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Follicular T Cell Regulation of Alloantibody Formation

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Abstract

Purpose of Review—To summarize recent studies elucidating the roles of follicular T cells in controlling allospecific antibody responses and antibody mediated rejection.

Recent Findings—The field of antibody regulation has provided an in depth identification of the T cell subsets involved in regulation of antibody responses. In addition, tools have been developed to study these cells during disease. Over the past few years, these strategies have been implemented in the field of transplantation to study the roles of T cells in mediating pathogenic antibody responses.

Summary—Antibody mediated rejection (AbMR) is largely responsible for long-term graft failure after solid organ transplantation and is induced by allospecific antibodies. In vaccination and infection, antibody responses are controlled by humoral immunoregulation in which T follicular helper (Tfh) cells promote, and T follicular regulatory (Tfr) cells inhibit, antibody responses. Recent studies have suggested multifaceted roles for follicular T cell subsets in regulating allospecific antibody responses and AbMR during organ transplantation. In addition, we discuss research priorities for the field to help elucidate mechanisms used by these cells so that new targeted therapeutics can be developed to prevent AbMR in human organ transplantation.

Keywords

Antibody mediated rejection; Donor specific antibody; T follicular helper cell; Tfh; T follicular regulatory cell; Tfr

INTRODUCTION

Tfh and Tfr Cells

Antibody responses are tightly controlled by the immune system. Production of most antibodies result from interactions between T follicular helper (Tfh) cells and B cells in germinal centers (GCs). Upon differentiation, Tfh cells migrate to the B cell follicle and interact with cognate B cells. During this interaction, called linked recognition, the Tfh cell supplies costimulation (CD40L) and cytokines (IL-4 and IL-21) to B cells. The B cell

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Conflict of interest

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presents cognate antigen and supplies costimulation to Tfh cells, causing full effector differentiation. During this interaction, B cells undergo class switch recombination, somatic hypermutation, and affinity maturation which promote the affinity and effector potential of antibody. Some Tfh cells that do not receive full effector differentiation can leave the lymph node into the circulation as memory-like cells, persist for long periods of time, and contribute to antibody response after antigen re-exposure. Tfh cells have been extensively studied in a number of normal and disease settings and are essential mediators of antibody responses¹.

T follicular regulatory (Tfr) cells are a specialized regulatory T (Treg) cell subset that is thought to control Tfh-mediated B cell responses^{2, 3, 4}. Tfr, Tfh and Treg cells are distinct cell subsets which have unique transcriptional programs for functionality^{5, 6}. Recent data has suggested that the transcription factor FoxP3 coopts the Tfh transcriptional program to promote a Tfr transcriptional program facilitating regulation of B cell responses. The tissue microenvironment can fine-tune the transcriptional programs of Tfh and Tfr cells to alter the functionality of these cells⁶. Like Tfh cells, a population of Tfr cells leave the lymph node before full activation, gain access to the circulation, and persist until antigen re-exposure⁷.

Altered Tfr cell proportions (or function) have been associated with autoimmune diseases (such as lupus/nephritis, myasthenia gravis, arthritis, IPEX, ankylosing spondylitis), viral infection (such as HIV, SIV and Hepatitis), gut microbiota, age-related defects in humoral immunity, and progression of graft versus host disease (GVHD)³. However, many of these studies are only correlative due to the lack of assays to assess the functionality of Tfr cells directly. Early adoptive transfer approaches into lymphopenic

mice suggested that Tfr cells potentially inhibit antibody responses to foreign antigens^{8, 9, 10}. However, these assays do not fully reconstitute Tfr cells for long periods of time. In addition, adoptively transferring small numbers of cells into lymphopenic hosts may alter immunoregulation. Therefore, more specific tools need to be developed to study Tfr cells in settings of disease.

