacept showed reduction of dnDSA via two mechanisms: acting directly on the B cell reducing plasmablast differentiation and antibody production, and by modulating the B cell-Tfh interaction, as belatacept blocked CD28-mediated activation of cTfh in a Bmem-Tfh co-culture [100]. In vivo, belatacept-treated patients had less activated cTfh (PD1 + ICOS+) than belatacept-untreated patients. These results are however contradictory to the study by Graav et al., in which they showed that in vitro belatacept did not inhibit Tfh generation nor plasmablast differentiation in co-cultures, and it only inhibited partially Tfh activation [94]. In kidney transplant patients randomized to a belatacept- or tacrolimus-based immunosuppressive regimen, no differences were observed regarding cTfh generation or activation [94]. Further studies are necessary to fully understand the effect of costimulation blockade by belatacept on Tfh and DSA generation in humans.

Current treatment of AMR includes plasmapheresis and IGIV to reduce circulating levels of DSA, as well as rituximab. The effect of rituximab on cTfh is again controversial, as one study has found that rituximab reduced cTfh, serum IL-21, IL-6 and Bcl6mRNA [57], while in a different study rituximab did not affect cTfh, suggesting that remaining cTfh could allow rapid reconstitution of the pathological GC response once the B cell pool recovered [101].

There seems to be contradictory results regarding the effects of the different immunosuppressive drugs on Tfh. This may be due to the small number of patients in some cohorts, different concomitant immunosuppressive agents used and different pre-transplantation immunological risk of the patients. Nevertheless, it seems evident that the current available therapies are insufficient to block Tfh function

and prevent DSA formation. Therefore, there is the need for new therapies targeted against Tfh to be developed.

8. Novel therapies targeted to Tfh

When considering targeting Tfh to increase graft survival, a plausible strategy would be therapies blocking interleukins relevant for Tfh differentiation and function (see Fig. 1). Both IL-6 and IL-21 regulate Bcl6 expression required for the generation of Tfh [15]. Data from animal and human studies indicate a critical role for IL-6 in cell-mediated rejection, AMR and chronic allograft vasculopathy, suggesting that blocking the IL-6/IL-6R interaction with the anti-IL-6 receptor tocilizumab could prevent allorecognition of the graft and improve long term survival. IL-6 has an important role in inflammation, and inhibition of this pathway could reduce the inflammatory cascade induced by alloantibody [102]. Mouse models of transplantation have shown the relevance of IL-6 in allograft rejection [103] and the effectiveness of anti-IL6R antibodies to limit alloantibody responses [104]. In humans, tocilizumab is proving to be useful to desensitize kidney transplant patients [105,106] and to treat chronic AMR [107]. The therapeutic potential of blocking the IL-21/IL-21R axis has been tested in vitro, in a co-culture of cTfh and memory B cells from transplant patients, stimulated with allogeneic donor PBMCs [69]. Addition of an anti-IL21R antibody decreased considerably plasmablast differentiation and immunoglobulin production, and a dose-dependent inhibition of STAT3 phosphorylation in T and B cells was observed.

Another approach to targeting Tfh would be costimulation blockade. Tfh rely on the CD28 costimulatory pathway for survival and function. Instead of blocking CD80/86 by using CTLA4-Ig we could use an antibody against CD28, with the advantage of preserving CTLA4 binding to CD80/86 to assure the avoidance of effector responses. Selective block of CD28 in a mouse model has shown improved allograft survival in sensitized recipients compared with CTL4-Ig, by attenuation of CD8+ memory T cell effector function [108] and by inhibition of PD1+ donor-reactive Tfh and of DSA formation [109]. Similarly, the CD40/CD40L axis could be blocked. Tfh need to receive signalling through surface CD40L for their differentiation and activation, as patients with CD40 mutations have low numbers of cTfh [110]. Different variations of anti-CD40L antibodies have been tested with success in murine and non-human primate models of autoimmunity and transplantation with success at inhibiting immune responses [111,112].

Blockade of ICOS-L in mice has been shown to revert Tfh phenotype, relocating these cells from the B cell follicle to the T cell zone, and to reduce antigen-specific GC B cells and serum levels of antigen-specific IgG [113]. Patients with mutations in ICOS suffer common variable immunodeficiency, a disorder characterized by low numbers of class-switched memory B cells and diminished IgG [114]. A blocking humanized monoclonal antibody against ICOS-L has proven to be safe in SLE patients, selectively blocking neoantigen IgG responses, although without improvement in SLE-related biomarkers or clinical measures [115]. OX40 is a member of the tumour necrosis factor receptor superfamily and is a potent T-cell costimulatory molecule. Its ligand, OX40L, is expressed on antigen presenting cells and participates in the pathogenesis of autoimmune diseases by promoting Tfh responses [116]. An anti-OX40 fusion protein has been used in mouse model of heart allograft helping to prevent allograft rejection [117]. In vitro blocking of OX40-OX40L reduced IFNg and IL-4 secretion by T cells from kidney transplant patients suffering acute rejection [118].

A less developed alternative to modulate Tfh would be targeting of PD-1. The effect of PD-1 stimulation inhibiting T cell activation, proliferation and cytokine production in CD4+ T cells is well established, however, its role in Tfh is still unknown. Mice deficient in PD-1 or its ligands PD-L1 and PD-L2 have increased numbers of Tfh with altered function [119,120]. On the one hand we could speculate that blocking PD-1 could therefore alter Tfh function in transplantation. Administering anti-PD-1 treatment to transplant patients, with the subsequent T cell activation effect of this therapy, seems too dangerous. Still, it would be interesting to study in patients receiving immune checkpoint inhibitors the effect of this therapy on Tfh. A probably more viable option would be to assess the effect of a PD-L1/PD-L2 fusion protein in an animal model of allotransplantation. A potent engagement of PD-1 induced by this fusion protein could perhaps inhibit Tfh function and reduce antibody production.

