HHS Public Access

Author manuscript

J Immunol. Author manuscript; available in PMC 2022 June 15.

Published in final edited form as:

J Immunol. 2021 June 15; 206(12): 2775–2783. doi:10.4049/jimmunol.2100082.

CXCR5^{+}CD8^{+} T cells: A Review of their Antibody Regulatory Functions and Clinical Correlations

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Abstract

CD8^{+} T cells have conventionally been studied in relationship to pathogen or tumor clearance. Recent reports have identified novel functions of CXCR5^{+}CD8^{+} T cells that can home to lymphoid follicles, a key site of antibody production. In this review we provide an in-depth analysis of conflicting reports regarding the impact of CXCR5^{+}CD8^{+} T cells on antibody production and examine the data supporting a role for antibody-enhancement (B cell "helper") and antibody-downregulation (antibody-suppressor) by CXCR5^{+}CD8^{+} T cell subsets. CXCR5^{+}CD8^{+} T cell molecular phenotypes are associated with CD8-mediated effector functions including distinct subsets that regulate antibody responses. Co-inhibitory molecule PD-1, among others, distinguish CXCR5^{+}CD8^{+} T cell subsets. We also provide the first in-depth review of human CXCR5^{+}CD8^{+} T cells in the context of clinical outcomes and discuss the potential utility of monitoring the quantity of peripheral blood or tissue infiltrating CXCR5^{+}CD8^{+} T cells as a prognostic tool in multiple disease states.

Keywords

CXCR5^{+}CD8^{+}T cells; Antibody

Introduction:

A review of the subset of CXCR5^{+}CD8^{+} T cells must first acknowledge the abundance of work that identifies the critical role of CD8^{+} T cells in pathogen clearance. Cytotoxic type 1 CD8^{+} T cells (Tc1) that produce pro-inflammatory cytokines such as IFN-γ and TNF-α and are among the most well-defined CD8^{+} T cell subsets involved in clearance

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Disclosure: The authors have no financial conflicts of interest.

of intracellular pathogens and tumors. Tc1 cells kill target cells via release of cytotoxic molecules, such as perforin and granzyme, and comprise the majority of $CD8^+$ T cell subsets in the peripheral blood of healthy human blood donors (1). These cells are critical to the defense against intracellular bacteria, viruses, and protozoa, and clear immunogenic targets in a variety of conditions including autoimmune, tumor, transplant, and other inflammatory milieus. In addition to the Tc1 cell subset, other less-abundant $CD8^+$ T effector cell subsets include inducible IL-4- and IL-5-producing (Tc2; active in mediating allergy, asthma, as well as defense against helminths and venoms), IL-9-producing [Tc9; active in preventing $CD4^+$ T cell-mediated inflammation in the small intestine (2), exacerbating asthma and allergic inflammation in atopic dermatitis (3), and mediating anti-tumor effects (4)], IL-17-producing (Tc17; active in the defense against extracellular bacteria and fungi), and FoxP3-expressing regulatory (active in maintaining immune homeostasis) $CD8^+$ T cells (5, 6). Given the disparate effector functions and plasticity of $CD8^+$ T cell subsets, a more in-depth understanding of $CD8^+$ T cell subpopulations is critical to their phenotypic and functional classification and to determination of their potential clinical utility in monitoring, preventing, and/or treating immune mediated disease.

Recent work in various models and disease states has identified unique subsets of $CD8^+$ T cells expressing the chemokine receptor CXCR5, which directs these cells into secondary lymphoid follicles (7). Within these secondary follicles, $CXCR5^+CD8^+$ T cells may encounter a rich milieu of antigens, antigen presenting cells, and other follicular cells, and are reported to display a range of effector functions in distinct viral, tumor, and autoimmune settings. The bulk of literature to date has focused on $CXCR5^+CD8^+$ T cell-mediated clearance of viruses that have predilection for sequestration in lymphoid follicles, including HIV/SIV (8–17) and LCMV (18–20), as well as prognostic associations of $CXCR5^+CD8^+$ T cells detected in tumors and draining lymph nodes with improved clinical outcomes for malignancies such as colorectal (21, 22) and hepatocellular carcinoma (23, 24).

This review builds upon prior analyses of $CXCR5^+CD8^+$ T cells that have examined their role in viral immunity, autoimmunity and tumor immunity (7, 25–27), by focusing on this subset's divergent roles on humoral immunity and by categorizing molecular phenotypes reported for subsets with distinct effector functions. While the full spectrum of $CXCR5^+CD8^+$ T cell function is included, we focus on an analysis of the data supporting a role for $CXCR5^+CD8^+$ T cell subsets on enhancement of antibody production (B cell "helper" function) versus downregulation of antibody-production (antibody-suppressor). We also provide an in-depth analysis of $CXCR5^+CD8^+$ T cells in a variety of clinical conditions (including viral or bacterial infection, malignancy, autoimmunity, and transplantation) and discuss their potential clinical utility.

$CXCR5^+CD8^+$ T Cells that Enhance Antibody Production

Similar to $CXCR5^+PD\text{-}1^{hi}CD4^+$ T follicular helper cells (T_{FH}) that support antibody production (28, 29), $CXCR5^+PD\text{-}1^{hi}CD8^+$ T subsets have also been reported to exhibit a B cell helper function. $CXCR5^+CD8^+$ T cells in various disease states and models, ranging from colorectal malignancy to viral hepatitis to LCMV infection, have been reported to

enhance antibody production by 1) direct interaction with B cells in co-culture (23, 30–37), or 2) synergistic interactions with $CD4^+$ T cells and B cells in co-culture (38, 39).

In the first such study to examine $CXCR5^+CD8^+$ T cell enhancement of antibody through direct interaction with B cells, *Quigley et al.* demonstrated that $CXCR5^+CD8^+$ T cells from human tonsillectomy specimens increase both the survival and non-specific IgG production of $CD19^+$ B cells when co-cultured for seven days. While specific cellular mechanisms of B cell help were not explored in this study, it was noted that *in vitro* stimulated $CXCR5^+CD8^+$ T cells exhibited upregulated expression of molecules necessary for T cell-dependent humoral immune responses (32), including CD70 (40), OX40 (41), and ICOS (42). The capacity of human $CXCR5^+CD8^+$ T cells to upregulate antibody production nearly 2-fold or greater in culture has been reported in a number of other studies utilizing human-derived cells from patients with various diseases including chronic hepatitis B infection (33), classical Hodgkin lymphoma (34), nasal polyps (35), and hepatocellular carcinoma (23). Since then, several groups have identified $CXCR5^+CD8^+$ T cells as direct B cell helpers in murine models.

In an IL-2 KO murine model of autoimmunity (autoimmune hemolytic anemia) that develops due to the absence of $CD4^+$ T regulatory cells (T_{Reg}), the authors observed an increase in $CXCR5^+PD\text{-}1^{hi}CD4^+$ T cells and $CXCR5^+PD\text{-}1^{hi}CD8^+$ T cells in lymphoid tissue. Both subsets upregulated ICOS and Bcl6, known to be important for interactions between $CD4^+$ T_{FH} and follicular B cells (38). Depletion of either $CD4^+$ T cells or $CD8^+$ T cells resulted in increased survival, improved anemia, decreased frequency of B cells, and decreased IgG autoantibody binding of erythrocytes. Interestingly, the reduction of IgG was predominantly for IgG1 (IL-4 dependent) for $CD4^+$ T cell depletion and both IgG1 and IgG3 (IFN-γ-dependent) for $CD8^+$ T cell depletion, suggesting some differences in CD4- and CD8-mediated enhancement of autoantibody production in this model. Furthermore, supernatant from *in vitro* stimulated $CXCR5^+PD\text{-}1^{hi}CD8^+$ T cells significantly upregulated IgG production by *in vitro* stimulated (anti-CD40, anti-IgM) wild type B cells. Notably, these $CD8^+$ T cells supported IgG production to levels comparable to those observed with supernatants from $CD4^+$ T_{FH} cells (38). In contrast to these studies, co-cultures consisting of B cells and $CXCR5^+PD\text{-}1^+CD8^+$ T cells from LCMV-infected wild type mice resulted in only mild increases in the percentage of $IgG1^+$ B cells and *in vitro* production of IgG1 in the supernatant compared to B cells cultured alone (and significantly less than co-cultures of B cells with $CXCR5^+PD\text{-}1^+CD4^+$ T_{FH} cells) (31).

In Bcl6 KO mice, that have functional B cells but abrogated endogenous $CD4^+$ T_{FH} cell responses, the adoptive transfer of Runx3-deficient transgenic (LCMV GP33/D^b-reactive) P14 $CD8^+$ T cells enriched for $CXCR5^+CD8^+$ T cells promoted antibody production in response to KLH-GP33 immunization (but to half the extent when compared to adoptive transfer of $CD4^+$ T_{FH} cells) (30). However, the increase in IgG antibody production occurred in the absence of germinal center (GC) formation, suggesting an extrafollicular mechanism of B cell help. This observation is consistent with studies in the IL-2 KO mouse model, where adoptive transfer of IL-2 KO $CD8^+$ T cells (enriched for $CXCR5^+PD\text{-}1^{hi}CD8^+$ T cells) into TCRα KO mice resulted in increased plasma cells but not GC B cells. In contrast,

adoptive transfer of $CD4^+$ T_{FH} cells resulted in expansion of both GC B cells and plasma cells (30).

