

Antigen affinity and antigen dose exert distinct influences on CD4 T-cell differentiation

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Cumulative T-cell receptor signal strength and ensuing T-cell responses are affected by both antigen affinity and antigen dose. Here we examined the distinct contributions of these parameters to CD4 T-cell differentiation during infection. We found that high antigen affinity positively correlates with T helper (Th)1 differentiation at both high and low doses of antigen. In contrast, follicular helper T cell (T_{FH}) effectors are generated after priming with high, intermediate, and low affinity ligand. Unexpectedly, memory T cells generated after priming with very low affinity antigen remain impaired in their ability to generate secondary Th1 effectors, despite being recalled with high affinity antigen. These data challenge the view that only strongly stimulated CD4 T cells are capable of differentiating into the T_{FH} and memory T-cell compartments and reveal that differential strength of stimulation during primary T-cell activation imprints unique and long lasting T-cell differentiation programs.

follicular helper | infection | lymphocytes

Following infection, T-cell receptor (TCR) interactions with foreign peptide/MHC (pMHC) drive the rapid clonal expansion and differentiation of T cells into distinct effector subsets specialized against different classes of microbes. An early bifurcation in CD4 T-cell responses results in the generation of T helper (Th)1 effectors, which regulate innate cell microbicidal function and follicular helper T (T_{FH}) cells, which migrate to B-cell follicles to regulate germinal center (GC) responses and antimicrobial antibody production (1). After pathogen is cleared, T cells undergo a contraction phase during which the majority of effectors die by apoptosis, leaving behind a population of long-lived memory cells to provide protection upon subsequent reinfection. The decision to differentiate into Th1 and T_{FH} lineages appears to occur very early in the immune response (2, 3). Initial T-cell priming by dendritic cells (DCs) is sufficient to induce fate-committed Th1 and T_{FH} cells as early as 3 d after infection, whereas maintenance and further expansion of the T_{FH} compartment depends on T-cell interactions with B cells (2). Similarly, memory T-cell differentiation occurs very early after infection and is critically dependent on B-cell interactions for optimal priming (4, 5). Importantly, CD4 T-cell differentiation is coupled to division, and unlike CD8 T-cell differentiation, requires constant antigen recognition (6, 7).

Although the strength of TCR–pMHC interactions has been shown to directly modulate T-cell expansion and clonal dominance within the Th cell compartment (8, 9), how this influences CD4 T-cell fate is not well understood. Cumulative TCR signaling can be influenced by both antigen affinity and antigen dose (10). In terms of proliferation, higher antigen dose can compensate for lower antigen affinity to some extent, but several reports have shown independent effects on T-cell responses both in vitro and in vivo (10–12). These data indicate that antigen affinity and antigen dose may promote qualitatively distinct TCR signals. Recently, modulation of the overall TCR signal by varying either TCR affinity or antigen dose was shown to influence the pattern of effector T-cell differentiation, with higher affinity ligands or higher antigen dose promoting T_{FH} generation

(13–15). However, another study examining high and low avidity CD4 T-cell responses during viral infection found significant differences in Th1 but not T_{FH} generation (16). Sustained TCR–pMHC interactions have also been shown to promote memory T-cell differentiation, which is associated with increased TCR avidity (17, 18). These studies, however, have focused on the development of the Th1 memory compartment, which is phenotypically and functionally distinct from the T_{FH} memory compartment (19, 20). Thus, although strong TCR signals resulting from high antigen affinity or high antigen dose can clearly affect the extent and quality of T-cell differentiation, whether or not T cells can discriminate these signals, and how this contributes to T-cell differentiation during infection, has not been determined.

To address this question, we infected mice with varying concentrations of *Listeria* expressing either high or low affinity antigens for the TCR. By normalizing the degree of proliferation induced by high and low affinity antigens we were able to discern distinct influences of antigen affinity and antigen dose on Th cell differentiation. We observed a strong positive correlation between antigen affinity and Th1 differentiation that occurs early and is dose independent. Importantly, high antigen dose does not compensate for the low efficiency of Th1 differentiation induced by low affinity antigen. In contrast, early T_{FH} effector generation was observed after priming with high, intermediate, and low affinity antigen, but was not maintained at later time points under conditions of low antigen dose. In addition, we found that T cells activated by either high or low affinity antigen are equally capable of memory T-cell differentiation. Surprisingly, memory T cells generated by either low antigen affinity or low antigen dose maintained their biased effector lineages following recall activation with high affinity antigen. These data indicate that differential strength of

Significance

T-cell receptor recognition of antigen is an essential first step in the initiation of a T-cell response. This report demonstrates that CD4 T cells responding during an infection can discriminate between antigen affinity and antigen dose, resulting in distinct types of effector and memory cell generation. Moreover, memory T cells “remember” the strength of primary T-cell activation and maintain a biased recall response upon secondary infection. These data reveal that antigen affinity exerts an important influence on T-cell differentiation that is not compensated for by high antigen dose. Understanding the rules of CD4 T-cell differentiation is integral to effective vaccine design.

