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## **B Cells and TCR Avidity Determine Distinct Functions of CD4<sup>+</sup> T Cells in Retroviral Infection**

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# B Cells and TCR Avidity Determine Distinct Functions of CD4<sup>+</sup> T Cells in Retroviral Infection

Mickaël J.-Y. Ploquin, Urszula Eksmond, and George Kassiotis

The T cell-dependent B cell response relies on cognate interaction between B cells and CD4<sup>+</sup> Th cells. However, the consequences of this interaction for CD4<sup>+</sup> T cells are not entirely known. B cells generally promote CD4<sup>+</sup> T cell responses to pathogens, albeit to a variable degree. In contrast, CD4<sup>+</sup> T cell responses to self- or tumor Ags are often suppressed by B cells. In this study, we demonstrated that interaction with B cells dramatically inhibited the function of virus-specific CD4<sup>+</sup> T cells in retroviral infection. We have used Friend virus infection of mice as a model for retroviral infection, in which the behavior of virus-specific CD4<sup>+</sup> T cells was monitored according to their TCR avidity. We report that avidity for Ag and interaction with B cells determine distinct aspects of the primary CD4<sup>+</sup> T cell response to Friend virus infection. Virus-specific CD4<sup>+</sup> T cells followed exclusive Th1 and T follicular helper (Tfh) differentiation. High avidity for Ag facilitated expansion during priming and enhanced the capacity for IFN- $\gamma$  and IL-21 production. In contrast, Tfh differentiation was not affected by avidity for Ag. By reducing or preventing B cell interaction, we found that B cells promoted Tfh differentiation, induced programmed death 1 expression, and inhibited IFN- $\gamma$  production by virus-specific CD4<sup>+</sup> T cells. Ultimately, B cells protected hosts from CD4<sup>+</sup> T cell-mediated immune pathology, at the detriment of CD4<sup>+</sup> T cell-mediated protective immunity. Our results suggest that B cell presentation of vaccine Ags could be manipulated to direct the appropriate CD4<sup>+</sup> T cell response. *The Journal of Immunology*, 2011, 187: 3321–3330.

Protective immunity against infection is provided by the multipartite immune system, in which CD4<sup>+</sup> Th cells play a coordinating role. CD4<sup>+</sup> T cell priming is initiated by dendritic cells in secondary lymphoid organs. However, the CD4<sup>+</sup> T cell response can be modulated by additional APC types and B cells in particular (1). Although studies in several experimental systems failed to detect a role for B cells in CD4<sup>+</sup> T cell priming (2–5), the overwhelming majority suggest that B cell deficiency generally leads to reduced CD4<sup>+</sup> T cell priming following viral infection or immunization (6–15). Thus, Ag presentation by B cells can enhance CD4<sup>+</sup> T cell priming, particularly when Ag is limiting (1). B cells have also been shown to reduce CD4<sup>+</sup> T cell responses, mostly through induction of regulatory T cells or IL-10 production (1), but also directly (16, 17). However, interpretation of many of these studies is confounded by established requirements for B cells in additional physiological processes, including the organization of lymphoid organ architecture (1). Thus, despite the potential of B cell Ag presentation to either suppress or enhance CD4<sup>+</sup> T cell responses, the factors that determine the ultimate effect of B cells on CD4<sup>+</sup> T cells are not completely understood.

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B cell Ag presentation to CD4<sup>+</sup> T cells is a necessary step for the T cell-dependent B cell response, helped by T follicular helper (Tfh) cells, a specialized class of CD4<sup>+</sup> T cells (18, 19). In turn, B cells have been implicated as APCs in Tfh differentiation (18, 19). Interaction with B cells has been found necessary for differentiation of Ag-specific CD4<sup>+</sup> T cells into Tfh cells (20, 21), whereas early acquisition of Tfh markers or follicular localization have been shown in other studies to be independent of B cell interaction (22, 23). Continuous Ag presentation has recently been suggested to overcome the requirement for B cell interaction for Tfh differentiation (5), and Ag presentation by plasma cells negatively regulated the Tfh program (24).

In addition to the type of priming APC, CD4<sup>+</sup> T cell expansion is profoundly affected also by the strength of TCR signaling, which is enhanced by high TCR affinity for Ag (25, 26). Thus, selection following priming of CD4<sup>+</sup> T cells favors clones with high TCR affinity (27–29). As well as expansion, CD4<sup>+</sup> T cell differentiation is also affected by TCR affinity and high-affinity CD4<sup>+</sup> T cell clones have been suggested to preferentially develop into Tfh cells (30). Thus, both the overall T cell avidity for Ag and the nature of the cell presenting it can influence expansion and differentiation of CD4<sup>+</sup> T cells, albeit to a variable degree and often with opposite outcomes, largely determined by the experimental system.

To gain insights into the CD4<sup>+</sup> T cell response to chronic retroviral infection we have been using, as a model system, persistent infection of mice with Friend virus (FV), a retroviral complex of Friend murine leukemia virus (F-MuLV) and spleen focus-forming virus (SFFV) (31). We have also been using polyclonal TCR $\beta$ -transgenic CD4<sup>+</sup> T cells, in which usage specifically of endogenous TCRV $\alpha$ 2 chains creates high avidity for the A<sup>b</sup>-restricted env<sub>122–141</sub> product of the F-MuLV *env* gene (32, 33), thus allowing analysis of the impact of T cell avidity on expansion and differentiation. In addition to helping B cells in this system, env-specific CD4<sup>+</sup> T cells provide significant protection against FV-induced disease, independently of a cytotoxic T cell or Ab

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Abbreviations used in this article: F-MuLV, Friend murine leukemia virus; FV, Friend virus; Fv2<sup>+</sup>, Fv2 susceptibility; HEL, hen egg lysozyme; *Ighm*<sup>−/−</sup>, B cell-deficient; NIMR, National Institute for Medical Research; PD-1, programmed death 1; *Rag1*<sup>−/−</sup>, Rag1-deficient; SAP, signaling lymphocyte activation molecule-associated protein; SFFV, spleen focus-forming virus; *Sh2d1a*<sup>−/−</sup>, signaling lymphocyte activation molecule-associated protein-deficient; SLAM, signaling lymphocyte activation molecule; *Tcr $\alpha$* <sup>−/−</sup>, TCR $\alpha$ -deficient; Tfh, T follicular helper; wt, wild-type.

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response (33), but can also cause bone marrow pathology, particularly when T cell regulation is incomplete (32). We have previously observed severe immune pathology mediated by virus-specific CD4<sup>+</sup> T cells during FV infection of lymphocyte-deficient, Rag1-deficient (*Rag1*<sup>-/-</sup>) or B cell-deficient *Ighm*<sup>-/-</sup> hosts (32). Furthermore, experimental B cell activation during FV infection, either by coinfection or by direct stimulation, dramatically enhances FV replication (34), which is associated with loss of immune control of FV and premature contraction of env-specific CD4<sup>+</sup> T cells (33, 34). These findings raised the possibility that in addition to mounting an FV-neutralizing Ab response, B cells may directly inhibit CD4<sup>+</sup> T cell function.

## Materials and Methods

### Mice

Inbred C57BL/6 (B6) and CD45.1<sup>+</sup> B6 (B6.SJL-*Ptprca*<sup>a</sup> *Pep3*<sup>b</sup>/BoyJ) mice were originally obtained from The Jackson Laboratory (Bar Harbor, ME) and were subsequently maintained at the National Institute for Medical Research (NIMR) animal facilities. The B6 TCR-transgenic strain EF4.1, expressing a transgenic TCRβ-chain from a T cell clone specific to F-MuLV env<sub>122-141</sub> presented by MHC class II A<sup>b</sup>, has been described (32). B6-backcrossed *Rag1*<sup>-/-</sup> mice (35), B cell-deficient (*Ighm*<sup>-/-</sup>) mice (36), signaling lymphocyte activation molecule-associated protein (SAP)-deficient (*Sh2d1a*<sup>-/-</sup>) mice (37), Fv2 susceptibility (*Fv2*<sup>s</sup>)-congenic *Rag1*<sup>-/-</sup> (*Fv2*<sup>s</sup> *Rag1*<sup>-/-</sup>) mice (34), hen egg lysozyme (HEL)-specific BCR-transgenic (MD4) mice (38), TCRα-deficient (*Tcrα*<sup>-/-</sup>) mice (39), and IL-10-deficient (*Il10*<sup>-/-</sup>) mice (40) were also maintained at the NIMR animal facilities. All animal experiments were conducted according to United Kingdom Home Office regulations and local guidelines.

