TIGIT-positive circulating follicular helper T cells and sickle cell alloimmunization

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Transfusion therapy remains an important treatment modality for patients with sickle cell disease (SCD). Despite its therapeutic benefits, red blood cell transfusion results in the development of antibodies against disparate antigens on transfused red cells (alloimmunization) in approximately 20-50% of patients with SCD. Alloantibodies can cause delayed hemolytic transfusion reactions, which in SCD patients can trigger hyperhemolysis, a life-threatening poorly understood phenomenon in which the transfused and the patient's own red blood cells are destroyed.[1] In addition, finding compatible units for patients with alloantibodies can be difficult, causing potentially life-threatening transfusion delays. Matching for key immunogenic antigens disparate between patients (of African ancestry) and blood donors (mostly white), namely Rh-C, -E and K, has been a strategy used to reduce alloimmunization in patients with SCD; however, patients transfused with C,E,K-matched donor RBC (from mostly black donors), can still develop pathogenic Rh antibodies due to the genetic diversity of the *RH* locus in individuals of African ancestry.[2,3] This highlights the need for better characterization of alloimmunization triggers and risk factors/biomarkers in this highly vulnerable population.

The etiology of alloimmunization in patients with SCD remains unclear. Our group has recently studied antigen-specific CD4^{+} helper T-cell responses in peripheral blood of transfused patients with SCD.[4] We found that in alloantibody responder patients, antigen-specific CD4^{+} T-cell responses were predominantly T_H17, and that interleukin(IL)-21, the prototypic follicular helper T-cell (T_{FH})-associated cytokine driving B-cell help, was only expressed by antigen-specific T_{FH}

derived from responders.[4] These data are consistent with the role of heightened CD4^{+} T-cell effector functions driving alloantibody production in alloimmunized SCD patients.

T_{FH} cells have emerged as the main effector CD4^{+} T cells specialized in providing help to support responses of antigen-engaged B cells to generate the initial wave of antibody response as well as in supporting B-cell differentiation into high affinity antibody-producing B cells and long-lasting IgG antibodies.[5] T_{FH} cells are universally characterized by: (i) the expression of the chemokine (C-X-C motif) receptor 5 (CXCR5) on memory (CD45RA^{-}) cells which directs them to the B-cell follicles in response to the specific ligand CXCL13;[6-8] (ii) expression of IL-21, a potent cytokine promoting growth, differentiation, and class switching of B cells although they also secrete other B-cell help cytokines including IL-4;[9,10] and (iii) co-stimulatory molecules specialized in providing B-cell help including inducible T-cell co-stimulatory (ICOS) and CD40L.[5,11] Given the key role of T_{FH} cells in providing help to B cells to support their activation, expansion and differentiation, these cells have attracted particular attention as potential targets for developing therapeutic strategies not only to enhance vaccination responses but also to prevent antibody-mediated autoimmunity.[5] Our studies of antigen-specific T_{FH} responses in alloimmunized SCD patients support the notion that T_{FH} cells are also likely to be critical in alloimmunization biology.[4] More recently, T_{FH}-related cells with ability to promote antibody responses were identified in the peripheral circulation of humans and mice,[12-19] opening up the possibility of studying T_{FH} cell responses in blood samples.

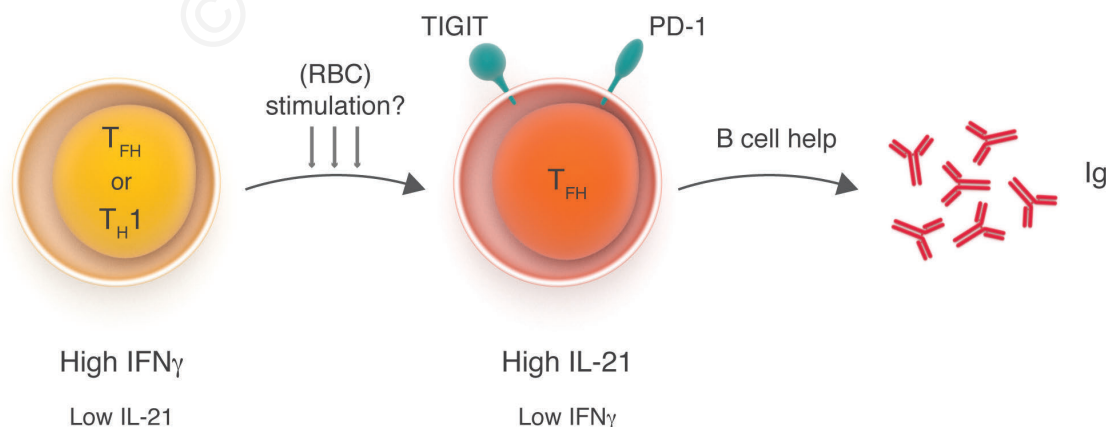In the report from Yazdanbakhsh's group published in this