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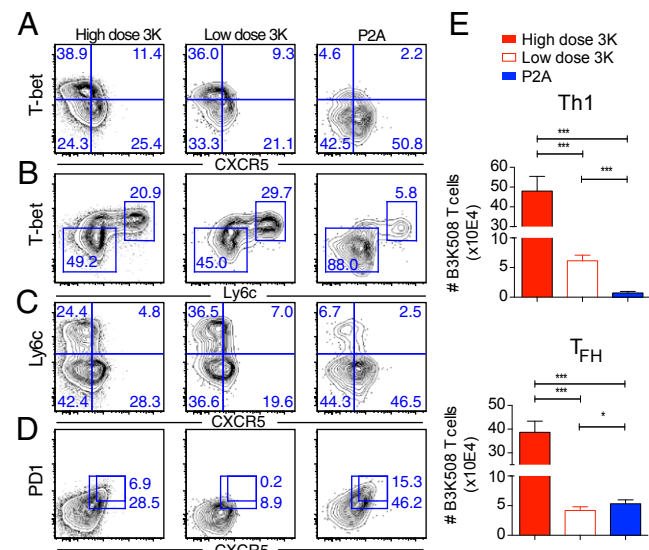
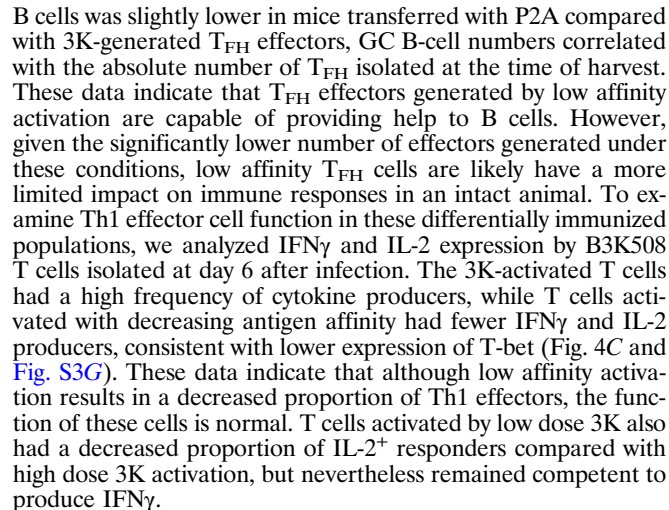


Fig. 2. Antigen affinity and effector T-cell differentiation. Ten thousand B3K508 T cells were transferred into congenic mice followed by infection with Lm.3K or Lm.P2A. (A–D) Representative flow cytometry plots of B3K508 T cells at day 6. (E) Absolute number of Th1 (T-bet^{high}CXCR5⁺) and T_{FH} (T-bet^{low}CXCR5⁺) effectors recovered from spleen and lymph nodes at day 6. Data represent $n \geq 3$ and are representative of three independent experiments. (* $P < 0.05$. *** $P < 0.0001$).

and could be observed under all conditions (Figs. S3C and S4C). T cells activated in vitro demonstrated a similar correlation between antigen affinity and early IL-2 and T-bet expression at a wide range of antigen doses (Fig. S5). In contrast, T cells activated in vitro with low affinity antigen expressed more Bcl6, possibly due to decreased production of IL-2, which can negatively regulate Bcl6 expression (33). Indeed, blocking IL-2 in vitro led to further increased Bcl6 expression in low affinity activated T cells, particularly at antigen concentrations where IL-2 production was evident (Fig. S5). Taken together, these data reveal that antigen affinity is key to setting the early Th1 differentiation potential, whereas T_{FH} differentiation is minimally impacted.