Recently, newer models have been developed to study Tfr cells. For instance, mice with conditional deficiency of Bcl6 in Treg cell subsets has been developed. In these mice, loss of Bcl6 results in lower expression of PD-1 and/or CXCR5 in Treg cell subsets, and therefore, are thought to have reduced Tfr cells. Studies with these mice have revealed a role for Tfr cells in controlling autoantibody responses, but revealed either no, or a positive role, in antigen specific antibody responses^{11, 12}. However, these models may be more complex than originally thought because Tfr cells may not be completely dependent on Bcl6/CXCR5 for positioning/functionality, Bcl6 on non-Tfr Treg cells may have roles in humoral immunoregulation, and compensatory mechanisms for humoral immunoregulation may develop over time. As an alternative approach, we recently developed a Tfr-deleter mouse to specifically study Tfr cells at distinct times. In Tfr deleter mice, deletion of Tfr cells specifically before GC formation resulted in augmented levels of antigen specific antibody after vaccination, expansion of autoreactive antibodies, and increased vaccine specific antibodies during memory responses¹³. Interestingly, the increased vaccine specific antibody formed after Tfr deletion had lower affinity. Therefore, we hypothesize a new paradigm in

which Tfr cells set activation thresholds, and do not simply inhibit, B cell responses. This paradigm suggests that Tfr cells have more complex roles in regulating antibody responses; Tfr cells may regulate the quantity, but simultaneously promote affinity, of antigen specific antibody. Therefore, the precise roles of Tfr cells in disease depends if pathogenicity of antibody is determined by quantity, affinity, or both. The development of newer and more specific tools to study Tfr cells should facilitate a deeper understanding of these cells in the context of immune responses, including organ transplantation.

Donor Specific Antibody and AbMR

Over the past 10 years, the development of new broad immunosuppressive drugs has improved short-term graft survival in settings of solid organ transplantation. However, long-term survival has not substantially improved. Long-term graft rejection has largely been attributed to formation of donor specific antibodies (DSA) which cause antibody-mediated rejection (AbMR). Despite the development of treatments to limit AbMR, such as immunosorption, ~33% of de novo DSA positive transplant recipients still proceed to AbMR in settings of kidney transplantation¹⁴. DSA can be pre-existing due to prior sensitization (during pregnancy, blood transfusion, etc.) or can arise *de novo* after transplantation. Since pre-existing DSA results in faster graft rejection, transplantation into patients with pre-existing DSA is avoided, if possible. However it is important to note that DSA quantification, although generally highly sensitive and robust, has some technical limitations and can vary between studies and clinical centers¹⁵. Recent studies in settings of kidney transplantation showed that in the absence of biopsy-proven rejection, human leukocyte antigen (HLA) DSAs are not associated with graft failure¹⁶. Therefore, in some, but not all, settings DSA can lead to AbMR. This has led to the presence of DSA being only one factor in diagnosing AbMR. The reasons why some DSA leads to AbMR is unclear but may involve the specificity of antibody, isotype of antibody, and effector functions of the antibody governed by glycosylation of the Fc region¹⁷. For instance, patients with DSA that can bind C1q have increased frequency of AbMR compared to patients in which DSA does not bind to C1q. More specific assays need to be developed to study the role of antibody functionality in AbMR. Nevertheless, a fundamental strategy to prevent AbMR is to limit the formation of de novo pathogenic DSA. To achieve this goal, mechanisms controlling pathogenic DSA formation need to be elucidated in detail.

TFH cells in alloantibody responses

Tfh cell subsets are thought to have unique functions based on the cytokines they produce¹⁸. For instance, in humans, Tfh2 (Tfh cells producing IL-4) and Tfh17 (Tfh cells producing IL17A) cells can efficiently promote antibody responses in vitro much better than Tfh1 (Tfh cells producing IFN γ) cells¹⁹. However, frequencies of Tfh1 cells correlate with antibody responses during human influenza vaccination. In murine models, Tfh21 cells (Tfh cells that produce IL-21) seem to be specialized in promoting high affinity antibody responses in vivo²⁰. Recently, we and others discovered a new subset of Tfh cells called Tfh13 cells that produce IL-13 and control IgE responses^{13, 21}. Therefore, Tfh cells actually consist of a number of specialized (and potentially overlapping) subsets that can have unique functions in controlling antibody responses. However, inconsistencies in nomenclature, identification strategies (chemokine receptor, cytokine or transcription factor), gating strategies, and

technical approaches have led to substantial debate and confusion in the field as to the existence and functionality of some individual Tfh cell subsets. More in depth functional and transcriptomic studies need to be performed to determine which Tfh cell subsets have specialized functions, and which are a reflection of other factors such as activation state, microenvironment and age.