In these murine and human studies, antibody-enhancing $CXCR5^+CD8^+$ T cells have routinely been reported to express IL-21, CD40L, ICOS, PD-1, and Bcl-6, though not necessarily to the extent expressed by $CD4^+$ T_{FH} cells. When comparing $CXCR5^+CD8^+$ T cells isolated from peritumoral tissue versus peripheral blood, hepatocellular carcinoma-infiltrating $CXCR5^+CD8^+$ T cells were noted to express higher levels of IL-21, ICOS, and PD-1, illustrating the heterogeneity of $CXCR5^+CD8^+$ T cell subsets' phenotypic expression and function not only between various disease states, but even in different immune locales within the same patient (23).

A few studies have investigated the specific cellular mechanisms by which $CXCR5^+CD8^+$ T cell-mediated upregulation of antibody production occurs. $CXCR5^+CD8^+$ T cells isolated from human tonsillectomy specimens and co-cultured with $CD19^+$ B cells alone significantly increased levels of IgG, IgM, and IgA in a dose-dependent manner, albeit not to the level of $CD4^+$ T cell:B cell co-cultures (36). Interestingly, the addition of anti-IL-21 or anti-CD40L to $CXCR5^+CD8^+$ T:B cell co-cultures reduced production of IgG and IgM by 40–60%, supporting a contact-dependent (CD40L-dependent) and soluble factor-mediated (IL-21-dependent) mechanism of antibody upregulation by these cells (36). The importance of CD40/CD40L interactions in $CXCR5^+CD8^+$ T cell-mediated help to B cells has also been reported in an LCMV mouse model, where addition of anti-CD40L neutralizing antibody to $CXCR5^+PD\text{-}1^+CD8^+$ T:B cell cultures, or co-culture with CD40-deficient B cells, abrogated IgG1 production similar to levels observed in cultures of B cells alone (31). In this study, contact dependent B cell help for *in vitro* (and *in vivo*) antibody production provided by $CXCR5^+PD\text{-}1^+CD8^+$ T cells was further supported by the requirement for MHC-I matching between $CD8^+$ T cells and B cells.

In contrast to the preceding data suggesting provision of direct help to B cells by $CXCR5^+PD\text{-}1^+CD8^+$ T cells, two groups have demonstrated $CD8^+$ T cell-mediated help to B cells through synergistic interactions with $CD4^+$ T_{FH} cells to enhance antibody production (38, 39). In one study, when $CXCR5^+CD8^+$ T cells from human patients (with hepatitis B) were incubated with either $CD19^+CD27^-$ naïve B cells or $CD19^+CD27^+$ memory B cells alone (stimulated with staphylococcal enterotoxin B), they failed to upregulate *in vitro* antibody production (despite expressing IL-21, a cytokine known to induce B cell antibody production). However, addition of the $CXCR5^+CD8^+$ T cells to $CXCR5^+CD4^+$ T:B cell co-culture elicited significantly increased IgG antibody production (~2-fold) compared to $CXCR5^+CD4^+$ T:B cell co-culture alone (39). In TCRα KO mice immunized with keyhole limpet hemocyanin (KLH), followed by adoptive transfer of IL-2 KO $CD8^+$ T cells ($CXCR5^+PD\text{-}1^{hi}Bcl\text{-}6^+IL\text{-}21^+CD8^+$ T cells) alone, was associated with increased frequency of $B220^{int}CD138^+$ plasma cells but not $B220^+GL\text{-}7^+Fas^+$ GC B cells in recipient spleens, and did not promote IgG production. However, when IL-2 KO $CD8^+$ T cells were transferred along with IL-2 KO $CD4^+$ T_{FH} cells, a synergistic effect resulting in increased IgG2b titer (to a greater extent than IgG1 and no effect on IgG2a titer) was observed (38). The results of these studies suggest that $CXCR5^+CD8^+$T cell subsets under some circumstances may act

synergistically with $CD4^+$ T_{FH} cells (rather than through independent stimulation of B cells) to enhance antibody production.

It remains elusive whether antigen immunogenicity, genetic, and/or acquired immune conditions or immune locales play a key role in driving $CXCR5^+CD8^+$ T cell subsets' differentiation, effector function, and capacity to influence antibody production. Phenotypic markers, cytokines, effector molecules, and transcription factors characterizing $CXCR5^+CD8^+$ T cell subsets investigated in specific models are shown in Table 1. Most $CXCR5^+CD8^+$ T cell subsets that are reported to enhance humoral immunity or regulate viral, tumor, and autoimmunity express some similar transcription factors, co-inhibitory molecules, co-stimulation molecules, cytokines, and effector molecules. In contrast, antibody-suppressor $CXCR5^+CD8^+$ T cells identified in transplantation models are distinguished from all of these other subsets by the absence of the co-inhibitory molecule PD-1, absence of the co-stimulatory molecules ICOS/ICOSL, and lack of production of IL-10 or IL-21 (43). Furthermore, antibody-suppressor $CXCR5^+CD8^+$ T cells are distinguished from $CD8^+$ T regulatory cells (44–49) by their expression of IFN-γ, perforin/granzyme, and CD28 and the lack of expression of PD-1, IL-10, and the transcription factor FoxP3 (Table 1). Further investigation of $CXCR5^+CD8^+$ T cells with complex immunophenotyping may improve the categorization of these heterogeneous subsets, clarify mechanisms of action, and implicate lineage relationships.

$CXCR5^+CD8^+$ T Cells that Mediate Downregulation of Antibody Production

In contrast to the literature supporting an antibody enhancing function of $CXCR5^+CD8^+$ T cells, our research group has identified a novel subset of antibody-suppressor $CXCR5^+IFN\text{-}\gamma^+CD8^+$ T cells ($CD8^+$ $T_{Ab\text{-}supp}$ cells) using *in vivo* transplant experimental murine models. We have reported that this subset suppresses *in vivo* alloantibody production 5- and 10-fold following allogeneic kidney (50) and hepatocyte transplant (51), respectively. This reduction in alloantibody formation is associated with significant enhancement of graft survival following $CD8^+$ $T_{Ab\text{-}supp}$ adoptive cellular therapy in both kidney (15 days to 52 days) (50) and hepatocyte (14 days to 35 days) (51) transplant recipients using models in which graft loss occurs by antibody-mediated rejection. Alloantibody suppression is mediated, in part, by CD8-mediated clearance of antibody-producing B cells through both FasL and perforin mechanisms (52). This cytotoxic clearance is antigen-specific, as $CD8^+$ $T_{Ab\text{-}supp}$ cells do not kill naïve or third-party primed IgG^+ B cells *in vitro* or in *vivo* (51, 52). Recently, our group has identified $CXCR5^+CD8^+$ T cells in the peripheral blood of human kidney transplant recipients. The quantity of both $CXCR5^+CD8^+$ and $CXCR5^+IFN\text{-}\gamma^+CD8^+$ T cells inversely associates with the incidence of *de novo* donor-specific antibody (53), suggesting a human correlate of murine $CD8^+$ $T_{Ab\text{-}supp}$ cells. In experimental murine models, we found that IFN-γ is critical to antibody-suppressor $CD8^+$ T cell function (54), since $CD8^+$ T cells isolated from IFN-γ KO mice do not develop antibody-suppressor function. Approximately 70% of $CD44^+CXCR5^+CD8^+$ T cells in both murine (51) and human (53) transplant recipients express IFN-γ. The $CD8^+$ $T_{Ab\text{-}supp}$ cells that our group has investigated lack expression of PD-1, ICOS/ICOSL, and $CD8^+$ T regulatory cell ($CD8^+$ T_{Reg}) markers IL-10, CD103, and FoxP3 (51), and thus represent a subset of $CXCR5^+CD8^+$ T cells that are distinct from $CD8^+$ T_{Reg} cells (44–49) as well as $PD\text{-}1^+CXCR5^+CD8^+$ T cell subsets with, antiviral,

anti-tumor, anti-autoimmune (Qa-1 restricted) and antibody-enhancing functions (Table 1). Furthermore, while antibody suppressor $CXCR5^+CD8^+$ T cells are antigen-specific, the antigen specificity of the $CXCR5^+PD\text{-}1^{hi}CD8^+$ T cells that enhance antibody production has not been tested. Collectively, these findings raise the prospect of further refining and developing this $CD8^+$ T cell subset as a cellular therapy for the prevention or treatment of antibody mediated rejection.

Another subset with antibody suppressive function, identified as $CD3^+CD8^+CXCR5^{hi}CD44^{hi}$ T "follicular regulatory" (T_{FR}) cells, has been reported to inhibit *ex vivo* $CD4^+$ T_{FH} IL-21 production and GC B cell (CPG-TLR9 stimulated $CD19^+CD38^+$) IgG production in the setting of HIV infection (55). These $CD8^+$ T_{FR} cells exhibit enhanced expression of IL-10, as well as Tim-3, CD122, and GITR, but reduced expression of perforin compared to conventional $CD8^+$ T cells. Many other markers shown in Table 1 have not been reported for this subset. $CD8^+$ T_{FR} cell suppression of $CD4^+$ T_{FH} IL-21 production in culture was Tim-3 dependent, since addition of anti-Tim3 antibody abrogated the effect. Virus-primed tonsillar $CD8^+$ T_{FR} cells reduced IgG production in co-culture greater than 3-fold when compared to IgG production by virus infected GC B cells alone (55). However, the exact mechanism of human $CXCR5^+CD8^+$ T cell-mediated inhibition of antibody production in these studies, whether via cytokine-facilitated suppression or direct cytotoxicity to B cells, was not explored. $CD8^+$ T_{FR} cells are distinguished from $CD8^+$ $T_{Ab\text{-}supp}$ cells by expression of IL-10.