Location and Function of T_{FH} and Th1 Effector Cells Generated by Low Affinity Ligand. Following infection, Th1 effector cells migrate to

splenic T-cell zones and red pulp, and T_{FH} effector cells are sequestered in the B-cell follicles (1). To determine the location of CD4 T cells activated by high or low affinity ligands we analyzed frozen splenic sections by immunofluorescence microscopy. At day 6 after infection, T cells activated by high dose 3K ligand were identified in all three splenic areas (Fig. 4A). T cells activated by low dose 3K were predominantly located in splenic T-cell zones and were less abundant in B-cell areas (Fig. 4A), consistent with their decreased expression of PD-1. Consistent with high expression of T_{FH} markers, P2A activated T cells were predominantly observed in splenic B-cell zones or at the B-cell–T-cell area border, although a reduced proportion could also be detected in the red pulp and T-cell zones (Fig. 4A). Although it is possible that Th1 effectors generated after low affinity activation emigrate prematurely from lymphoid organs, we did not observe a higher proportion of these cells in the splenic red pulp, a major site of T-cell trafficking to the blood. As the primary function of T_{FH} effector cells is to help B cells, we also examined T_{FH} helper function. T_{FH} cells generated after Lm.3K or Lm.P2A infection were sorted based on high expression of CXCR5 and PD-1 and transferred into CD3ε^{-/-} mice that had been s.c. immunized with 3K-OVA one day earlier. At day 6 after transfer, both 3K and P2A generated T_{FH} effectors maintained high expression of CXCR5 and PD-1 and induced GC B cells in CD3ε^{-/-} mice (Fig. 4B and Fig. S6A and B). Although the proportion of GC



Memory T Cells Generated by Low-Affinity pMHC-TCR Interactions Exhibit Distinct Recall Properties.

tion is suggested to be driven by the strength of TCR signaling during the primary response (17, 18). However, CXCR5⁺ T cells present at early time points after infection have also been suggested to be precursors to the long-lived central memory T-cell compartment (3). As low affinity P2A activation resulted in efficient differentiation of CXCR5⁺ CD4 T-cell effectors, we wondered if low affinity interactions were sufficient to generate long-lived memory T cells. Similar to 3K-activated T cells, P2A-activated T-cell numbers peaked at ~5–6 d after infection followed by a rapid contraction phase between days 6 and 25 (Fig. S7A). P2A-activated T cells underwent less contraction compared with high dose 3K-activated T cells, and both populations could be identified in the long-lived memory T-cell compartment. Unlike polyclonal memory T cells, which have been shown to differentiate into distinct T-bet^{high}CXCR5[−] and T-bet^{low}CXCR5⁺ populations (3), the majority of B3K508 memory T cells had high expression of CXCR5 regardless of the nature of their primary stimulation (Fig. S7B), possibly due to the intrinsic nature of the B3K508 TCR (15). However, 3K memory T cells generated by both high and low antigen doses had higher overall expression of T-bet and a larger proportion of Ly6c^{high}T-bet^{high} cells, while

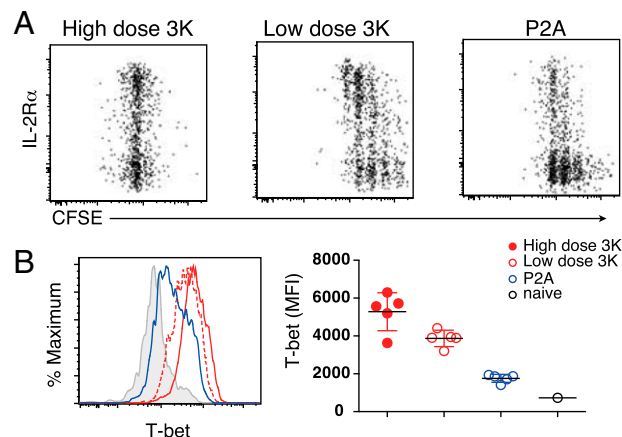


Fig. 3. Antigen affinity and early IL-2 induction. Two hundred thousand carboxyfluorescein succinimidyl ester (CFSE)-labeled B3K508 T cells were followed by infection with Lm.3K or Lm.P2A. CD25 proliferation (A) and T-bet expression (B) were assessed by flow cytometry after 2 (A) and 3 (B) d. T-bet MFI is shown for T cells that have undergone at least one division. Data represent $n = 3$ for each data point and are representative of two independent experiments. (** $P < 0.001$, *** $P < 0.0001$).