### Viruses and infections

The FV used in this study was a retroviral complex of a replication-competent B-tropic F-MuLV and a replication-defective SFFV. Stocks were propagated in vivo and prepared as 10% w/v homogenate from the spleen of 12-d infected BALB/c mice. Mice received an inoculum of ~1000 spleen focus-forming units of FV. The B-tropic helper F-MuLV stock was prepared as culture supernatant harvested from chronically infected *Mus dunni* cells. Mice received an inoculum of ~10<sup>4</sup> infectious units F-MuLV. All viral stocks were free of Sendai virus, murine hepatitis virus, parvoviruses 1 and 2, reovirus 3, Theiler's murine encephalomyelitis virus, murine rotavirus, ectromelia virus, murine CMV, K virus, polyomavirus, Hantaan virus, murine norovirus, lymphocytic choriomeningitis virus, murine adenoviruses FL and K87, and lactate dehydrogenase-elevating virus. Viruses were injected via the tail vein in 0.1 ml PBS. For the assessment of anemia, mice were bled by a small incision of the tail vein, and blood was collected into heparinized capillary tubes. Complete blood counts were measured on a VetScan HMII hematology analyzer (Abaxis), following the manufacturer's instructions. RBC counts of uninfected mice ranged between  $9.56 \times 10^6$  and  $10.49 \times 10^6/\text{mm}^3$  blood. FV-induced splenomegaly in infected mice was expressed as spleen index, which is the ratio of the weight of the spleen (in milligrams) to the weight of the rest of the body (in grams).

### T cell purification and adoptive transfer

Single-cell suspensions were prepared from the spleens and lymph nodes of donor CD45.1<sup>+</sup> TCRβ-transgenic mice by mechanical disruption. Spleen suspensions were treated with ammonium chloride for erythrocyte lysis. CD4<sup>+</sup> T cells were enriched using immunomagnetic-positive selection (StemCell Technologies, Vancouver, BC, Canada) according to the manufacturer's instructions. Purity of the isolated CD4<sup>+</sup> T cell population was routinely >92%. In some experiments, purified cells were labeled with CFSE (Molecular Probes) prior to cell transfer. A total of  $\sim 3 \times 10^6$  TCRβ-transgenic CD4<sup>+</sup> T cells were injected in recipient mice via the tail vein in 0.1 ml air-buffered IMDM. This resulted in engraftment of 5000–10,000 env-specific CD4<sup>+</sup> T cells (4% of all donor CD4<sup>+</sup> T cells) per spleen (~0.025% of total host CD4<sup>+</sup> T cells), which was routinely confirmed by staining for donor-type (CD45.1<sup>+</sup>) T cells 1 d posttransfer. When adoptive transfer of CD4<sup>+</sup> T cells was combined with FV infection, purified CD4<sup>+</sup> T cells and virus stocks were injected separately into recipient mice within a 24 h-period. In indicated experiments, enriched TCRβ-transgenic CD4<sup>+</sup> T cells were stained with Abs to surface markers and then further purified by cell sorting, performed on MoFlo cell sorters (Dako-Cytomation, Fort Collins, CO), according to TCRVα2 expression. A total

of  $\sim 0.35 \times 10^6$  TCRVα2<sup>+</sup> or  $1.30 \times 10^6$  TCRVα2<sup>-</sup> purified TCRβ-transgenic CD4<sup>+</sup> T cells were injected in recipient mice, resulting in engraftment of a similar number (2000–4000) of env-specific CD4<sup>+</sup> T cells. T cells used for transcriptional profiling were first enriched for total CD4<sup>+</sup> or donor-type CD45.1<sup>+</sup> T cells, stained with Abs to surface markers, and then further purified by cell sorting. Typical cell purity following cell sorting was >98%.

### Flow cytometry

Spleen-cell suspensions were stained with directly conjugated Abs to surface markers obtained from eBioscience (San Diego, CA), Caltag Laboratories/Invitrogen (Carlsbad, CA), BD Biosciences (San Jose, CA), or BioLegend (San Diego, CA). Donor-type env-specific CD4<sup>+</sup> T cells were gated either as CFSE<sup>-</sup>CD45.1<sup>+</sup> or CD44<sup>hi</sup>CD45.1<sup>+</sup> CD4<sup>+</sup> T cells with comparable results (33). CXCR5 expression was detected with a PE-conjugated anti-mouse CXCR5 (clone 2G8; BD Biosciences) and was further amplified with a biotinylated anti-PE Ab (BioLegend) followed by incubation with a streptavidin-PE conjugate (BioLegend). For detection of cytokine synthesis, cells were stained for surface markers and stimulated for 4 h with phorbol 12,13-dibutyrate and ionomycin (both at 500 ng/ml) in the presence of monensin (1 μg/ml). Cells were then fixed and permeabilized using buffers from eBioscience before intracellular staining with Abs specific to IL-17A, IFN-γ (eBioscience), or IL-21 (R&D Systems, Minneapolis, MN). FV-infected cells were detected by flow cytometry using surface staining for the glycosylated product of the viral *gag* gene (glyco-Gag) using the matrix-specific mAb 34 (mouse IgG2b), followed by an anti-mouse IgG2b-FITC secondary reagent (BD Biosciences). Four- and eight-color cytometry was performed on FACSCalibur (BD Biosciences) and CyAn (DakoCytomation) flow cytometers, respectively, and analyzed with FlowJo v8.7 (Tree Star, Ashland, OR) or Summit v4.3 (DakoCytomation) analysis software, respectively.

### FV-neutralizing Ab assay

Serum titers of FV-neutralizing Abs were measured as previously described (34). The dilution of serum, which resulted in 50% neutralization, was taken as the neutralizing titer.

### TCR re-expression assay

CD45.1<sup>+</sup> TCRβ-transgenic env-specific CD4<sup>+</sup> T cells were transferred into FV-infected wild-type (wt) recipients, and total CD4<sup>+</sup> T cells were isolated from the spleens 7 d later using immunomagnetic-positive selection (StemCell Technologies). Purified cells were subsequently cultured in the presence of 10 ng/ml recombinant human IL-2 and 50 ng/ml recombinant mouse IL-7. At the indicated time points, host and donor CD4<sup>+</sup> T cells were assessed for expression of TCRβ and TCRVα2 by flow cytometry.

### RNA extraction and gene expression profiling

Donor-type env-specific effector CD4<sup>+</sup> T cells were identified as CD45.1<sup>+</sup> CD44<sup>hi</sup>CD4<sup>+</sup> (TCRVα2<sup>+</sup> or TCRVα2<sup>-</sup>) and purified by cell sorting on day 7 posttransfer into FV-infected recipients. Naive TCRβ-transgenic CD4<sup>+</sup> T cell precursors were purified as CD44<sup>lo</sup>CD25<sup>-</sup>CD4<sup>+</sup> T cells from uninfected control mice. Between  $1.5 \times 10^6$  and  $2 \times 10^6$  purified CD4<sup>+</sup> T cells were used for RNA extraction using the RNeasy mini kit (Qiagen, Crawley, U.K.) according to the manufacturer's instructions. For quantitative RT-PCR analysis, RNA samples were reverse-transcribed with a High Capacity cDNA Reverse Transcription kit (Applied Biosystems, Foster City, CA) and used as template for the amplification of target gene transcripts with a SYBR Green PCR Master Mix (Applied Biosystems) and the ABI Prism 7000 Detection System (TaqMan; Applied Biosystems). Primers used for *Il2*, *Il4*, *Il10*, and *Tnfr* amplification (30), *Tbx21*, *Bcl6*, *Gata3*, *Rorc*, *Foxp3*, and *Il21* amplification (21), and *Ifng* amplification (41) have been previously described. The housekeeping gene *Hprt* was amplified with forward 5'-TTGTATACCTAATCATATGCCGAG-3' and reverse 5'-CATCTCGAGCAAGTCTTTCA-3' primers and was used to normalize the critical threshold values for the genes of interest. For microarray analysis, RNA samples were checked for quality using an Agilent bioanalyzer (Agilent Technologies, Santa Clara, CA). Synthesis of cDNA, probe labeling, and hybridization were performed using MouseGene 1.0 ST oligonucleotide arrays (Affymetrix, Santa Clara, CA). Primary microarray data were analyzed with GeneSpring GX (Agilent Technologies) and deposited at <http://www.ebi.ac.uk/arrayexpress> (E-MEXP-2950).

### Statistical analysis

ANOVA and statistical comparisons were made using SigmaPlot 11.0 (Systat Software). Parametric comparisons of normally distributed values

that satisfied the variance criteria were made by unpaired Student *t* tests. Linear percentages of FV-infected cells, spleen indices, and nAb titers, which did not pass the variance test, were compared with nonparametric two-tailed Mann–Whitney rank-sum or Wilcoxon signed-rank tests.