An additional subset of antibody downregulating $CXCR5^+CD8^+$ T cells has been identified in the setting of murine autoimmunity models and is characterized by the triad of CD44, CD122, and Ly49 (a MHC-I inhibitory receptor) surface marker expression (56). These cells express ICOSL, PD-1, and are restricted to interaction with $Qa\text{-}1^+CD4^+$ T cells and protect against autoimmunity (many other markers shown in Table 1 have not been reported for this subset). Qa-1, a murine homolog of HLA-E, is expressed on activated T and B lymphocytes and dendritic cells, and promotes expansion of antigen-specific $CD8^+$ T cells when bound to their TCR (57). Adoptive transfer of Qa-1-restricted effector $CD8^+$ T cells ($CD44^+CD122^+Ly49^+$) into $Rag2^{-/-}$ hosts reconstituted with $Qa\text{-}1^+$ B and $Qa\text{-}1^+CD4^+$ T cells inhibits primary autoantibody responses by 10-fold (58). In the setting of rheumatoid arthritis, cellular therapy with Qa-1-restricted effector $CD8^+$ T cells resulted in clearance of autoreactive $CD4^+Qa\text{-}1^+$ T_{FH} cells, suppression of downstream autoantibody, and amelioration of disease progression (as evidenced by assessment score of limb swelling and erythema) (56). Recently, $CXCR5^+CD44^+CD122^+Ly49^+CD8^+$ Tregs were reported to mediate key protection against lethal autoimmunity that is distinct from $CD4^+$ T_{Reg} cells (59). In this study, genetic deletion of TGF-β receptor (TGF-βR) and the transcription factor Eomesodermin (Eomes) resulted in enhanced spontaneous GCs in the spleen, increased percentage of GC B cells, increased $CD4^+$ T_{FH} cells, increased anti-double stranded DNA autoantibodies, and reduced overall survival. These double knockout (DKO) mice had unaltered $CD4^+$ T_{FR} cells but significantly decreased quantity of $CXCR5^+CD44^+CD122^+Ly49^+CD8^+Helios^+$ Tregs, and only adoptive transfer of the latter $CD8^+$ Treg population could rescue the autoimmune phenotype. $CD122^{hi}Ly49^{hi}CD8^+$ T cells from DKO mice displayed increased effector molecules and markers such as granzyme A, granzyme B, and KLRG-1, consistent with their lower expression of the transcription

factor Helios that is associated with T_{Reg} identity. Furthermore, these authors reported that while TGF-βR is critical for expression of the transcription factor Helios and $CD8^+$ T_{Reg} development, the transcription factor Eomes regulates the expression of CXCR5 critical for follicular localization and GC regulation. However, both TGF-βR and Eomes contribute to expression of pro-survival Bcl-2 and *in vivo* $CD8^+$ T_{Reg} cell survival and homeostasis. Interestingly, *Choi et al* reported that host mice with a mutation impairing Qa1 interaction with the $CD8^+$ T cell receptor (60), resulted in robust alloantibody response after full MHC disparate heart transplant and accelerated rejection despite peri-transplant treatment with CTLA-4 Ig, owing to disruption of Qa-1 restricted $CD8^+$ T_{Reg} cell inhibition of $CD4^+$ T_{FH} expansion (61). In contrast, $CD8^+$ $T_{Ab\text{-}supp}$ cells that also significantly suppress alloantibody production after transplant express KLRG-1 (associated with effector cell function) and mediate antigen specific antibody suppression, features that distinguish them from KLRG-1-negative Qa-1-restricted $CD8^+$ T follicular regulatory cells ($CD8^+$ T_{FR}) that downregulate excessive or dysregulated $Qa1^+$-expressing $CD4^+$ T_{FH} cells (59). Furthermore, while these Qa-1-restricted $CD8^+$ T_{FR} have been reported to eliminate target $Qa\text{-}1^+CD4^+$ T_{FH} cells in a perforin-dependent manner, their inhibitory effect on antibody production does not occur through direct interaction with B cells. Thus, while Qa-1-restricted $CD8^+$ T_{FR} have capacity to inhibit antibody production, their exclusive targeting of $CD4^+$ T_{FH} cells is decidedly different from B cell helper $CXCR5^+PD\text{-}1^{hi}CD8^+$ T cells and antibody-suppressor $CXCR5^+PD\text{-}1^-CD8^+$ T cells that exert direct effects on B cells (Figure 1).

Clinical Correlations of Human $CXCR5^+CD8^+$ T Cells

Given the identification of $CXCR5^+CD8^+$ T cells in a wide array of both clinical diseases and murine models, their potential to serve as indicators of disease severity and/or as useful predictive tools warrants consideration. Table 2 provides a comprehensive listing of all reports of human $CXCR5^+CD8^+$ T cells and their clinical correlations in patients with viral infection, bacterial infection, malignancy, autoimmune disease or after kidney transplantation. Owing to their unique $CD8^+$ T cell follicular homing capabilities, several groups have noted a direct correlation between the quantity of $CXCR5^+CD8^+$ T cells with the suppression of viral load for viruses known to sequester in lymphoid follicles, such as HIV and SIV (8, 14, 18, 33, 62, 63). $CXCR5^+CD8^+$ T cell quantity is increased in HIV infected patients compared to healthy controls and their frequency is inversely correlated with HIV viral load (8). In addition, $CXCR5^+CD8^+$ T cell quantity has been introduced as an indicator of disease severity since among HIV infected patients, those with lower frequencies of circulating $CXCR5^+CD8^+$ T cells also have lower $CD4^+$ T cell counts (8). None of the aforementioned studies reported concurrent anti-viral antibody titers. However, *Roider et al*. identified a significant positive correlation between the frequency of circulating $CXCR5^+CD8^+$ T cells and HIV neutralization by plasma from HIV infected and antiretroviral therapy naïve children who reportedly have higher titers of broadly neutralizing antibody compared to adults (64). Perinatally infected HIV-positive children exhibit significantly lower $CXCR5^+CD8^+$T cell frequencies compared to HIV-negative unexposed children (65).

Qiu et al. noted, in a study of 36 patients infected with Dengue virus 2 (DENV2), that higher quantity of peripheral blood $PD\text{-}1^+CXCR5^+CD8^+$ T cells correlated with decreased

viral load at time of diagnosis. In addition, their quantity was protective for virally mediated kidney injury (a common complication of DENV2 infection), as patients without kidney injury exhibited significantly higher quantities of $CXCR5^+CD8^+$ T cells compared to those with kidney injury (66). These results were not correlated with anti-viral antibody responses.

In a study of 65 patients hospitalized for bacterial pneumonia, *Shen et al.* found that quantity of peripheral blood $CXCR5^+CD8^+$ T cells correlated with the severity of community-acquired and hospital-acquired pneumonia, noting a nearly 3-fold or greater increase in circulating $CXCR5^+CD8^+$ T cells during community-acquired and hospital-acquired pneumonia exacerbations, respectively, compared to patients with controlled disease. Furthermore, in assessing the diagnostic correlation associated with monitoring of these cells, the AUC for the ratio of $CXCR5^+CD8^+$ T/$CD8^+$ T cells was determined to be 0.944 (Sensitivity = 90.5%; Specificity = 93%), leading the authors to suggest that CXCR5-expressing $CD8^+$ T cell quantity alone can predict exacerbation of community- or hospital-acquired pneumonia (67). This direct correlation with disease severity suggests a deleterious pro-inflammatory immunity or an inhibitory impact of these cells on protective immunity. However, the correlation of $CXCR5^+CD8^+$ T cell quantity with the strength of humoral immunity to specific pathogens was not assessed in these studies.

The prevalence and phenotypic characteristics of $CXCR5^+CD8^+$ T cell subsets also correlate with patient outcomes in non-viral disease states, such as with malignancy. In patients with hepatocellular carcinoma (HCC), a high percentage of tumor-infiltrating $CXCR5^+CD8^+$ T cells correlated with a lower risk of early recurrence (within three years) and metastasis (23). Interestingly, the authors of this study also noted a co-localization of $IL\text{-}21^+CXCR5^+CD8^+$ T cells and $CD19^+$ B cells in some patients and a positive correlation between the frequency of tumor infiltrating $CXCR5^+CD8^+$ T cells and tumor-infiltrating $CD19^+$ B cells. Furthermore, co-culture of tumor-infiltrating $CXCR5^+CD8^+$ T cells with autologous $CD19^+$ B cells resulted in significantly higher *in vitro* IgG production than co-culture of $CXCR5^+CD8^+$ T cells and B cells from healthy donors. The frequency of tumor-infiltrating $CD138^+$ plasmablasts in these HCC patients also positively correlated with overall and disease-free survival. However, these authors did not investigate tumor-antigen specific antibody production in these patients.