The role of Tfh cells in mediating human disease, including DSA and AbMR, is only beginning to be elucidated. Because of the relative accessibility of peripheral blood (compared to lymphoid tissue) in human patients, there has been great effort to correlate changes in circulating Tfh cell subset frequencies with disease progression in transplantation. The goals of these strategies are not only to provide conceptual proof that Tfh cells may have roles in AbMR, but also to identify biomarkers for disease. Recently, a number of studies have assessed Tfh subsets in the blood after solid organ transplant with the goal of identifying early biomarkers of DSA and AbMR. The presence of circulating PD-1+ Tfh cells precedes DSA levels in mouse heart transplant models suggesting that activated Tfh cells may be an early biomarker for AbMR²². However, in human liver and kidney transplantation, all circulating Tfh cell subsets seem to decrease after transplantation, likely due to the start of immunosuppression which affects Tfh cell populations^{23, 24}. Interestingly, in humans, Tfh2 and Tfh17, but not total Tfh, cells correlate with AbMR years after kidney transplantation²⁵. In newer studies, PD-1+, but not total, circulating Tfh cells correlate with DSA at 1 year post kidney transplantation²⁶. Therefore, some Tfh cell subsets show promise as biomarkers for early DSA and AbMR, such as activated Tfh cells. However, variability in CXCR5 staining and inconsistent gating of Tfh cell subsets are significant hurdles preventing the broad use of Tfh cell subsets as clinical biomarkers for AbMR. Nevertheless, these correlation studies also suggest that individual Tfh cell subsets may have specialized roles in mediating (or inhibiting) DSA and AbMR. However, as discussed above, changes in circulating Tfh cells in humans may provide only a glimpse of changes occurring in lymph nodes where DSA is thought to develop. As the relationship between lymph node, lymph-transiting, and circulating

Tfh cells becomes more defined transcriptionally and functionally, changes in circulating Tfh cells may better predict events occurring in lymph nodes^{6, 27}. Definitive mechanistic and functional studies will require the development of new tools to specifically track and perturb individual Tfh cell subsets in both lymphoid organs and the circulation during solid organ transplantation.

Recent studies have begun to assess the functional role of Tfh cells in solid organ transplant rejection using murine models. In a recent study, the authors adoptively transferred allo-specific WT or SAP (which is essential for Tfh and Tfr development) deficient T cells into lymphopenic mice followed by heart transplantation and found that T cells incapable of differentiating into Tfh cells caused less DSA and prolonged survival of heart transplants²⁸. These data point to a fundamental role for Tfh cells in controlling

DSA and AbMR. However, these experimental approaches do have limitations. For instance, these models likely eliminate immunoregulatory pathways (such as Tfr cells), T cell functionality may be superphysiological because of homeostatic expansion which occurs in

lymphopenic mice, and lack of a full TCR repertoire may alter the biology of the GC reaction and DSA responses. Therefore, more specific models need to be developed to specifically perturb Tfh cells in intact mice with full polyclonal repertoires to elucidate how and when Tfh cells regulate AbMR of solid organ transplants.