## Results

### *Virus-specific CD4<sup>+</sup> T cell differentiation during FV infection*

To study the expansion and differentiation of virus-specific CD4<sup>+</sup> T cells as a function of functional avidity, we used TCRβ-transgenic CD4<sup>+</sup> T cells, containing a polyclonal population of env-specific precursors. Approximately 25% of env-specific precursors in these TCRβ-transgenic CD4<sup>+</sup> T cells use an endogenous TCRVα2 chain, which creates >30-fold higher functional avidity than the use of other endogenous TCRVα-chains in the remaining 75% of env-specific precursors (ED<sub>50</sub> 0.04 μM and >1.24 μM env<sub>122–141</sub>, respectively) (32). TCRβ-transgenic CD4<sup>+</sup> T cells were adoptively transferred into allotypically marked syngeneic wt hosts that were infected with FV within a 24-h period. We monitored a cohort of 5000–10,000 env-specific CD4<sup>+</sup> T cells, which also excluded changes arising from recruitment of new naive precursors throughout the response. Env-specific CD4<sup>+</sup> T cells were identified among donor CD4<sup>+</sup> T cells by dilution of prior CFSE labeling or by upregulation of CD44 expression, with comparable results (33). CFSE-labeled CD45.1<sup>+</sup> TCRβ-transgenic T cells divided and accumulated substantially (>200-fold) following adoptive transfer into acutely FV-infected but not into uninfected CD45.2<sup>+</sup> B6 wt recipients (Fig. 1A). At this time point of peak expansion (33), gene expression analysis of env-specific donor CD4<sup>+</sup> T cells revealed a transcriptional program compatible with exclusive Th1 and Tfh differentiation (Fig. 1B).

The frequency of high-avidity TCRVα2<sup>+</sup> cells in env-specific donor CD4<sup>+</sup> T cells rose during the first 7 d from 25% in the naive repertoire to >65% (Fig. 1C, *left panel*) due to significantly higher expansion of high-avidity cells (500-fold) in comparison with low-avidity ones (50-fold) (Fig. 1C, *right panel*). Examination of the earliest time points of CD4<sup>+</sup> T cell expansion revealed that the numerical advantage of high-avidity TCRVα2<sup>+</sup> cells manifested between days 1 and 3 of the response, during which cohorts of low- and high-avidity env-specific cells had already significantly diluted the CFSE label and after which expansion of both low- and high-avidity env-specific cells followed similar kinetics to reach near peak numbers by day 5 (Supplemental Fig. 1). Although the potential effect of differential rate of death could not be excluded, the early numerical advantage of high-avidity TCRVα2<sup>+</sup> cells indicated faster recruitment and/or proliferation during the expansion phase. Both low- and high-avidity cells that responded were fully activated, suggested by high CD43 expression (Supplemental Fig. 2A), and expressed comparably higher levels than those in naive T cells of transcription factors *Tbx21* and *Bcl6*, specific to the Th1 and Tfh lineages, respectively (19), and of Th-specific cytokine genes encoding IFN-γ or IL-21 (Fig. 1D). Differences between low- and high-avidity subsets were also noted, with high-avidity TCRVα2<sup>+</sup> cells expressing lower levels of programmed death 1 (PD-1) and higher levels of ICOS than low-avidity cells (Supplemental Fig. 2B, 2C), and a higher frequency of high-avidity TCRVα2<sup>+</sup> cells could be further stimulated in vitro to produce IFN-γ, alone or in combination with IL-21 (Fig. 1E). Importantly, low- and high-avidity populations contained similar frequencies of Tfh cells, defined by high coexpression of PD-1 and the B cell follicle-homing chemokine receptor CXCR5 (Fig. 1F). Furthermore, purified TCRVα2<sup>+</sup> or TCRVα2<sup>−</sup> naive TCRβ-transgenic CD4<sup>+</sup> T cells, transferred separately into FV-infected T cell-deficient *Tcra*<sup>−/−</sup> hosts, induced comparably high levels of FV-neutralizing Abs (Fig. 1G), demonstrating that both high- and

low-avidity CD4<sup>+</sup> T cells provided sufficient help for the T cell-dependent B cell response. To control for any potential effects of the TCRVα2 Ab staining used to purify CD4<sup>+</sup> T cells, in additional experiments, CD4<sup>+</sup> T cells were either stained with the TCRVα2 Ab or not and were then transferred separately into hosts that were either acutely infected with FV or left uninfected. This comparison revealed that TCRVα2 Ab staining did not cause any measurable activation of CD4<sup>+</sup> T cells in the absence of infection, nor did it affect their response to FV infection (Supplemental Fig. 3). Collectively, these results indicated that high-avidity for Ag of TCRVα2<sup>+</sup> env-specific CD4<sup>+</sup> T cells conferred a substantial numerical advantage and significantly enhanced cytokine production potential but did not affect Tfh differentiation or function.

### *Impact of B cell deficiency on virus-specific CD4<sup>+</sup> T cell expansion and differentiation*

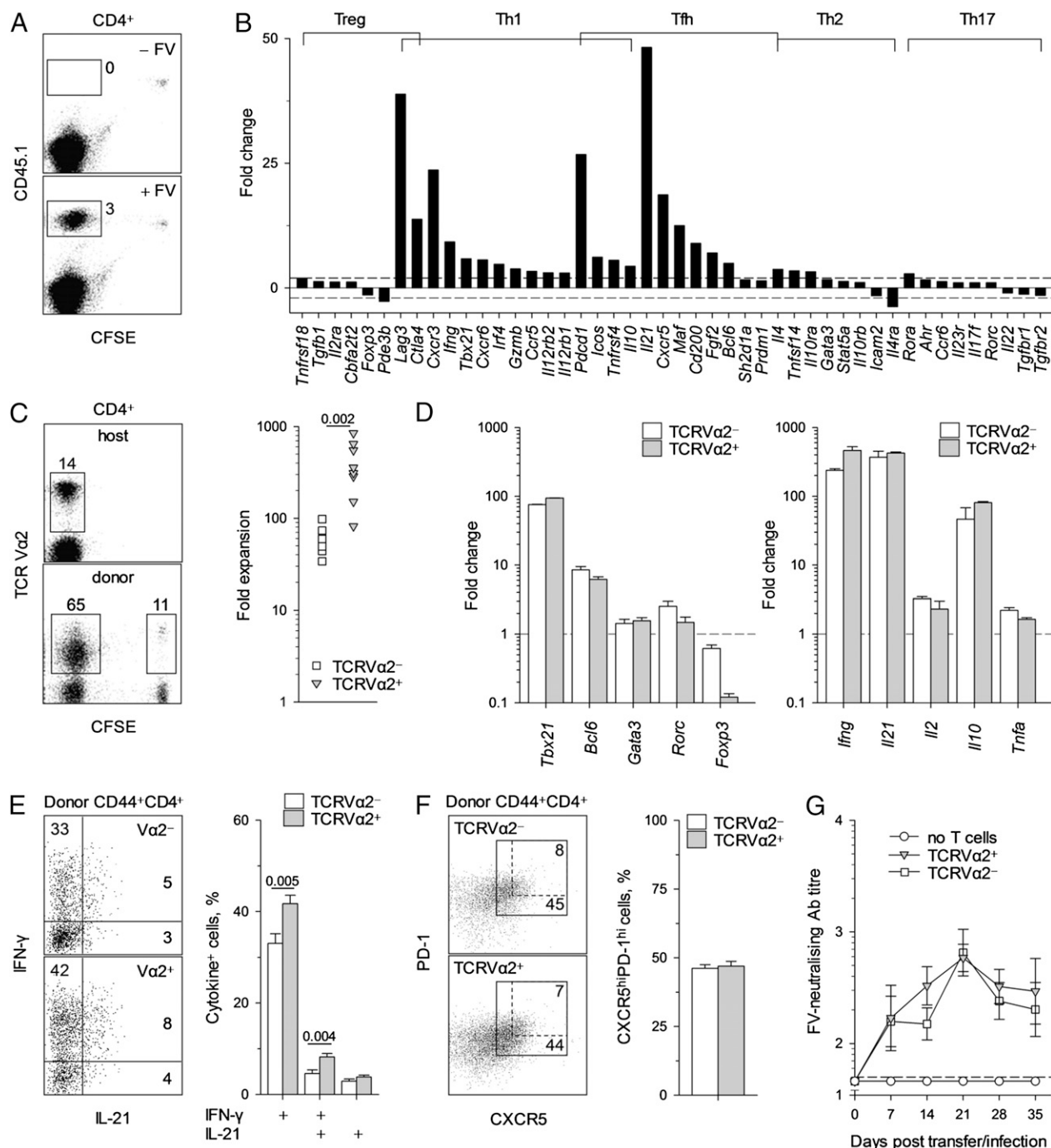
We next explored whether virus-specific CD4<sup>+</sup> T cell expansion and differentiation was shaped by interaction with B cells by comparing wt and B cell-deficient *Ighm*<sup>−/−</sup> hosts. Due to the absence of an FV-neutralizing Ab response, *Ighm*<sup>−/−</sup> hosts exhibited dramatically elevated levels of FV replication (Fig. 2A). Nevertheless, expansion of env-specific CD4<sup>+</sup> T cells was comparable in wt and *Ighm*<sup>−/−</sup> hosts (Fig. 2B). Notably, the proportion of Tfh cells, identified by high expression of CXCR5 and PD-1 in env-specific CD4<sup>+</sup> T cells (Fig. 2C), was significantly reduced in *Ighm*<sup>−/−</sup> hosts (Fig. 2D). In line with transcriptional analysis, IFN-γ and IL-21 were the major cytokines that could be recalled in env-specific CD4<sup>+</sup> T cells, whereas no IL-17A production could be detected (Fig. 2E). Analysis of cytokine production by env-specific CD4<sup>+</sup> T cells from either wt or *Ighm*<sup>−/−</sup> hosts revealed that B cell deficiency did not affect IL-21 production (Fig. 2F). In contrast, both the frequency of IFN-γ-producing cells and the amount of IFN-γ produced per cell were significantly increased in env-specific CD4<sup>+</sup> T cells from *Ighm*<sup>−/−</sup> hosts (Fig. 2G). Thus, in the absence of B cells, env-specific CD4<sup>+</sup> T cells displayed a preference for Th1 rather than Tfh differentiation. However, in addition to lack of FV-neutralizing Abs, B cell deficiency affects additional physiological processes, which could indirectly affect CD4<sup>+</sup> T cell differentiation. We therefore employed a system in which CD4<sup>+</sup> T cell interaction specifically with B cells could be impaired in an otherwise normal host.