In a study of twelve patients with pancreatic cancer, high quantities of tumor-infiltrating $CXCR5^+CD8^+$ T cells (at the time of tumor resection) correlated with prolonged disease free survival time (68). $CXCR5^+CD8^+$ T cells are a small population of total $CD8^+$ T cells in the peripheral blood of healthy controls (0.4–5.0%). However, in pancreatic cancer patients, circulating $CXCR5^+CD8^+$ T cells were significantly increased and comprised 7–21% of total peripheral $CD8^+$ T cells. Furthermore, $CXCR5^+CD8^+$ T cells comprised more than 50% of total $CD8^+$ T cells in pancreatic tumor specimens. $CXCR5^+CD8^+$ T cells in this study were $PD\text{-}1^+Tim\text{-}3^+$ and mediated higher *in vitro* cytotoxicity to target cells compared to $CXCR5^-CD8^+$ T cells. Interestingly, these investigators utilized autologous human B cells as targets in these *in vitro* cytotoxicity assays which is analogous to the CD8:B cell cytotoxicity assays used in our murine studies to investigate $CD8^+_{Ab\text{-}supp}$ T cell mediated B cell killing.

In patients with bladder cancer, high quantities of tumor-infiltrating $CXCR5^+CD8^+$ T cells also correlated with higher overall and disease-free survival (69). In another study involving 44 patients with Stage II colorectal cancer, patients with high quantity of $CXCR5^+CD8^+$ T cells in tumors or tumor-draining lymph node and patients with high IFN-γ, perforin, and granzyme B expression by tumor $CXCR5^+CD8^+$ T cells exhibited significantly longer disease-free survival (22). Taken together, these studies highlight an important positive correlation between quantity of $CXCR5^+CD8^+$ T cells detected in tumors and/or tumor draining lymph nodes with enhanced tumor immunity and raises the potential for including quantitative assessment of tumor-infiltration by $CXCR5^+CD8^+$ T cells as an additional prognostic tool for some malignancies. More studies are warranted to investigate the cellular and humoral immune mechanism(s) associated with these improved clinical outcomes.

One study investigated $CXCR5^+CD8^+$ T cells in human follicular lymphoma, a non-Hodgkin lymphoma that arises from malignant GC B cells (70). The authors found that $CXCR5^+CD8^+$ T cells were more abundant in follicular lymphoma compared to normal human tonsillar tissue. Co-culture of autologous flow-sorted $CXCR5^+CD8^+$ T cells (expanded *in vitro* with α−CD3/CD28, TGF-β and IL-23) with autologous follicular lymphoma tumor cells (activated with soluble CD40L and IL-4) revealed *in vitro* tumor killing. They also determined that autologous $CXCR5^+CD8^+$ T cells suppressed $CD4^+$ T_{FH} mediated differentiation of $CD38^+CD19^+$ plasmablasts (stimulated with staphylococcal enterotoxin B) in a cell contact independent mechanism. Thus, while this study focused on $CXCR5^+CD8^+$ T cell-mediated cytotoxic killing of tumor cells, these tumor cells were malignant GC B cells, and is consistent with the $CD8^+$ $T_{Ab\text{-}supp}$ cells that our group has reported has the functional capacity to kill alloprimed B cells and reduce alloantibody production after transplant in mice (51).

A recent study reported the critical roles of TFG-β receptor and transcription factor Eomes on development, homeostasis and survival of $Ly49^+CD122^{hi}CXCR5^+CD8^+$ Tregs that control GC reactions and mediate protection against lethal autoimmunity in mice. These authors also analyzed peripheral blood mononuclear cells (PBMCs) from patients with the autoimmune disease systemic lupus erythematosus (SLE) and found that SLE patients compared to healthy controls had reduced quantity of $CD158e^+CD8^+$ T cells with reduced expression of Helios, a key Treg associated transcription actor. Corresponding autoantibody titers in these SLE patients were not reported (59). The CD158 gene family encodes killer cell immunoglobulin-like receptors (KIR). In these studies, $CD158e^+CD8^+$ T cells were analyzed based on their presumed similarity to human $CD122^{hi}KIR^+Eomes^+CD8^+$ T cells described in other studies (71) and resemblance to murine anti-autoimmune Qa-1-restricted $Ly49^+CD122^{hi}CD8^+$ T cells.

In a prospective study of 95 first-time kidney transplant recipients, our group demonstrated an inverse association between the quantity of peripheral blood $CXCR5^+CD8^+$ T cells (and $CXCR5^+IFN\text{-}\gamma^+CD8^+$ T cells) and risk for development of *de novo* donor-specific antibody (DSA) after transplant. Transplant recipients who developed *de novo* DSA exhibited 2-fold lower quantity of $CXCR5^+CD8^+$ T cells (and $CXCR5^+IFN\text{-}\gamma^+CD8^+$ T cells) both pre-transplant as well as post-transplant compared to recipients who did not develop DSA. Furthermore, pre-transplant quantity of $CXCR5^+IFN\text{-}\gamma^+CD8^+$ T cells less than 3,300 per

million peripheral blood mononuclear cells was highly associated with the development of *de novo* DSA [Area Under the Curve (AUC) = 0.81; Sensitivity = 93%; Specificity = 62%] (53). This pre-transplant immune assessment suggests that prospective kidney transplant recipients could be stratified into groups that are low or high risk for *de novo* DSA production. This is the only prospective study reporting a direct correlation between the quantity of human $CXCR5^+CD8^+$ T cells with *in vivo* antibody responses.

While the clinical utility of monitoring $CXCR5^+CD8^+$ T cells is promising, several potential barriers exist to their routine use in assessing clinical disease or for their development as a cellular therapy. First, the relatively low percentage of $CXCR5^+CD8^+$ T cells, comprising 1.9% of all peripheral blood mononuclear cells (PBMC) in healthy individuals (32) and ranging from less than 1% of all PBMC (22, 37) to 5% of $CD3^+$ T cells even in active disease (68, 72) renders the isolation, consistent quantification, and characterization of these cells challenging. Second, although expression of CXCR5 differentiates a subset of effector $CD8^+$ T cells in various models and diseases, its expression in peripheral blood lymphocytes may fluctuate over time as $CXCR5^+CD8^+$ T cells traffic in and out of the circulation from their functional niches in lymphoid follicles and other sites. More research is needed to understand how the quantity of peripheral blood $CXCR5^+CD8^+$ T cells correlates with their quantity and differentiated phenotype in lymphoid depots or other tissues. Third, the marked phenotypic heterogeneity of $CXCR5^+CD8^+$ T cell subsets, as highlighted in this review, further complicates the categorization and separation of disease related $CXCR5^+CD8^+$ T cell subsets by effector (including regulatory) functions. Finally, factors that contribute to expansion of the various $CXCR5^+CD8^+$ T cell subsets (*in vivo* or *in vitro*) require further investigation. Despite these and other challenges ahead, significant potential exists to enhance existing knowledge and to develop the prognostic and therapeutic potential of $CXCR5^+CD8^+$ T cells. Promising results from murine models in which $CXCR5^+CD8^+$ T cells tested as a cellular therapy reduced autoantibody (56, 58) or alloantibody (50, 51) levels and associated antibody-mediated tissue damage encourage the clinical translation of these studies. This review highlights the need for further studies to elucidate the biology of this newly emerging subset including its role in modulating antibody responses in a variety of clinical conditions, including transplantation.

Conclusions:

Emerging interest in immune properties of $CXCR5^+CD8^+$ T cells has led to investigation of their role in a variety of experimental and clinical settings in the past few years. These cells, owing to their follicular-homing capabilities unique amongst known $CD8^+$ T cell subsets, participate in a wide variety of functions in lymphoid-associated viral, tumor, auto- and allo-immune disease processes. $CXCR5^+CD8^+$ T cell subsets in some studies have been reported to enhance antibody production, while in others have been reported to downregulate antibody production. It is likely then that phenotypic and functional differentiation of distinct $CXCR5^+CD8^+$ T cell subsets account for these differences. It remains to be determined how the diverse phenotypes and effector functions reported to date for $CXCR5^+CD8^+$ T cells correlate with their lineage, differentiation, and plasticity. Knowledge gained from these future studies is critical to refine the application of $CXCR5^+CD8^+$ T cell subsets for potential diagnostic, prognostic, and therapeutic purposes.

Acknowledgements:

BioRender was used to generate Figure 1.

Support:

This work was supported by The Ohio State University Department of Surgery, Division of Transplant Surgery, Comprehensive Transplant Center, The Ohio State University College of Medicine, and the National Institutes of Health grant AI139913. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

Nonstandard Abbreviations:

Ab	Antibody
DSA	Donor specific antibody
GC	Germinal center
Tc1	Cytotoxic type 1 $CD8^+$ T cell
T_{FH}	Follicular helper $CD4^+$ T cell
T_{FR}	Follicular regulatory $CD8^+$ T cell

References:

1. Amancha PK, Ackerley CG, Duphare C, Lee M, Hu YJ, Amara RR, and Kelley CF. 2019. Distribution of Functional CD4 and CD8 T cell Subsets in Blood and Rectal Mucosal Tissues. Sci. Rep9: 6951. [PubMed: 31061442]
2. Chang SY, Song JH, Guleng B, Cotoner CA, Arihiro S, Zhao Y, Chiang HS, O'Keeffe M, Liao G, Karp CL, Kweon MN, Sharpe AH, Bhan A, Terhorst C, and Reinecker HC. 2013. Circulatory antigen processing by mucosal dendritic cells controls CD8(+) T cell activation. Immunity. 38: 153–165. [PubMed: 23246312]
3. Visekruna A, Ritter J, Scholz T, Campos L, Guralnik A, Poncette L, Raifer H, Hagner S, Garn H, Staudt V, Bopp T, Reuter S, Taube C, Loser K, and Huber M. 2013. Tc9 cells, a new subset of CD8(+) T cells, support Th2-mediated airway inflammation. Eur. J. Immunol43: 606–618. [PubMed: 23254356]
4. Lu Y, Hong B, Li H, Zheng Y, Zhang M, Wang S, Qian J, and Yi Q. 2014. Tumor-specific IL-9-producing CD8+ Tc9 cells are superior effector than type-I cytotoxic Tc1 cells for adoptive immunotherapy of cancers. Proc. Natl. Acad. Sci. USA111: 2265–2270. [PubMed: 24469818]
5. Mittrucker HW, Visekruna A, and Huber M. 2014. Heterogeneity in the differentiation and function of CD8(+) T cells. Arch. Immunol. Ther. Exp. (Warsz)62: 449–458. [PubMed: 24879097]
6. Annunziato F, Romagnani C, and Romagnani S. 2015. The 3 major types of innate and adaptive cell-mediated effector immunity. J. Allergy Clin. Immunol135: 626–635. [PubMed: 25528359]
7. Fousteri G, and Kuka M. 2020. The elusive identity of CXCR5(+) CD8 T cells in viral infection and autoimmunity: Cytotoxic, regulatory, or helper cells?Mol. Immunol119: 101–105. [PubMed: 32007752]
8. Jiao YM, Yang HG, Huang HH, Tu B, Xing SJ, Mao L, Xia W, He R, Zhang JY, Xu RN, Jin L, Shi M, Xu Z, Qin EQ, Wang XC, Wu H, Ye L, and Wang FS. 2017. Dichotomous Roles of Programmed Cell Death 1 on HIV-Specific CXCR5(+) and CXCR5(–) CD8(+) T Cells during Chronic HIV Infection. Front. Immunol8: 1786. [PubMed: 29312314]
9. Leong YA, Chen Y, Ong HS, Wu D, Man K, Deleage C, Minnich M, Meckiff BJ, Wei Y, Hou Z, Zotos D, Fenix KA, Atnerkar A, Preston S, Chipman JG, Beilman GJ, Allison CC, Sun L, Wang P, Xu J, Toe JG, Lu HK, Tao Y, Palendira U, Dent AL, Landay AL, Pellegrini M, Comerford I, McColl SR, Schacker TW, Long HM, Estes JD, Busslinger M, Belz GT, Lewin SR, Kallies A, and

Yu D. 2016. CXCR5(+) follicular cytotoxic T cells control viral infection in B cell follicles. Nat. Immunol17: 1187–1196. [PubMed: 27487330]

10. Yang HG, Jiao YM, Huang HH, Zhang C, Zhang JY, Xu RN, Song JW, Fan X, Jin L, Shi M, and Wang FS. 2020. Transforming growth factor-beta promotes the function of HIV-specific CXCR5(+) CD8 T cells. Microbiol. Immunol64: 458–468. [PubMed: 32221997]
11. Velu V, Mylvaganam G, Ibegbu C, and Amara RR. 2018. Tfh1 Cells in Germinal Centers During Chronic HIV/SIV Infection. Front. Immunol9: 1272. [PubMed: 29928280]
12. Perdomo-Celis F, Feria MG, Taborda NA, and Rugeles MT. 2019. Induction of Follicular-Like CXCR5(+) CD8(+) T Cells by TGF-beta1/IL-23 Is Limited During HIV Infection. Viral Immunol. 32: 278–288. [PubMed: 31274389]
13. Reuter MA, Del Rio Estrada PM, Buggert M, Petrovas C, Ferrando-Martinez S, Nguyen S, Sada Japp A, Ablanedo-Terrazas Y, Rivero-Arrieta A, Kuri-Cervantes L, Gunzelman HM, Gostick E, Price DA, Koup RA, Naji A, Canaday DH, Reyes-Teran G, and Betts MR. 2017. HIV-Specific CD8(+) T Cells Exhibit Reduced and Differentially Regulated Cytolytic Activity in Lymphoid Tissue. Cell Rep. 21: 3458–3470. [PubMed: 29262326]
14. Starke CE, Vinton CL, Ladell K, McLaren JE, Ortiz AM, Mudd JC, Flynn JK, Lai SH, Wu F, Hirsch VM, Darko S, Douek DC, Price DA, and Brenchley JM. 2020. SIV-specific CD8+ T cells are clonotypically distinct across lymphoid and mucosal tissues. J. Clin. Invest130: 789–798. [PubMed: 31661461]
15. Xiao M, Chen X, He R, and Ye L. 2018. Differentiation and Function of Follicular CD8 T Cells During Human Immunodeficiency Virus Infection. Front. Immunol9: 1095. [PubMed: 29872434]
16. Mylvaganam GH, Chea LS, Tharp GK, Hicks S, Velu V, Iyer SS, Deleage C, Estes JD, Bosinger SE, Freeman GJ, Ahmed R, and Amara RR. 2018. Combination anti-PD-1 and antiretroviral therapy provides therapeutic benefit against SIV. JCI Insight3: e122940.
17. Mylvaganam GH, Rios D, Abdelaal HM, Iyer S, Tharp G, Mavigner M, Hicks S, Chahroudi A, Ahmed R, Bosinger SE, Williams IR, Skinner PJ, Velu V, and Amara RR. 2017. Dynamics of SIV-specific CXCR5+ CD8 T cells during chronic SIV infection. Proc. Natl. Acad. Sci. USA114: 1976–1981. [PubMed: 28159893]
18. He R, Hou S, Liu C, Zhang A, Bai Q, Han M, Yang Y, Wei G, Shen T, Yang X, Xu L, Chen X, Hao Y, Wang P, Zhu C, Ou J, Liang H, Ni T, Zhang X, Zhou X, Deng K, Chen Y, Luo Y, Xu J, Qi H, Wu Y, and Ye L. 2016. Follicular CXCR5- expressing CD8(+) T cells curtail chronic viral infection. Nature. 537: 412–428. [PubMed: 27501245]
19. Im SJ, Konieczny BT, Hudson WH, Masopust D, and Ahmed R. 2020. PD-1+ stemlike CD8 T cells are resident in lymphoid tissues during persistent LCMV infection. Proc. Natl. Acad. Sci. USA117: 4292–4299. [PubMed: 32034098]
20. Huang Z, Zak J, Pratumchai I, Shaabani N, Vartabedian VF, Nguyen N, Wu T, Xiao C, and Teijaro JR. 2019. IL-27 promotes the expansion of self-renewing CD8(+) T cells in persistent viral infection. J. Ex. Med216: 1791–1808.
21. Lofroos AB, Kadivar M, Resic Lindehammer S, and Marsal J. 2017. Colorectal cancer-infiltrating T lymphocytes display a distinct chemokine receptor expression profile. Eur. J. Med. Res22: 40. [PubMed: 29020986]
22. E J, Yan F, Kang Z, Zhu L, Xing J, and Yu E. 2018. CD8(+)CXCR5(+) T cells in tumor-draining lymph nodes are highly activated and predict better prognosis in colorectal cancer. Human Immunol. 79: 446–452. [PubMed: 29544815]
23. Ye L, Li Y, Tang H, Liu W, Chen Y, Dai T, Liang R, Shi M, Yi S, Chen G, and Yang Y. 2019. CD8+CXCR5+T cells infiltrating hepatocellular carcinomas are activated and predictive of a better prognosis. Aging. (Albany NY)11: 8879–8891. [PubMed: 31663864]
24. Jin Y, Lang C, Tang J, Geng J, Song HK, Sun Z, and Wang J. 2017. CXCR5(+)CD8(+) T cells could induce the death of tumor cells in HBV-related hepatocellular carcinoma. Int. Immunopharmacol53: 42–48. [PubMed: 29032029]
25. Perdomo-Celis F, Taborda NA, and Rugeles MT. 2017. Follicular CD8(+) T Cells: Origin, Function and Importance during HIV Infection. Front. Immunol8: 1241. [PubMed: 29085360]
26. Yu D, and Ye L. 2018. A Portrait of CXCR5(+) Follicular Cytotoxic CD8(+) T cells. Trends Immunol. 39: 965–979. [PubMed: 30377045]