Figure 1. Hypothesized model of TIGIT[+] circulating follicular helper T-cell differentiation following chronic stimulation. T_H1 or T_{FH}-type 1 cells express high levels of type 1 cytokines such as interferon-gamma but no or low levels of IL-21. Upon chronic stimulation such as following regular transfusions, these T cells become exhausted, losing the ability to produce interferon-gamma. At the same time, immune checkpoint molecules, including TIGIT and PD-1, are up-regulated and the cells express the prototypic B-cell help cytokine IL-21, which subsequently drives antibody production by B cells.

issue of the journal,[20] Godefroy et al. examine the role of two immune checkpoints[21] expressed by CD4+/CD45RA-/CXCR5+ circulating follicular helper T (cT$_{FH}$) cells: the programmed cell death-1 (PD-1) and the T-cell immunoreceptor with Ig and immunoreceptor tyrosine-based inhibitory domains (TIGIT) surface molecules. The function of PD-1 on T$_{FH}$ cells remains controversial and has been described as both promoting[15,16] and reducing[17,22,23] humoral responses in different models. The recent discovery of regulatory follicular T cells (T$_{FR}$) expressing high levels of PD-1[17] may help to reconcile these divergent findings. Although TIGIT expression on T cells has been studied previously,[24-29] the function of TIGIT on T$_{FH}$ cells is being addressed for the first time in the study by Godefroy et al.[20]

The authors show that TIGIT-expressing cells represent almost half of the cT$_{FH}$ population, while PD-1+ cells, all co-expressing TIGIT, are significantly less frequent, accounting for about 9% of the cT$_{FH}$ cells.[20] Several subsets of cT$_{FH}$ have previously been characterized. Three main subsets, named T$_{FH}$1, T$_{FH}$2 and T$_{FH}$17, can be identified not only by their ability to produce type 1, 2 and 17 cytokines or express their respective transcription factors (T-bet, GATA3, RORc), but also by their expression of chemokine receptors CXCR3 and CCR6.[13] T$_{FH}$1 cells express CXCR3, but not CCR6; T$_{FH}$2 cells express neither; T$_{FH}$17 cells express CCR6, but not CXCR3.[13] Based on chemokine receptor expression, the authors show that TIGIT+ cT$_{FH}$ represent a subset phenotypically different from any of the three populations previously described.[20] Interestingly, TIGIT+ cT$_{FH}$ can produce IL-4 and to some extent interferon-gamma. Accordingly, both T$_{FH}$1 and T$_{FH}$2 can be found in TIGIT+ cT$_{FH}$ independently of the cells' PD-1 expression.[20] IL-17 secretion was not tested. Nonetheless, these findings establish that TIGIT+ cells, whether or not they express PD-1, represent a novel subset of cT$_{FH}$.

Importantly, TIGIT+ cT$_{FH}$ cells exerted strong B-cell help functions.[20] Compared to TIGIT- cT$_{FH}$, they were able to better promote differentiation of B cells into plasmablasts and IgG-producing cells.[20] Furthermore, the study of sorted cT$_{FH}$ subsets showed that TIGIT+/PD-1- cells provide similar help to B cells as TIGIT+/PD-1+ cells,[20] suggesting that PD-1 plays a limited role in promoting humoral responses in this model. Despite the fact that it still needs to be proven, TIGIT+ cT$_{FH}$ cells heightened help to B cells probably lie in their enhanced expression of CD40L and ICOS co-stimulation molecules and/or their capacity to produce large amounts of IL-21. Alternatively, B-cell help could be provided directly by signaling through TIGIT's ligand(s). Whether TIGIT+ cT$_{FH}$ cells influence isotype switching of Ig production, as shown for T$_{FH}$2 and T$_{FH}$17, remains to be determined.