In addition to initial integrin-dependent adhesion, CD4<sup>+</sup> T cell interaction with B cells is uniquely characterized by a longer second phase mediated by multiple signaling lymphocyte activation molecules (SLAMs) (20, 42). Signaling through SLAMs is transduced by SAP, encoded by *Sh2d1a*, and SAP deficiency in T cells shortens the duration of CD4<sup>+</sup> T cell contacts with B cells and impairs humoral immunity (5, 20, 22, 42). We generated SAP-deficient env-specific TCRβ-transgenic CD4<sup>+</sup> T cells, which we compared with wt env-specific TCRβ-transgenic CD4<sup>+</sup> T cells, both transferred into wt hosts infected with FV at the time of T cell transfer. Importantly, FV replication followed a similar course in wt hosts of both types of CD4<sup>+</sup> T cells (Fig. 2H), and peak expansion was also comparable between SAP-deficient or wt CD4<sup>+</sup> T cells (Fig. 2I).

### *Differentiation of high- and low-avidity virus-specific CD4<sup>+</sup> T cells when B cell interaction is absent or shortened*

To examine the impact of high- or low-avidity interaction of virus-specific CD4<sup>+</sup> T cells with B cells during FV infection, we compared the differentiation of wt env-specific TCRβ-transgenic CD4<sup>+</sup> T cells transferred into either wt or *Ighm*<sup>−/−</sup> hosts with that of *Sh2d1a*<sup>−/−</sup> env-specific TCRβ-transgenic CD4<sup>+</sup> T cells transferred into wt hosts. Indicated by the frequency of CXCR5<sup>hi</sup>PD-1<sup>hi</sup>

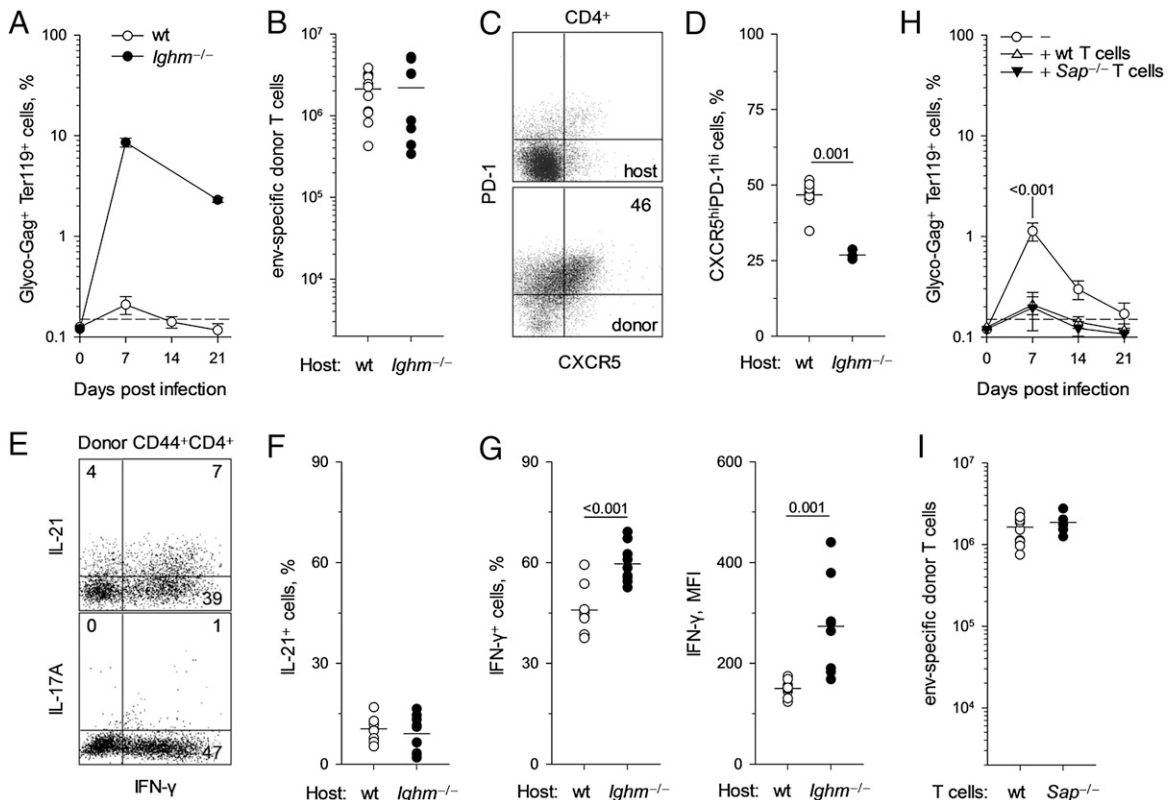




**FIGURE 1.** Virus-specific CD4<sup>+</sup> T cell differentiation during FV infection. **A–F**, Cohorts of CD45.1<sup>+</sup> TCRβ-transgenic CD4<sup>+</sup> T cells were adoptively transferred into wt recipients infected with FV the same day and recovered for analysis from the spleen of the recipients 7 d later. **A**, Dilution of prior CFSE label in gated CD4<sup>+</sup> T cells from acutely FV infected (+ FV) or uninfected (– FV) wt recipients. **B**, Fold change in the transcription of indicated genes, grouped according to Th class, in env-specific CD4<sup>+</sup> effector T cells compared with naive CD4<sup>+</sup> TCRβ-transgenic T cells ( $n = 3–6$ ). **C**, Frequency of TCR Va2<sup>+</sup> cells in host (CD45.1<sup>–</sup>) or env-specific (CFSE<sup>–</sup>CD45.1<sup>+</sup>) or nonspecific (CFSE<sup>+</sup>CD45.1<sup>+</sup>) donor CD4<sup>+</sup> T cells (left panel) and fold expansion of TCR Va2<sup>+</sup> or TCR Va2<sup>–</sup> env-specific donor (CD44<sup>hi</sup>CD45.1<sup>+</sup>) CD4<sup>+</sup> T cells between days 1 and 7 after transfer (right panel). Each symbol is an individual mouse. **D**, Fold change in the transcription of the indicated Th class-specific transcription factor (left panel) or cytokine (right panel) genes in TCR Va2<sup>+</sup> or TCR Va2<sup>–</sup> env-specific donor (CD44<sup>hi</sup>CD45.1<sup>+</sup>) CD4<sup>+</sup> T cells. **E**, Flow cytometric example (left panel) and mean frequency ( $\pm$  SEM;  $n = 9–12$ ) (right panel) of IFN-γ and IL-21 production by TCR Va2<sup>+</sup> or TCR Va2<sup>–</sup> env-specific donor CD4<sup>+</sup> T cells. **F**, CXCR5 and PD-1 expression in TCR Va2<sup>+</sup> or TCR Va2<sup>–</sup> env-specific donor CD4<sup>+</sup> T cells (left panel). Values within each gate denote the frequency of CXCR5<sup>hi</sup>PD-1<sup>hi</sup> cells in that gate, with the dashed gate restricted to cells coexpressing CXCR5 and PD-1 at the highest level. Mean frequency ( $\pm$  SEM;  $n = 17$ ) of CXCR5<sup>hi</sup>PD-1<sup>hi</sup> Tfh cells in either TCR Va2<sup>+</sup> or TCR Va2<sup>–</sup> env-specific donor CD4<sup>+</sup> T cells (right panel). **G**, Serum titers (medians  $\pm$  SEM;  $n = 5$  to 6) of FV-neutralizing Abs in *Tcrα*<sup>–/–</sup> hosts that received no T cells and those that received either TCR Va2<sup>+</sup> or TCR Va2<sup>–</sup> TCRβ-transgenic CD4<sup>+</sup> T cells.