27. Valentine KM, and Hoyer KK. 2019. CXCR5+ CD8 T Cells: Protective or Pathogenic?Front. Immunol10: 1322. [PubMed: 31275308]
28. Breitfeld D, Ohl L, Kremmer E, Ellwart J, Sallusto F, Lipp M, and Forster R. 2000. Follicular B helper T cells express CXC chemokine receptor 5, localize to B cell follicles, and support immunoglobulin production. J. Exp. Med192: 1545–1552. [PubMed: 11104797]
29. Schaerli P, Willimann K, Lang AB, Lipp M, Loetscher P, and Moser B. 2000. CXC chemokine receptor 5 expression defines follicular homing T cells with B cell helper function. J. Exp. Med192: 1553–1562. [PubMed: 11104798]
30. Shan Q, Zeng Z, Xing S, Li F, Hartwig SM, Gullicksrud JA, Kurup SP, Van Braeckel-Budimir N, Su Y, Martin MD, Varga SM, Taniuchi I, Harty JT, Peng W, Badovinac VP, and Xue HH. 2017. The transcription factor Runx3 guards cytotoxic CD8(+) effector T cells against deviation towards follicular helper T cell lineage. Nat. Immunol18: 931–939. [PubMed: 28604718]
31. Chen Y, Yu M, Zheng Y, Fu G, Xin G, Zhu W, Luo L, Burns R, Li QZ, Dent AL, Zhu N, Cui W, Malherbe L, Wen R, and Wang D. 2019. CXCR5(+)PD-1(+) follicular helper CD8 T cells control B cell tolerance. Nat. Commun10: 4415. [PubMed: 31562329]
32. Quigley MF, Gonzalez VD, Granath A, Andersson J, and Sandberg JK. 2007. CXCR5+ CCR7- CD8 T cells are early effector memory cells that infiltrate tonsil B cell follicles. Eur. J. Immunol37: 3352–3362. [PubMed: 18000950]
33. Li Y, Tang L, Guo L, Chen C, Gu S, Zhou Y, Ye G, Li X, Wang W, Liao X, Wang Y, Peng X, Liu G, Zhang X, Sun J, Peng J, and Hou J. 2020. CXCL13-mediated recruitment of intrahepatic CXCR5(+)CD8(+) T cells favors viral control in chronic HBV infection. J. Hepatol72: 420–430. [PubMed: 31610223]
34. Le KS, Ame-Thomas P, Tarte K, Gondois-Rey F, Granjeaud S, Orlanducci F, Foucher ED, Broussais F, Bouabdallah R, Fest T, Leroux D, Yadavilli S, Mayes PA, Xerri L, and Olive D. 2018. CXCR5 and ICOS expression identifies a CD8 T-cell subset with TFH features in Hodgkin lymphomas. Blood. Adv2: 1889–1900. [PubMed: 30087107]
35. Xiao L, Jia L, Bai L, He L, Yang B, Wu C, and Li H. 2016. Phenotypic and functional characteristics of IL-21-expressing CD8(+) T cells in human nasal polyps. Sci. Rep6: 30362. [PubMed: 27468819]
36. Shen J, Luo X, Wu Q, Huang J, Xiao G, Wang L, Yang B, Li H, and Wu C. 2018. A Subset of CXCR5(+)CD8(+) T Cells in the Germinal Centers From Human Tonsils and Lymph Nodes Help B Cells Produce Immunoglobulins. Front. Immunol9: 2287. [PubMed: 30344522]
37. Xing J, Zhang C, Yang X, Wang S, Wang Z, Li X, and Yu E. 2017. CXCR5(+)CD8(+) T cells infiltrate the colorectal tumors and nearby lymph nodes, and are associated with enhanced IgG response in B cells. Exp. Cell Res356: 57–63. [PubMed: 28412245]
38. Valentine KM, Davini D, Lawrence TJ, Mullins GN, Manansala M, Al-Kuhlani M, Pinney JM, Davis JK, Beaudin AE, Sindi SS, Gravano DM, and Hoyer KK. 2018. CD8 Follicular T Cells Promote B Cell Antibody Class Switch in Autoimmune Disease. J. Immunol201: 31–40. [PubMed: 29743314]
39. Jiang H, Li L, Han J, Sun Z, Rong Y, and Jin Y. 2017. CXCR5(+) CD8(+) T Cells Indirectly Offer B Cell Help and Are Inversely Correlated with Viral Load in Chronic Hepatitis B Infection. DNA Cell biol. 36: 321–327. [PubMed: 28157399]
40. Agematsu K, Kobata T, Yang FC, Nakazawa T, Fukushima K, Kitahara M, Mori T, Sugita K, Morimoto C, and Komiyama A. 1995. CD27/CD70 interaction directly drives B cell IgG and IgM synthesis. Eur. J. Immunol25: 2825–2829. [PubMed: 7589078]
41. Stuber E, and Strober W. 1996. The T cell-B cell interaction via OX40-OX40L is necessary for the T cell-dependent humoral immune response. J. Exp. Med183: 979–989. [PubMed: 8642301]
42. Wikenheiser DJ, and Stumhofer JS. 2016. ICOS Co-Stimulation: Friend or Foe?Front. Immunol7: 304. [PubMed: 27559335]
43. Yu Y, Ma X, Gong R, Zhu J, Wei L, and Yao J. 2018. Recent advances in CD8(+) regulatory T cell research. Oncol. Letters15: 8187–8194.
44. Agle K, Vincent BG, Piper C, Belle L, Zhou V, Shlomchik W, Serody JS, and Drobyski WR. 2018. Bim regulates the survival and suppressive capability of CD8(+) FOXP3(+) regulatory T cells during murine GVHD. Blood. 132: 435–447. [PubMed: 29769260]

45. Churlaud G, Pitoiset F, Jebbawi F, Lorenzon R, Bellier B, Rosenzwajg M, and Klatzmann D. 2015. Human and Mouse CD8(+)CD25(+)FOXP3(+) Regulatory T Cells at Steady State and during Interleukin-2 Therapy. Front. Immunol6: 171. [PubMed: 25926835]
46. Elizondo DM, Andargie TE, Haddock NL, da Silva RLL, de Moura TR, and Lipscomb MW. 2019. IL-10 producing CD8(+) CD122(+) PD-1(+) regulatory T cells are expanded by dendritic cells silenced for Allograft Inflammatory Factor-1. J. Leukoc. Biol105: 123–130. [PubMed: 30512224]
47. Barbon CM, Davies JK, Voskertchian A, Kelner RH, Brennan LL, Nadler LM, and Guinan EC. 2014. Alloanergization of human T cells results in expansion of alloantigen-specific CD8(+) CD28(–) suppressor cells. Am. J. Transplant14: 305–318. [PubMed: 24410845]
48. Vuddamalay Y, Attia M, Vicente R, Pomie C, Enault G, Leobon B, Joffre O, Romagnoli P, and van Meerwijk JP. 2016. Mouse and human CD8(+) CD28(low) regulatory T lymphocytes differentiate in the thymus. Immunology. 148: 187–196. [PubMed: 26924728]
49. Uss E, Rowshani AT, Hooibrink B, Lardy NM, van Lier RA, and ten Berge IJ. 2006. CD103 is a marker for alloantigen-induced regulatory CD8+ T cells. J. Immunol177: 2775–2783. [PubMed: 16920912]
50. Han JL, Zimmerer JM, Zeng Q, Ringwald BA, Cassol C, Warren RT, Abdel-Rasoul M, Breuer CK, and Bumgardner GL. 2020. Antibody-suppressor CD8+ T cells ameliorate antibody-mediated rejection following kidney transplant in mice. J. Immunol204: 87.16. [PubMed: 31776205]
51. Zimmerer JM, Ringwald BA, Elzein SM, Avila CL, Warren RT, Abdel-Rasoul M, and Bumgardner GL. 2019. Antibody-suppressor CD8+ T Cells Require CXCR5. Transplantation. 103: 1809–1820. [PubMed: 30830040]
52. Zimmerer JM, Pham TA, Wright CL, Tobin KJ, Sanghavi PB, Elzein SM, Sanders VM, and Bumgardner GL. 2014. Alloprimed CD8(+) T Cells Regulate Alloantibody and Eliminate Alloprimed B Cells Through Perforin- and FasL-Dependent Mechanisms. Am. J. Transplant14: 295–304. [PubMed: 24472191]
53. Zimmerer JM, Basinger MW, Ringwald BA, Abdel-Rasoul M, Pelletier RP, Rajab A, El-Hinnawi A, Parekh H, Washburn K, and Bumgardner GL. 2020. Inverse Association Between the Quantity of Human Peripheral Blood CXCR5+IFN-γ+CD8+T cells with De Novo DSA Production in the First Year After Kidney Transplant. Transplantation. 104: 2424–2434. [PubMed: 32032292]
54. Zimmerer JM, Pham TA, Sanders VM, and Bumgardner GL. 2010. CD8+ T cells negatively regulate IL-4-dependent, IgG1-dominant posttransplant alloantibody production. J. Immunol185: 7285–7292. [PubMed: 21084663]
55. Miles B, Miller SM, Folkvord JM, Levy DN, Rakasz EG, Skinner PJ, and Connick E. 2016. Follicular Regulatory CD8 T Cells Impair the Germinal Center Response in SIV and Ex Vivo HIV Infection. PLoS Pathog. 12: e1005924. [PubMed: 27716848]
56. Leavenworth JW, Tang X, Kim HJ, Wang X, and Cantor H. 2013. Amelioration of arthritis through mobilization of peptide-specific CD8+ regulatory T cells. J. Clin. Invest123: 1382–1389. [PubMed: 23376792]
57. Kim HJ, and Cantor H. 2011. Regulation of self-tolerance by Qa-1-restricted CD8(+) regulatory T cells. Sem. Immunol23: 446–452.
58. Kim HJ, Verbinnen B, Tang X, Lu L, and Cantor H. 2010. Inhibition of follicular T-helper cells by CD8(+) regulatory T cells is essential for self tolerance. Nature. 467: 328–332. [PubMed: 20844537]
59. Mishra S, Liao W, Liu Y, Yang M, Ma C, Wu H, Zhao M, Zhang X, Qiu Y, Lu Q, and Zhang N. 2021. TGF-beta and Eomes control the homeostasis of CD8+ regulatory T cells. J. Exp. Med218: e20200030. [PubMed: 32991667]
60. Choi JY, Eskandari SK, Cai S, Sulkaj I, Assaker JP, Allos H, AlHaddad J, Muhsin SA, Alhussain E, Mansouri A, Yeung MY, Seelen MAJ, Kim HJ, Cantor H, and Azzi JR. 2020. Regulatory CD8 T cells that recognize Qa-1 expressed by CD4 T-helper cells inhibit rejection of heart allografts. Proc. Natl. Acad. Sci. USA117: 6042–6046. [PubMed: 32111690]
61. Lu L, Kim HJ, Werneck MB, and Cantor H. 2008. Regulation of CD8+ regulatory T cells: Interruption of the NKG2A-Qa-1 interaction allows robust suppressive activity and resolution of autoimmune disease. Proc. Natl. Acad. Sci. USA105: 19420–19425. [PubMed: 19047627]