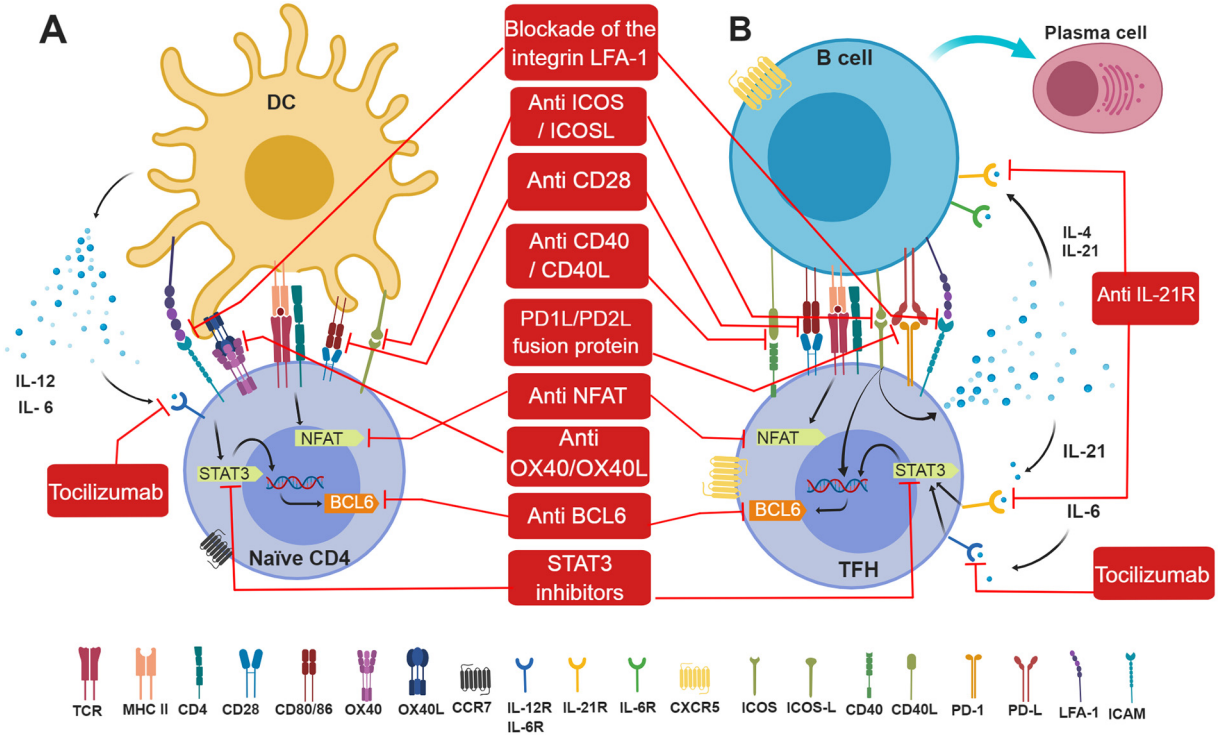

Fig. 1. Tfh-targeted immunotherapies. Targeted molecules by Tfh-specific immunotherapies in two key stages of a Tfh cell: during its priming by a dendritic cell in a secondary lymphoid organ (A) and the subsequent T:B crosstalk resulting in the differentiation of the B cell into an antibody-secreting cell (B). *DC: Dendritic Cell, Tfh: Follicular helper T cell.*

A different strategy would be blockade of the integrin LFA-1 to prevent the immune synapse and AMR. Monoclonal antibodies against LFA-1 have been used in a mouse model of alemtuzumab-induced chronic AMR, in which the treatment with anti-LFA-1 resulted in reduced DSA, allo-specific B cells and signs of graft rejection [91].

Finally, one more alternative could be targeting transcription factors relevant for the differentiation or function of Tfh. STAT3 is an important regulator of B cell differentiation, directly acting inside the B cell as STAT3 mutations dramatically reduce the capacity of B cells to differentiate into plasma cells [121] and by being involved in the upregulation of Bcl6 required for Tfh differentiation [24] and in the signalling downstream of IL-21R. Severe toxicities were reported in clinical trials using STAT3 inhibitors for solid tumours, possibly due to the ubiquitous tisular expression and many functions of STAT3, including modulation of the immune system [122]. Despite newer inhibitors are being developed with more favourable toxicity profiles [123], so far there is no data on the use of STAT3 inhibitors in transplantation. Nuclear factor of activated T cells (NFAT) binding sites have been found near the transcriptional starting sites of many differentially expressed genes in Tfh, including ICOS and CXCR5, suggesting that NFAT is an important transcription regulator of Tfh [124]. Targeting the transcription factor NFAT with small-molecule inhibitors is an emerging strategy for immune modulation [125], which would be worth pursuing in models of allotransplantation. Recently, a small-molecule BCL6 inhibitor has been successfully used to treat a murine model of nonsclerodermatous chronic GVHD (cGVHD), demonstrating that BCL6 expression in donor T and B cells is necessary for cGVHD and that alloantibodies are associated with the pathogenesis of cGVHD [126]. This suggests that a similar approach targeting BCL6 could be useful to prevent AMR in solid organ transplantation.

All of these novel therapeutic options are providing exciting results. Molecules to inhibit IL21/IL21R, ICOS/ICOSL or BCL6 seem very promising to prevent Tfh function and production of DSA, and further studies in animal models of allotransplantation would be useful before moving into clinic. Other molecules are already approved for human use, like Tocilizumab, and more controlled trials would be required to evaluate its effect on the prevention or treatment of AMR in transplant patients.

Declarations of competing interest

None.

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