cells, Tfh differentiation of *Sh2d1a*<sup>–/–</sup> env-specific CD4<sup>+</sup> T cells in wt hosts was intermediate between that of wt env-specific CD4<sup>+</sup> T cells in wt hosts and *Ighm*<sup>–/–</sup> hosts (Fig. 3A, 3B). Importantly,

the frequency of CXCR5<sup>hi</sup>PD-1<sup>hi</sup> cells remained comparable between TCR Va2<sup>+</sup> and TCR Va2<sup>–</sup> CD4<sup>+</sup> T cells (Fig. 3A, 3B), indicating that higher avidity did not favor Tfh differentiation even



**FIGURE 2.** Role of B cells in virus-specific CD4<sup>+</sup> T cell expansion and differentiation. **A–G,** Cohorts of CD45.1<sup>+</sup> TCRβ-transgenic CD4<sup>+</sup> T cells were adoptively transferred into wt or B cell-deficient *Ighm*<sup>-/-</sup> hosts and analyzed 7 d later. **A,** Mean frequency (± SEM; *n* = 5–9) of FV-infected (glyco-Gag<sup>+</sup>) Ter119<sup>+</sup> cells in the spleens of FV-infected wt or *Ighm*<sup>-/-</sup> hosts. **B,** Absolute number of splenic env-specific donor (CD44<sup>hi</sup>CD45.1<sup>+</sup>) CD4<sup>+</sup> T cells 7 d after transfer. **C,** Example of CXCR5 and PD-1 expression in host or donor CD4<sup>+</sup> T cells from wt hosts. **D,** Frequency of CXCR5<sup>hi</sup>PD-1<sup>hi</sup> cells in env-specific donor CD4<sup>+</sup> T cells from wt or *Ighm*<sup>-/-</sup> hosts. **E,** Example of IFN-γ, IL-21, and IL-17A production by env-specific donor CD4<sup>+</sup> T cells from wt hosts. **F** and **G,** Frequency of IL-21<sup>+</sup> cells (**F**) or IFN-γ<sup>+</sup> cells (**G, left panel**) and mean fluorescence intensity (MFI) of IFN-γ staining (**G, right panel**) in env-specific donor CD4<sup>+</sup> T cells from wt or *Ighm*<sup>-/-</sup> hosts. **H,** Mean frequency (± SEM, *n* = 8 to 9) of FV-infected (glyco-Gag<sup>+</sup>) Ter119<sup>+</sup> cells in the spleens of FV-infected wt hosts that received no T cells (–) in comparison with those that received wt (+ wt T cells) or *Sh2d1a*<sup>-/-</sup> (+ *Sap*<sup>-/-</sup> T cells) TCRβ-transgenic env-specific CD4<sup>+</sup> T cells. **I,** Absolute number of either wt or *Sh2d1a*<sup>-/-</sup> (*Sap*<sup>-/-</sup>) splenic env-specific donor (CD44<sup>hi</sup>CD45.1<sup>+</sup>) CD4<sup>+</sup> T cells 7 d after transfer into FV-infected wt hosts. In **B, D, F, G,** and **I,** each symbol is an individual mouse.

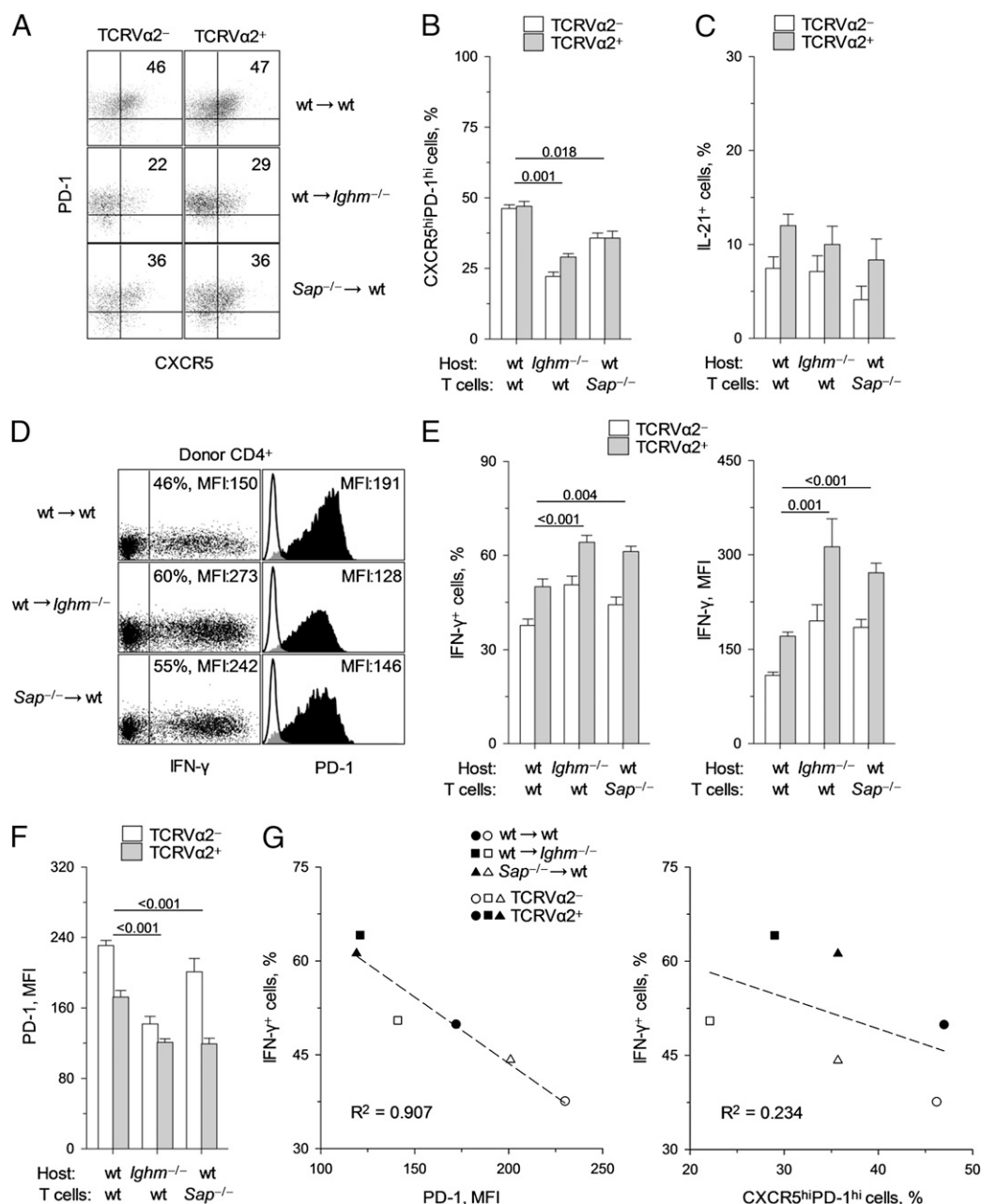
under suboptimal conditions. IL-21 production by env-specific CD4<sup>+</sup> T cells was not significantly affected by the reduction in B cell interaction, and high-avidity TCRVα2<sup>+</sup> CD4<sup>+</sup> T cells produced higher IL-21 levels than their low-avidity TCRVα2<sup>-</sup> counterparts in all three conditions (Fig. 3C). In comparison with wt CD4<sup>+</sup> T cells, *Sh2d1a*<sup>-/-</sup> CD4<sup>+</sup> T cells from wt hosts showed a significant increase in IFN-γ production, comparable with that of wt CD4<sup>+</sup> T cells from *Ighm*<sup>-/-</sup> hosts (Fig. 3D). Notably, this increase in IFN-γ production was accompanied by a reciprocal decrease in PD-1 expression levels (Fig. 3D). When analyzed separately, high-avidity env-specific CD4<sup>+</sup> T cells maintained increased potential for IFN-γ production than low-avidity CD4<sup>+</sup> T cells, independently of B cell interaction (Fig. 3E). Importantly, however, both the frequency of IFN-γ-producing cells and amount of IFN-γ produced per cell were comparably increased in both high- and low-avidity *Sh2d1a*<sup>-/-</sup> CD4<sup>+</sup> T cells from wt hosts and in wt CD4<sup>+</sup> T cells from *Ighm*<sup>-/-</sup> hosts, in comparison with wt CD4<sup>+</sup> T cells from wt hosts (Fig. 3E). These results indicated that low avidity for Ag and B cell interaction independently and comparably reduced IFN-γ production by env-specific CD4<sup>+</sup> T cells.