62. Connick E, Folkvord JM, Lind KT, Rakasz EG, Miles B, Wilson NA, Santiago ML, Schmitt K, Stephens EB, Kim HO, Wagstaff R, Li S, Abdelaal HM, Kemp N, Watkins DI, MaWhinney S, and Skinner PJ. 2014. Compartmentalization of simian immunodeficiency virus replication within secondary lymphoid tissues of rhesus macaques is linked to disease stage and inversely related to localization of virus-specific CTL. J. Immunol193: 5613–5625. [PubMed: 25362178]

63. Perdomo-Celis F, Taborda NA, and Rugeles MT. 2018. Circulating CXCR5-Expressing CD8+ T-Cells Are Major Producers of IL-21 and Associate With Limited HIV Replication. J. Acquir. Immune Defic. Syndr78: 473–482. [PubMed: 29649077]

64. Roider J, Maehara T, Ngoepe A, Ramsuran D, Muenchhoff M, Adland E, Aicher T, Kazer SW, Jooste P, Karim F, Kuhn W, Shalek AK, Ndung'u T, Morris L, Moore PL, Pillai S, Kloverpris H, Goulder P, and Leslie A. 2018. High-Frequency, Functional HIV-Specific T-Follicular Helper and Regulatory Cells Are Present Within Germinal Centers in Children but Not Adults. Front. Immunol9: 1975. [PubMed: 30258437]

65. McCarty B, Mwamzuka M, Marshed F, Generoso M, Alvarez P, Ilmet T, Kravietz A, Ahmed A, Borkowsky W, Unutmaz D, and Khaitan A. 2018. Low Peripheral T Follicular Helper Cells in Perinatally HIV-Infected Children Correlate With Advancing HIV Disease. Front. Immunol9: 1901. [PubMed: 30197641]

66. Qiu L, Wang H, Yu Q, Liu J, Chen S, and Zhao Z. 2019. Protective role of follicular CXCR5(+)CD8(+) T cells against dengue virus 2 infection. Int. J. Infect. Dis83: 12–19. [PubMed: 30904675]

67. Shen Y, Qu QX, Jin MN, and Chen C. 2019. Investigating the role of circulating CXCR5-expressing CD8+ T-cells as a biomarker for bacterial infection in subjects with pneumonia. Respiratory Res. 20: 54.

68. Bai M, Zheng Y, Liu H, Su B, Zhan Y, and He H. 2017. CXCR5(+) CD8(+) T cells potently infiltrate pancreatic tumors and present high functionality. Exp. Cell Res361: 39–45. [PubMed: 28965867]

69. Huang Q, Zhou Q, Zhang H, Liu Z, Zeng H, Chen Y, Qu Y, Xiong Y, Wang J, Chang Y, Xia Y, Wang Y, Liu L, Zhu Y, Xu L, Dai B, Guo J, Wang Z, Bai Q, and Zhang W. 2020. Identification and validation of an excellent prognosis subtype of muscle-invasive bladder cancer patients with intratumoral CXCR5(+) CD8(+) T cell abundance. Oncoimmunology. 9: 1810489. [PubMed: 32939328]

70. Chu F, Li HS, Liu X, Cao J, Ma W, Ma Y, Weng J, Zhu Z, Cheng X, Wang Z, Liu J, Jiang ZY, Luong AU, Peng W, Wang J, Balakrishnan K, Yee C, Dong C, Davis RE, Watowich SS, and Neelapu SS. 2019. CXCR5(+)CD8(+) T cells are a distinct functional subset with an antitumor activity. Leukemia. 33: 2640–2653. [PubMed: 31028278]

71. Jacomet F, Cayssials E, Basbous S, Levescot A, Piccirilli N, Desmier D, Robin A, Barra A, Giraud C, Guilhot F, Roy L, Herbelin A, and Gombert JM. 2015. Evidence for eomesodermin-expressing innate-like CD8(+) KIR/NKG2A(+) T cells in human adults and cord blood samples. Eur. J. Immunol45: 1926–1933. [PubMed: 25903796]

72. Xing J, Li X, E J, Wang C, and Wang H. 2020. Inverse relationship between CD40L expression and cytolytic molecule expression by CD8(+)CXCR5(+) T follicular cytotoxic cells in colorectal cancer. Exp. Cell Res389: 111892. [PubMed: 32035135]

73. Im SJ, Hashimoto M, Gerner MY, Lee J, Kissick HT, Burger MC, Shan Q, Hale JS, Lee J, Nasti TH, Sharpe AH, Freeman GJ, Germain RN, Nakaya HI, Xue HH, and Ahmed R. 2016. Defining CD8+ T cells that provide the proliferative burst after PD-1 therapy. Nature. 537: 417–421. [PubMed: 27501248]

74. Brummelman J, Mazza EMC, Alvisi G, Colombo FS, Grilli A, Mikulak J, Mavilio D, Alloisio M, Ferrari F, Lopci E, Novellis P, Veronesi G, and Lugli E. 2018. High-dimensional single cell analysis identifies stem-like cytotoxic CD8(+) T cells infiltrating human tumors. J. Exp. Med215: 2520–2535. [PubMed: 30154266]

75. Chatterjee B, Deng Y, Holler A, Nunez N, Azzi T, Vanoaica LD, Muller A, Zdimerova H, Antsiferova O, Zbinden A, Capaul R, Dreyer JH, Nadal D, Becher B, Robinson MD, Stauss H, and Munz C. 2019. CD8+ T cells retain protective functions despite sustained inhibitory receptor expression during Epstein-Barr virus infection in vivo. PLoS Pathog. 15: e1007748. [PubMed: 31145756]

76. Zhou Y, Guo L, Sun H, Xu J, and Ba T. 2018. CXCR5(+) CD8 T cells displayed higher activation potential despite high PD-1 expression, in tumor-involved lymph nodes from patients with thyroid cancer. Int. Immunopharmacol62: 114–119. [PubMed: 30005226]

77. Ma QY, Chen J, and Zhao J. 2020. Follicular cytotoxic CD8 T cells present high cytokine expression, and are more susceptible to Breg-mediated suppression in non-small cell lung cancer. Immunologic Res. 68: 54–62.

78. Tang J, Zha J, Guo X, Shi P, and Xu B. 2017. CXCR5(+)CD8(+) T cells present elevated capacity in mediating cytotoxicity toward autologous tumor cells through interleukin 10 in diffuse large B-cell lymphoma. Int. Immunopharmacol50: 146–151. [PubMed: 28662433]

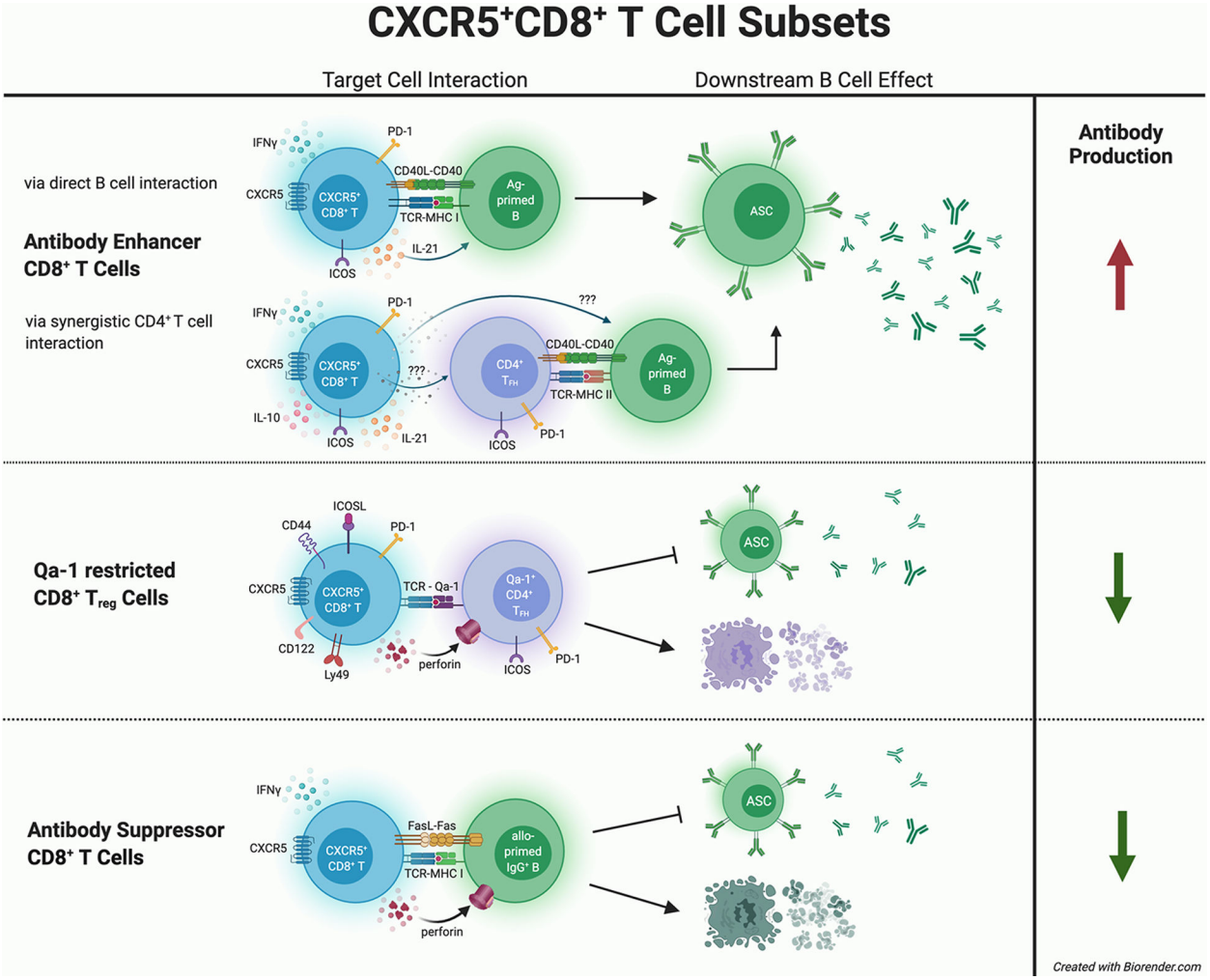

Figure 1. Distinct Mechanisms by which CXCR5$^+$CD8$^+$ T Cell Subsets Impact Humoral Immunity.