Using blocking antibodies, Godefroy et al. also demonstrated that whereas PD-1 did not seem to play a major role in this system, TIGIT was directly implicated in the function of these cells.[20] Indeed, blocking TIGIT not only decreased the expression levels of ICOS, CD40L and IL-21, but also prevented B cells from producing IgG.[10] Other authors proposed a direct role for PD-1 in T$_{FH}$ function.[15-17,22,23] In the study by Godefroy et al., the authors did not observe a significant effect after blocking PD-1.[20] This could be due to the use of different experimental systems or a mixture of opposite effects mediated by PD-1 can-

celling each other. Instead, the fact that all PD-1+ cT$_{FH}$ co-express TIGIT[20] could suggest that PD-1 represents a non-functional marker on cT$_{FH}$ and that TIGIT+/PD-1+ cT$_{FH}$ B-cell help functions are due, at least in part, to TIGIT.

The study suggests a role for TIGIT-expressing cT$_{FH}$ cells in alloimmunization in the context of SCD.[20] In alloimmunized patients, TIGIT+ cT$_{FH}$ cells offered more potent help to B cells than in non-alloimmunized patients: IL-21 and CD40L expression was higher in alloimmunized patients than in non-alloimmunized patients.[20] This could be explained either by faulty TIGIT signaling in non-alloimmunized patients or by an exacerbated response to TIGIT triggering in alloimmunized patients. Collectively, these data suggest involvement of various subsets of cT$_{FH}$ cells displaying different functions in the alloimmunization process of SCD patients.

Chronic antigen stimulation can result in T-cell exhaustion associated with lack of cytotoxic activity and failure to produce type 1 cytokines, interferon-gamma and IL-2 as well as up-regulation of several immune checkpoint molecules including TIGIT on the exhausted T cells.[21] Godefroy et al. propose an interesting model in which repeated exposure to allogeneic red blood cells in patients with SCD could lead to exhaustion of red blood cell-specific T cells, which would end up triggering the expression of immune checkpoints such as TIGIT. This brings into question whether exhausted type 1 CD4+ T cells could be skewed towards a T$_{FH}$ phenotype by expressing TIGIT (or other "exhaustion" markers) after repeated stimulation caused by chronic transfusions. Considering the latter question from a non-transfusion perspective, the authors propose an interesting hypothesis: if chronic stimulation induces T$_{H}$1 or T$_{FH}$1 cells to differentiate into T$_{FH}$ cells, could this process reflect an evolutionary mechanism by which TIGIT would be involved in reducing type 1 chronic inflammation as well as shifting immune responses from cellular to potentially more "suitable", humoral responses?

Overall, these studies provide new insight into T$_{FH}$ polarization, and help give rise to the development of therapeutic strategies, not only regarding alloimmunization, but also in vaccination as well as in a large array of disorders, including inflammatory diseases, autoimmunity and immune deficiencies.

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The origin of relapse in pediatric T-cell acute lymphoblastic leukemia

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Acute lymphoblastic leukemia (ALL) comprises a group of hematologic neoplasms that arise from the malignant transformation of lymphoid B-lineage or T-lineage progenitor cells. ALL is the most common malignancy in childhood, accounting for almost 30% of pediatric cancers.[1] Considerable advances in the treatment of childhood ALL have been made in the past decades, and 5-year survival rates now exceed 85% in children. However, approximately 15%-20% of those ALL cases relapse and have a poor prognosis.[2,3] Because most intensive chemotherapy regimens have reached the limit of tolerability, current research efforts are focusing on identifying new targets for the development of more effective therapies, a strategy which requires a detailed understanding of the biology of these leukemias.

ALL is characterized by a multistep oncogenic process, in which a plethora of genomic lesions accumulate and cooperate to alter normal mechanisms that control cell cycle, proliferation, differentiation and survival of lymphocytes. Over the last decade, studies using gene expression profiling, DNA copy-number analyses, and next-generation sequencing have provided new insights into the pathogenesis and clinical behavior of ALL.[4] Furthermore, sequencing studies of matched diagnostic, remission and relapse samples have provided important insights into the correlation of the different mutations, clonal evolution, and treatment resistance.[4]

Using single nucleotide polymorphism array technology, Charles Mullighan and colleagues performed genome-wide copy number analysis and loss-of-heterozygosity (LOH) analysis on matched diagnostic and relapse pediatric ALL samples.[5] They observed a significant increase in the number of chromosomal deletions in B-ALL samples taken at relapse, but no significant changes were observed in T-ALL.[5] Based on a comparison of the chromosomal deletions identified in paired diagnosis-relapse samples, these investigators were able to conclude that in about half of the cases of B-ALL the relapse clone was derived from the major leukemic clone at diagnosis. However, in the other half of B-ALL cases, the relapse clone was derived from a pre-leukemic clone or a minor clone present at diagnosis, since the relapse clone had very few dele-