Although PD-1 expression in *Sh2d1a*<sup>-/-</sup> CD4<sup>+</sup> T cells from wt hosts was reduced in comparison with wt CD4<sup>+</sup> T cells from wt hosts, low-avidity CD4<sup>+</sup> T cells expressed significantly higher levels of PD-1 than high-avidity CD4<sup>+</sup> T cells, independently of SAP expression (Fig. 3F). Contrastingly, PD-1 expression was

similarly reduced in both high- and low-avidity wt CD4<sup>+</sup> T cells from *Ighm*<sup>-/-</sup> hosts (Fig. 3F), suggesting that PD-1 expression in env-specific CD4<sup>+</sup> T cells was regulated by low-avidity interaction preferentially with B cells. Importantly, the frequency of IFN-γ-producing cells in either high- or low-avidity env-specific CD4<sup>+</sup> T cells followed a close inverse correlation with the level of PD-1 expression, but not with the proportion of CXCR5<sup>hi</sup>PD-1<sup>hi</sup> Tfh cells (Fig. 3G), indicating a mechanistic link. In contrast to regulation of PD-1 expression by B cell interaction, ICOS expression in several different conditions varying in FV replication levels was regulated strictly by Ag levels (Supplemental Fig. 4) and did not correlate with the overall Tfh phenotype. Lastly, the reduction of IFN-γ production by CD4<sup>+</sup> T cells that interacted with B cells did not depend on IL-10 production by B cells, as expansion, Tfh differentiation, and IFN-γ and IL-21 production by env-specific CD4<sup>+</sup> T cells were similar when these cells were transferred into wt or IL-10-deficient hosts (data not shown).

#### B cells induce TCR downregulation in virus-specific CD4<sup>+</sup> T cells

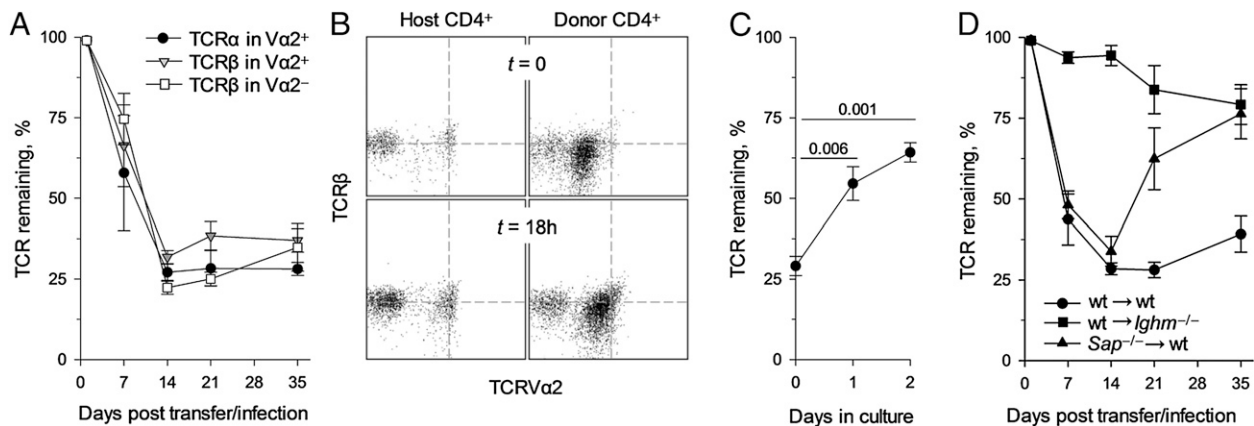
Regulation of the CD4<sup>+</sup> T cell response by B cells has been suggested to involve Ag-induced downregulation of the TCR (16, 43). We have previously observed significant TCR downregulation in env-specific CD4<sup>+</sup> T cells at the peak of their expansion during FV infection (33), and it was therefore of interest to determine



**FIGURE 3.** Effect of absent or reduced B cell interaction on the phenotype of virus-specific CD4<sup>+</sup> T cells. **A–G**, Cohorts of CD45.1<sup>+</sup> wt TCRβ-transgenic CD4<sup>+</sup> T cells were transferred into wt (wt → wt) or *Ighm*<sup>-/-</sup> hosts (wt → *Ighm*<sup>-/-</sup>), and CD45.1<sup>+</sup> *Sh2d1a*<sup>-/-</sup> TCRβ-transgenic CD4<sup>+</sup> T cells were transferred into wt hosts (*Sap*<sup>-/-</sup> → wt). All hosts were infected with FV the day of the T cell transfer, and donor CD4<sup>+</sup> T cells were recovered for analysis from the spleens of these hosts 7 d later. **A**, CXCR5 and PD-1 expression in either TCRVα2<sup>+</sup> or TCRVα2<sup>-</sup> env-specific donor CD4<sup>+</sup> T cells. Values within each gate denote the frequency of CXCR5<sup>hi</sup>PD-1<sup>hi</sup> cells. **B**, Frequency of CXCR5<sup>hi</sup>PD-1<sup>hi</sup> cells. **C**, Frequency of IL-21<sup>+</sup> cells. **D**, Flow cytometric measurement of the frequency of IFN-γ<sup>+</sup> cells and MFI of intracellular IFN-γ staining (left panel) and MFI of PD-1 surface staining (right panel) in env-specific donor CD4<sup>+</sup> T cells. **E**, Frequency of IFN-γ<sup>+</sup> cells (left panel) and MFI of intracellular IFN-γ staining (right panel) in either TCRVα2<sup>+</sup> or TCRVα2<sup>-</sup> env-specific donor CD4<sup>+</sup> T cells. **F**, MFI of PD-1 surface staining. **G**, Correlation between frequency of IFN-γ<sup>+</sup> cells and either the MFI of PD-1 expression (left panel) or the frequency of CXCR5<sup>hi</sup>PD-1<sup>hi</sup> cells (right panel) in TCRVα2<sup>+</sup> or TCRVα2<sup>-</sup> env-specific donor CD4<sup>+</sup> T cells from the hosts described above. Each symbol is the mean of each condition. In each panel, data show the means ± SEM (*n* = 6–9).

whether it was induced by B cells. TCR levels were substantially reduced at the peak of the response to FV in both high- and low-avidity env-specific CD4<sup>+</sup> T cells to a comparable degree (Fig. 4A). Notably, TCR levels remained significantly reduced throughout infection and were restored when T cells were separated from persisting Ag and cultured in vitro (Fig. 4B, 4C). In stark contrast, TCR levels were only minimally reduced in env-specific CD4<sup>+</sup> T cells recovered from infected *Ighm*<sup>-/-</sup> hosts (Fig. 4D). Interestingly, the shortened duration of interaction between

B cells and *Sh2d1a*<sup>-/-</sup> env-specific CD4<sup>+</sup> T cells (22) was sufficient to cause TCR downregulation, comparable to that in wt env-specific CD4<sup>+</sup> T cells, during acute FV infection, but not during chronic FV infection (Fig. 4D). Thus, increased Ag availability for presentation by B cells during the acute phase of infection could overcome the requirement for SAP expression in CD4<sup>+</sup> T cells for TCR downregulation induced by B cell interaction. Nevertheless, the finding that *Sh2d1a*<sup>-/-</sup> CD4<sup>+</sup> T cells from wt hosts produced elevated IFN-γ levels, similar to wt CD4<sup>+</sup> T cells from *Ighm*<sup>-/-</sup>