CXCR5$^+$CD8$^+$ T cell subsets are reported to mediate different effects upon antibody production. Antibody-enhancing CXCR5$^+$CD8$^+$ T cells express PD-1, ICOS, CD40L, and cytokines such as IFN-γ and IL-21. *In vitro* co-culture studies suggest that antibody-enhancing CXCR5$^+$CD8$^+$ T cells interact directly with antibody-producing B cells, or synergistically with CD4$^+$ T$_{FH}$ cells, to increase antibody production by antibody secreting cells (ASC). In contrast, one subset of CXCR5$^+$CD8$^+$ T cells that protects against autoimmunity is Qa-1 restricted (CD44$^+$ICOSL$^+$PD-1$^+$CD122$^+$Ly49$^+$) and exerts cytotoxic killing of autoreactive CD4$^+$ T$_{FH}$ cells resulting in decreased *auto*antibody production. Another subset of antibody-suppressor CXCR5$^+$CD8$^+$ T cells (CD44$^+$ICOSL$^-$PD-1$^-$IFN-γ$^+$) is MHC-I restricted, antigen-specific, and directly mediates killing of allo-primed IgG$^+$ B cells resulting in reduction of *allo*antibody production after transplant.

Table 1.

Phenotype of $CXCR5^{+}CD8^{+}$ T cell Subsets by Function

		CD8+ T cell subsets						
		CXCR5+CD8+ T cells Evidence for Antibody Regulation				CXCR5+CD8+ T Cells Antibody Regulation Not Reported		CD8+ T Regulatory cells (for reference)
Effector Function		Antibody-enhancer**	Antibody-Suppression			Anti-viral	Anti-tumor	CD8+ T regs
			Qa-1-restricted (Anti-autoimmune)	Follicular regulatory (Anti-Viral immunity)	Antibody-suppressor (Transplant Alloimmunity)			
Transcription factors	Bcl-6	(30, 31, 34–37)	nr	nr	*	(9, 12, 17, 20, 73)	(74)	nr
	Tbet	(30, 35)	nr	nr	*	(19)	nr	nr
	Tcf1	(30)	nr	nr	*	(19)	nr	nr
	Eomes	(30, 38)	(59)	nr	*	(19)	(74)	(44)
	FoxP3	nr	(58)	nr	(51)	nr	nr	(44, 45)
Co-inhibitory molecules	PD-1	(23, 31, 33–35)	(58)	nr	(51)	(8, 12, 18, 19, 66, 73, 75)	(24, 74, 76)	(46)
	Tim-3	(33)	nr	(55)	*	(9, 18, 19)	(24)	nr
	CTLA-4	(33)	(58)	nr	*	(17)	(76)	(44, 45)
Co-stimulatory molecules	CD27	(32, 36)	nr	nr	*	nr	(74, 76)	nr
	CD28	(32, 38)	nr	nr	*	(73)	(74)	(47, 48)
	CD40L	(31, 36)	nr	nr	*	(12)	nr	nr
	ICOS	(23, 31, 32, 34, 35)	nr	nr	*	(9, 20, 73)	nr	(45)
	ICOSL	nr	(58)	nr	(51)	(9)	nr	nr
Cytokines	IFN-γ	(30, 32, 33, 35–37, 39)	nr	nr	(51, 53, 54)	(12, 18, 66)	(22, 76, 77)	(49)
	IL-10	(39)	(57)	(55)	(51)	(17, 66)	(77)	(46)
	IL-21	(31, 33–36, 39)	nr	nr	*	(12)	nr	nr
Effector molecules	Perf	(32, 34, 36)	(58)	(55)	(52)	(18)	(76, 78)	(45)
	Gzmb	(34, 36)	nr	nr	*	(18)	(22, 24, 76, 78)	(45)
Chemokine Receptor	CXCR5	(30, 31, 34–37)	(58)	(55)	(51, 53)	(9, 12, 17, 19, 20, 73)	(20, 23, 24, 74, 76)	nr

Red highlight = Positive expression; Green highlight = Not expressed.

*Unpublished data; nr = Not reported

**$CXCR5^{+}CD8^{+}$ T cells enhance antibody in models of viral infection, autoimmunity, and tumor

Table 2.

Clinical Correlations and Phenotype of Human $CXCR5^+CD8^+$ T cell subsets

	Clinical Condition/ Number of Patients (n)	$CXCR5^+CD8^+$ T cell phenotype	$CXCR5^+CD8^+$ T cell Clinical Correlation	Reference
Anti-viral	HIV (n=36)	$IL\text{-}2^+IFN\text{-}\gamma^+IL\text{-}17^+$	High circulating $CXCR5^+CD8^+$ T cell quantity correlates with HIV neutralization by plasma from HIV-infected children	(64)
	HIV (n=115)	$CCR7^{-/lo}CD62L^{hi}IL\text{-}21^{hi}$ $TCF1^{hi}BCL6^{hi}BLIMP1^-$	High circulating $CXCR5^+CD8^+$ T cell quantity correlates with reduced HIV viral load	(63)
	HIV (n=101)	$PD\text{-}1^+$	High circulating HIV-specific $CXCR5^+CD8^+$ T cell quantity correlates with reduced HIV viral load	(8)
	HIV (n=14)	$PD\text{-}1^+CD44^+KLRG1^+$ $IFN\text{-}\gamma^+CD107a^+$	High circulating HIV-specific $CXCR5^+CD8^+$ T cell quantity correlates with reduced HIV viral load	(18)
	Dengue Virus 2 (n=57)	$PD\text{-}1^+$	High circulating anti-viral $CXCR5^+CD8^+$ T cell quantity correlates with reduced viral load and protection from associated kidney injury	(66)
Bacterial Infection	Bacterial Pneumonia (n=65)	$PD\text{-}1^+CD103^{lo}$	High circulating $CXCR5^+CD8^+$ T cell quantity correlates with exacerbation of pneumonia severity	(67)
Anti-tumor	Colorectal cancer (n=44)	$PD1^{hi}Tim3^{hi}LAG3^{hi}$ $IFN\text{-}\gamma^{hi}TNF\text{-}\alpha^{hi}IL\text{-}2^{hi}$ $PRF1^{hi}GZMB^{hi}$	High $CXCR5^+CD8^+$ T cell quantity in tumors or tumor draining lymph nodes correlates with increased cancer-free survival times	(22)
	HCC (n=40)	$PD\text{-}1^{hi}ICOS^{hi}$ $IL\text{-}21^{hi}$	High tumor-infiltrating $CXCR5^+CD8^+$ T cell quantity negatively correlates with microvascular invasion, TNM staging and early recurrence	(23)
	Pancreatic cancer (n=12)	$PD\text{-}1^+TIM\text{-}3^+$	High tumor-infiltrating and circulating $CXCR5^+CD8^+$ T cell quantity correlates with disease-free survival after tumor resection	(68)
	Bladder cancer (n=11, fresh tissue for flow cytometry; n=249 bioinformatics gene signature)	$PD\text{-}1^+CTLA\text{-}4^+$ $CD62L^+LAG3^+$ $IFN\text{-}\gamma^+GZMB^+$ $TNF\text{-}\alpha^+CD107a^+$	High tumor-infiltrating $CXCR5^+CD8^+$ T cell quantity correlates with lower tumor stage and prolonged overall survival and cancer-free survival	(69)
	Follicular (Germinal Center B cell) lymphoma (n=187, bioinformatics gene expression)	$PD\text{-}1^+IFN\text{-}\gamma^+TNF\text{-}\alpha^+$	High tumor expression of $CXCR5^+CD8^+$ T cell signature genes correlates with prolonged survival	(70)
Autoimmunity	SLE (n=79)	$CD158e^+$ (Inhibitory, Killer cell Ig-like receptor, KIR^+)	Reduced quantity of circulating $CD158e^+CD8^+$ T cells in SLE patients compared to healthy controls; KIR^+CD8^+ T cells potential human counterpart to murine $Ly49^+$ Qa-1-restricted $CD122^{hi}CD8^+$ T cell related to protection from autoimmunity	(59)
Transplantation	Kidney Transplant (n=95)	$CD44^+CD62L^-IFN\text{-}\gamma^+$	High circulating $CXCR5^+CD8^+$ T cell quantity correlates with lower incidence of *de novo* donor specific antibody production and improved clinical outcomes at 1 year post transplant	(53)