TFR cells in alloantibody responses

Since Tfh cells have been implicated in mediating DSA and AbMR in settings of solid organ transplantation in mice and humans, Tfr cells likely regulate these responses. In human kidney transplant patients, the frequency of circulating Tfr cells is substantially lower (and Tfh:Tfr ratio higher) in patients with AbMR, suggesting that Tfr cells may control DSA levels^{25, 29}. Moreover, circulating Tfr cell frequencies are lower in patients with chronic allograft dysfunction and biopsy proven rejection, suggesting Tfr cells can control pathogenic, and not just total, DSA²⁹. However, it is important to note that these studies are only correlative and rely on assessing circulating Tfr cells only. As with Tfh cells, circulating Tfr cells generally correlate with LN effector Tfr cell responses and may provide a glimpse into lymph node responses, but caution must be used since circulating Tfr cells have altered functionality and transcriptional program compared to effector lymph node Tfr cells in mice and humans^{6, 7}. Nevertheless, these studies provide rationale for the role of Tfr cells in controlling DSA and AbMR in settings of solid organ transplantation. To fully understand the role(s) of Tfr cells in controlling DSA and AbMR in solid organ transplant functional studies need to be performed. These include specific perturbation studies in intact mice (using either Tfr deficient or Tfr deleter mouse models). In addition, careful assessment of DSA needs to be performed to determine if altered antibody responses in the absence of Tfr cells is responsible for eliciting AbMR. Unfortunately, development of sensitive and standardized tools to quantify levels and functionality of DSA in murine systems are lagging behind the sophisticated tools already developed in human clinical settings. We hypothesize that Tfr cells control the formation of DSA and AbMR because of the potent role of Tfr cells in controlling autoantibody and foreign antibody responses. However, since Tfr cells may set thresholds on B cell responses, the role of Tfr cells may be more complex. For instance, the presence of suppressive Tfr cells may provide a high threshold for B cell responses resulting in lower titers of DSA with high affinity which may contribute to AbMR more so than higher titers of DSA with lower affinity. In addition, we recently described a small population of ex-Tfr cells which naturally downregulate FoxP3 resulting in altered functionality⁶. Without a fate marker allele, ex-Tfr cells phenotypically (but not transcriptionally) resemble Tfh cells and are therefore likely contained in traditional Tfh gating strategies. Although it has yet to be studied, ex-Tfr cells may also have roles in controlling DSA and AbMR. Studies need to be performed to address these issues in detail.

Additional Follicular T cell Subsets in alloantibodies

Tfh and Tfr cells are thought to be the dominant follicular T cell populations controlling antibody responses. However, a newly described population of CD8+CXCR5+ T follicular cytotoxic (Tfc) cells have been described that can gain access to the B cell follicle and modulate B cell responses in the context of viral infections³⁰. Using adoptive transfer techniques, a recent study has shown that CXCR5+CD8 T cells inhibit DSA levels in murine models, possibly through controlling GC B cell and Tfh cell populations³¹. Therefore Tfc

cells may regulate DSA. It is important to note that CXCR5 expression also identifies stem-like CD8 T cells with high replication potential which have roles beyond modulating B cell responses, such as target killing in tissues such as tumors. Therefore, in the context of solid organ transplantation, Tfc cells may have multifaceted roles and inhibit AbMR by controlling DSA, but promote TCMR by supplying cytotoxic cells in the graft. Untangling the interaction of Tfc, Tfh and Tfr cells will help delineate mechanisms by which the immune system controls DSA and AbMR and will provide a framework for new therapeutics that specifically control DSA and AbMR without the complications of broad immunosuppression.

Conclusion

Tfh and Tfr cells regulate antibody responses and have been strongly implicated in having fundamental roles in controlling DSA and AbMR in both mice and humans. However, more in depth studies need to be performed to fully elucidate the specific roles and mechanisms of how Tfh and Tfr cells control DSA and AbMR. These in depth studies will be essential for the development of both new early biomarkers to predict, and therapeutics to treat, AbMR during transplantation.

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Key Points:

- Antibody responses are controlling by Tfh and Tfr cells
- Tfh cells have recently been implicated as having fundamental roles in controlling DSA and AbMR.
- Tfr cells likely have roles in regulating DSA and AbMR but have not been studied in detail
- In depth functional studies are needed to fully determine the therapeutic potential of targeting Tfh and Tfr cells to prevent AbMR.