**FIGURE 4.** B cells induce profound but reversible TCR downregulation in virus-specific CD4<sup>+</sup> T cells. *A*, CD45.1<sup>+</sup> TCRβ-transgenic CD4<sup>+</sup> T cells were adoptively transferred into FV-infected wt recipients, and TCR expression was monitored throughout infection. Levels of TCRβ and TCRVα2 expression in TCRVα2<sup>+</sup> env-specific cells donor CD4<sup>+</sup> T cells and levels of TCRβ in TCRVα2<sup>-</sup> env-specific cells donor CD4<sup>+</sup> T cells are shown ( $n = 5-7$ ). *B* and *C*, Purified CD4<sup>+</sup> T cells (host and donor) recovered on day 7 from FV-infected wt recipients were subsequently cultured in vitro in the presence of IL-2 and IL-7. TCRβ and TCRVα2 expression in host or env-specific donor (CD45.1<sup>+</sup>CD44<sup>+</sup>) gated CD4<sup>+</sup> T cells at the time of ex vivo recovery ( $t = 0$ ) or after an 18 h in vitro culture ( $t = 18$  h) (*B*). Dashed lines indicate the MFI of TCRβ or TCRVα2 expression in host CD4<sup>+</sup> T cells. Levels of TCR remaining on TCRVα2<sup>+</sup> env-specific cells from the same env-specific donor CD4<sup>+</sup> T cells after the indicated length of in vitro culture (*C*) ( $n = 4$ ). *D*, Levels of TCRVα2 remaining on wt TCRβ-transgenic CD4<sup>+</sup> T cells transferred into wt (wt → wt) or *Ighm*<sup>-/-</sup> hosts (wt → *Ighm*<sup>-/-</sup>) and *Sh2d1a*<sup>-/-</sup> TCRβ-transgenic CD4<sup>+</sup> T cells were transferred into wt hosts (*Sap*<sup>-/-</sup> → wt), following FV infection ( $n = 6-9$ ). In *A*, *C*, and *D*, values are the means ( $\pm$  SEM) of TCRβ or TCRVα2 MFI in env-specific donor cells, expressed as a percentage of the same intensity in nonspecific donor or host cells.

hosts, despite profound TCR downregulation, suggested that TCR downregulation was not a requirement for inhibition of IFN- $\gamma$  production.

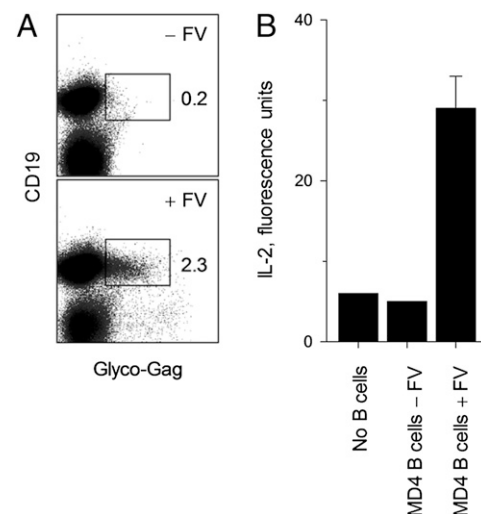
#### Virus-nonspecific B cells affect virus-specific CD4<sup>+</sup> T cell function

We next addressed whether the impact of B cell interaction on differentiation of env-specific CD4<sup>+</sup> T cells translated into an effect on either the protective or pathogenic function mediated directly by CD4<sup>+</sup> T cells. FV infection in B6 mice is relatively mild due to a strong FV-neutralizing Ab response and to genetic resistance at the *Fv2* locus, which prevents splenomegaly (34). We therefore examined if B cells could directly affect CD4<sup>+</sup> T cell-mediated virus control or immune pathology in a severe infection, in which FV-neutralizing Abs could also be precluded. We compared lymphocyte-deficient mice expressing the *Fv2* susceptibility (*Fv2*<sup>s</sup>) allele (*Rag1*<sup>-/-</sup> *Fv2*<sup>s</sup>) with those additionally harboring a monoclonal B cell population specific to the FV-unrelated Ag HEL (*Rag1*<sup>-/-</sup> *Fv2*<sup>s</sup> MD4). B cells are targets of FV infection and can present viral Ags to T cells in wt mice (44, 45). We further established that HEL-specific B cells were also infected by FV, and, when isolated from FV-infected MD4, BCR-transgenic mice presented Ag to and stimulated env-specific CD4<sup>+</sup> T cells (Fig. 5). However, MD4 BCR-transgenic B cells would not directly contribute to antiviral immunity. Transfer of env-specific TCRβ-transgenic CD4<sup>+</sup> T cells in *Rag1*<sup>-/-</sup> *Fv2*<sup>s</sup> hosts infected with F-MuLV alone (which does not cause splenomegaly without SFFV) led to significantly more severe anemia and weight loss than in *Rag1*<sup>-/-</sup> *Fv2*<sup>s</sup> MD4 hosts (Fig. 6*A*). When infected with FV, both types of hosts rapidly developed severe splenomegaly (Fig. 6*B*). Remarkably, however, transfer of TCRβ-transgenic T cells protected *Rag1*<sup>-/-</sup> *Fv2*<sup>s</sup> hosts, but failed to protect *Rag1*<sup>-/-</sup> *Fv2*<sup>s</sup> MD4 hosts against splenomegaly (Fig. 6*B*). Thus, the presence of FV-neutral B cells negated the antiviral effect and diminished the pathogenic effect of env-specific CD4<sup>+</sup> T cells during FV infection.

## Discussion

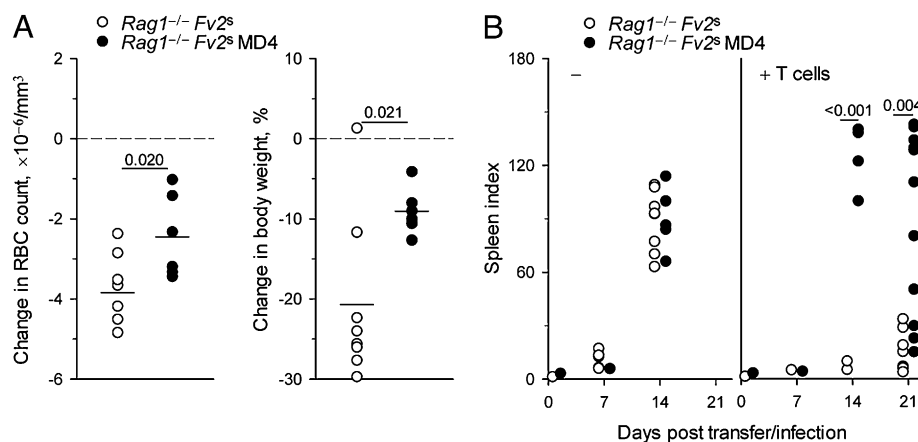
Upon Ag recognition, naive CD4<sup>+</sup> T cells expand and acquire one of many distinct sets of effector functions and migration prop-

erties. The final outcome of CD4<sup>+</sup> T cell expansion and differentiation is heavily influenced by T cell avidity for Ag as well as the type of APC. The results of the current study demonstrated that key aspects of the CD4<sup>+</sup> T cell response to a retroviral Ag were extensively shaped by interaction with B cells. We found that B cell interaction was dispensable for CD4<sup>+</sup> T cell priming following FV infection. It did, however, contribute significantly to the differentiation of virus-specific CD4<sup>+</sup> T cells toward the Tfh phenotype. More importantly, B cell interaction dramatically reduced the potential of virus-specific CD4<sup>+</sup> T cells to produce IFN- $\gamma$ , in proportion with induction of PD-1 expression,



**FIGURE 5.** Infection of MD4 BCR-transgenic B cells and presentation of viral Ags to env-specific CD4<sup>+</sup> T cells. Splenocytes from MD4 BCR-transgenic (*Ighm*<sup>-/-</sup> MD4) mice that were infected with FV 7 d earlier (+ FV) or left uninfected (- FV) were isolated. *A*, The frequency of FV-infected B cells (glyco-Gag<sup>+</sup>CD19<sup>+</sup>) in total splenocytes is shown. *B*, CD19<sup>+</sup> cells were further purified and used for the stimulation of an env-specific T cell hybridoma cell line. IL-2 production by the T cell hybridoma cells in culture supernatant is shown. Data are the means  $\pm$  SEM;  $n = 2-4$ .





**FIGURE 6.** B cells determine the pathogenic and protective effect of virus-specific CD4<sup>+</sup> T cells. *A*, Change in RBC count (left panel) and body weight (right panel) of *Rag1*<sup>-/-</sup> Fv2<sup>s</sup> or *Rag1*<sup>-/-</sup> Fv2<sup>s</sup> MD4 hosts 21 d post-adoptive transfer of TCR $\beta$ -transgenic CD4<sup>+</sup> T cells and infection with only the B-tropic helper virus F-MuLV, relative to the average RBC count and body weight of uninfected control groups (dashed lines). *B*, Spleen indices (spleen weight, milligrams; body weight, grams) of *Rag1*<sup>-/-</sup> Fv2<sup>s</sup> or *Rag1*<sup>-/-</sup> Fv2<sup>s</sup> MD4 hosts following FV infection in conjunction with (+ T cells) or without (-) adoptive transfer of TCR $\beta$ -transgenic CD4<sup>+</sup> T cells. In *A* and *B*, each symbol is an individual mouse from one representative of two experiments.

but not with Tfh differentiation, with profound consequences for CD4<sup>+</sup> T cell-mediated antiviral immunity and immune pathology.

B cells have been shown to impact on CD4<sup>+</sup> T cell function by influencing CD4<sup>+</sup> T cell expansion or differentiation in numerous experimental systems, although the results were highly variable and often conflicting (1). B cells have not been consistently found necessary for CD4<sup>+</sup> T cell priming, unless Ag may be limiting (1). However, several studies of a diverse range of parasites, bacteria, and viruses have shown that in most cases, B cell interaction is required for a full Th1 and Th2 effector response (6–15). Similarly, SAP-dependent B cell interaction has also been shown to contribute to Tfh differentiation, with the amount of this contribution reflecting Ag availability (5, 18–21, 46). Thus, B cells can contribute to a variable degree to CD4<sup>+</sup> T cell differentiation into many functionally distinct subsets.

In contrast, we find that B cell interaction during FV infection promoted Tfh, at the expense of Th1 differentiation of virus-specific CD4<sup>+</sup> T cells. The requirement for B cell interaction for Tfh differentiation of CD4<sup>+</sup> T cells responding to FV infection is seemingly at odds with the suggestion that continuous Ag presentation overcomes this requirement for B cell interaction (5). More recently, three separate reports demonstrated that although B cells may be dispensable for the initial differentiation of Tfh cells, they are critically involved in their maintenance and function (47–49). Although eventually controlled to low levels of replication, FV causes persistent infection in B6 mice and viral Ags are presented chronically. Furthermore, B cell-deficient mice are unable to control FV replication and thus constantly present very high levels of viral Ags. Nevertheless, both the presence of B cells and SAP expression in CD4<sup>+</sup> T cells were necessary for full Tfh differentiation in this model. Based on these seemingly opposing observations, we would propose that B cells promote Tfh differentiation when polarizing conditions are suboptimal, in a weakly proinflammatory environment and when Ag is the limiting factor for CD4<sup>+</sup> T cells to complete their differentiation program. This contribution of B cells may be overcome with increased Ag availability. At the other end of the spectrum, in a strongly proinflammatory environment and high Ag availability, the limiting factor for Tfh differentiation may be competition with alternative differentiation programs. This is particularly relevant to retroviral infection, in which we have observed exclusive Th1

and Tfh differentiation of virus-specific CD4<sup>+</sup> T cells. In this light, the contribution of B cells to Tfh differentiation of virus-specific CD4<sup>+</sup> T cells during FV infection may rely on their ability to suppress otherwise favored Th1 differentiation.

In addition to B cell interaction, the strength of TCR Ag binding has been suggested to regulate Tfh differentiation (30). This was studied during the CD4<sup>+</sup> T effector response to peptide immunization, in which CD4<sup>+</sup> T cells with stronger peptide–MHC class II complex binding showed increased expression of markers associated with the Tfh subset, although Tfh function was not investigated and was only inferred by phenotype (30). Our results following retroviral infection did not support this notion, and we failed to detect, by any of the readouts used, any preference for Tfh differentiation in either high- or low-avidity virus-specific CD4<sup>+</sup> T cells. Direct comparison of these results is complicated by experimental differences. For example, the relative TCR affinities for the respective Ags used in these studies may differ substantially. Also, kinetics of Ag availability are drastically different, peaking on day 0 following peptide immunization and on day 7 following viral infection, in relative synchrony with the CD4<sup>+</sup> T cell response. Lastly, preferential Tfh differentiation of high-avidity CD4<sup>+</sup> T cells may be particularly pronounced in conditions of limiting Ag availability. Indeed, preferential representation of high-avidity CD4<sup>+</sup> T cells in the Tfh subset was accentuated following immunization with less immunogenic peptides (30). These findings suggest that a preference for Tfh differentiation of high-avidity CD4<sup>+</sup> T cells may be a result of suboptimal conditions rather than a general feature of the CD4<sup>+</sup> T cell response. More importantly, we found that high- and low-avidity CD4<sup>+</sup> T cells provided comparable help for the T cell-dependent FV-neutralizing Ab response, which clearly demonstrated that in response to FV infection, low avidity for Ag hinders neither the acquisition nor expression of Tfh function.

Notably, our results revealed that the potential for IFN- $\gamma$  production by virus-specific CD4<sup>+</sup> T cells was significantly reduced if interaction with B cells was allowed, independently of an indirect effect of B cells on Ag availability. It could be argued that B cell-mediated suppression of the potential for IFN- $\gamma$  production is a consequence of their effect in promoting Tfh differentiation. Indeed, Tfh cells have been shown to produce reduced amounts of IFN- $\gamma$  in comparison with Th1 cells (50–52). However, our comparison of high- and low-avidity virus-specific CD4<sup>+</sup> T cells

revealed significant differences in IFN- $\gamma$  production, but not Tfh differentiation. Furthermore, the effect of B cells on IFN- $\gamma$  production by virus-specific CD4<sup>+</sup> T cells strongly correlated with PD-1 regulation, with T cells displaying the lowest PD-1 levels expressing the highest IFN- $\gamma$  amount, but correlated only weakly with Tfh differentiation. These results are in keeping with the established role of PD-1 in inhibiting T cell activation and effector cytokine production (53, 54). Lastly, PD-1 expression during FV infection was not restricted to Tfh cells, defined by high coexpression of CXCR5, but was also present in non-Tfh virus-specific CD4<sup>+</sup> T cells. Thus, B cell interaction reduced IFN- $\gamma$  production by CD4<sup>+</sup> T cells in line with PD-1 upregulation. These consequences of CD4<sup>+</sup> T cell interaction with B cells may be necessary for the optimization of the B cell response. Indeed, high PD-1 expression in Tfh cells has been shown to contribute to germinal center B cell survival and selection and long-lived plasma-cell generation (55).

Interaction with B cells also induced profound TCR downregulation in virus-specific CD4<sup>+</sup> T cells. This loss of surface TCR was reversible, indicating it was due to persistent antigenic stimulation during FV infection. B cell-mediated TCR downregulation in Ag-specific CD4<sup>+</sup> T cells has been previously seen in two studies using mice transgenically expressing the cognate Ag (16, 43). It is currently unclear whether Ag presentation by B cells is uniquely capable of inducing TCR downregulation or if this is a consequence of an overabundance (by a factor of 10) of B cells compared with other MHC class II-expressing cells in lymphoid organs. Although not always required, B cell-induced TCR downregulation has also been linked to CD4<sup>+</sup> T cell unresponsiveness to further stimulation (16, 43). This loss of TCR caused by B cells could indeed further compromise the ability of virus-specific CD4<sup>+</sup> T cells to mediate antiviral immunity or immune pathology.

It is interesting to note that, although B cells have been found to promote CD4<sup>+</sup> T cell effector responses against many pathogens, B cell interaction has also been shown to inhibit the CD4<sup>+</sup> T cell effector response against self-Ags or tumors (1, 16, 17). Indeed, B cell presentation of tumor Ags may induce a nonprotective Ab response instead of T cell-mediated antitumor immunity (17). Furthermore, B cell depletion has been shown to be beneficial in certain T cell-mediated autoimmune diseases (1). In this respect, our findings suggest that the effect of B cells on the CD4<sup>+</sup> T cell response to retroviral infection is more similar to that of the response to self-Ags than to pathogens. Thus, in the case of retroviral infection, efficient CD4<sup>+</sup> T cell–B cell collaboration provides help for B cells at the expense of B cell-independent CD4<sup>+</sup> T cell functions and can therefore be seen as a switch between cellular and humoral immunity. Such a switch could be exploited to reduce an excessive CD4<sup>+</sup> T cell response and associated immune pathology. It could also be manipulated to promote B cell-independent CD4<sup>+</sup> T cell-mediated immunity instead of an ineffective Ab response.

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## Disclosures

The authors have no financial conflicts of interest.

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