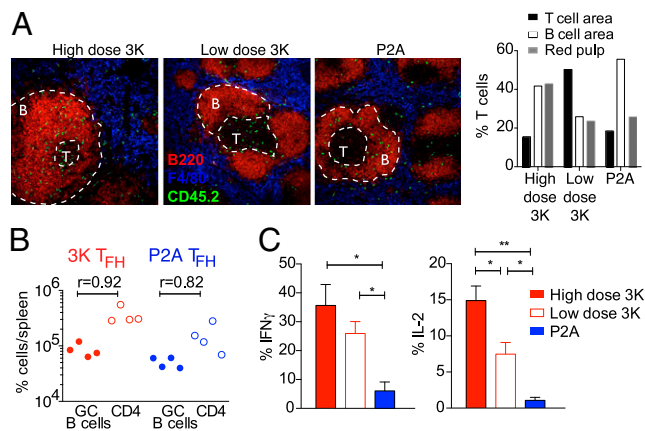


Fig. 4. Function and location of low affinity activated T cells. (A) Splenic sections were stained for B220 (red) to identify B-cell zones, F4/80 (blue) to identify red pulp area, and CD45.2 (green) to identify B3K508 donor T cells. Percentage of B3K508 T cells located in the indicated areas at day 6. (B) Th1 and T_{FH} cells were sorted from infected mice and transferred into CD3ε^{-/-} mice immunized 1 d earlier with 3K-OVA and alum. GC B-cell numbers correlate with T_{FH} cell numbers. (C) Intracellular cytokine production was assessed by flow cytometry. Data represent $n \geq 3$ mice per group and at least two independent experiments.

P2A memory T cells had an approximately threefold lower proportion of Ly6c^{low}T-bet^{low} cells that were mostly confined to the CXCR5⁺ compartment (Fig. S7B). Both CXCR5⁺T-bet^{low} and Ly6c^{low} memory T-cell subsets have been suggested to belong to the central memory compartment and have an increased ability to further differentiate following a recall infection (3, 20, 34). To determine whether recall capacity differed between 3K memory T cells and P2A memory T cells, mice were given a secondary infection with *Listeria* expressing high affinity 3K ligand. Four days after secondary infection, almost all high dose 3K memory T cells were T-bet^{high}Ly6c^{high} and approximately half of the T cells had down-regulated CXCR5 expression (Fig. S7C and Fig. S7C). Low dose 3K memory T cells also demonstrated a strong T-bet^{high}Ly6c^{high} bias, although a significant proportion of T-bet^{low}Ly6c^{low} cells were also present (Fig. 5 and Fig. S7C). Surprisingly, P2A memory T cells further down-regulated T-bet and Ly6c expression, and had a larger proportion of CXCR5⁺T-bet^{lo} cells, despite receiving a high affinity secondary stimulation (Fig. 5 and Fig. S7C). These data indicate that memory T cells generated by low affinity activation remain impaired in their ability to up-regulate T-bet, despite receiving a high affinity recall stimulus. Recall responses following infection with recombinant vaccinia virus expressing 3K ligand showed a similar trend, with a higher proportion of P2A memory T cells expressing low levels of T-bet and Ly6c compared with 3K memory T cells (Fig. S8). Consistent with its intermediate priming affinity, P5R memory T cells generated an intermediate proportion of Th1 and T_{FH} effectors (Fig. S9). Of note, memory T cells expanded after weak priming by low dose Lm.3K and Lm.P2A generated much more than memory T cells generated by high dose Lm.3K (Fig. S7D), indicating an inverse relationship between the magnitude of the primary and secondary responses. Taken together, these data show that robust and strikingly biased recall responses can be generated following primary infection with low antigen affinity versus low antigen dose, and suggest that a differentiated recall capacity is imprinted during the primary response.

T Cells Activated by Low Affinity Antigen Depend on B Cells for Differentiation. The observation that low affinity activation is sufficient to induce memory T-cell differentiation was unexpected, because decreased functional avidity has been previously correlated with a failure to differentiate into the memory compartment (17). In the previous study, however, mice were infected with a

virulent strain of *Listeria* that results in lower antigen presentation by B cells, weak T_{FH} generation, and decreased serum antibody titers. Given that both T_{FH} and CXCR5⁺ memory T-cell differentiation depends on B-cell antigen presentation (3, 35), we wondered if P2A activated T cells could differentiate and survive long term in the absence of B cells. To examine this, the differentiation of B3K508 T cells in B-cell deficient (mb1-cre) mice was assessed. At day 6 after infection, B3K508 T cells activated with either Lm.3K or Lm.P2A in B-cell-deficient hosts underwent less expansion compared with B3K508 T cells activated in wild-type controls (Fig. S10A). T cells activated with high dose Lm.3K had a higher proportion of Th1 effectors (Fig. 6) and a higher mean fluorescence intensity (MFI) of T-bet (Fig. S10B), suggesting that B cells may play a role in limiting Th1 differentiation. In contrast, T cells weakly activated with either low dose Lm.3K or Lm.P2A in B-cell-deficient hosts had a lower T-bet MFI compared with priming in wild-type hosts (Fig. S10B), indicating that B cells are required for both optimal expansion and T-bet expression in response to weak TCR stimulation. At late time points after infection, memory B3K508 T cells were nearly undetectable in B-cell-deficient hosts infected with either Lm.3K or Lm.P2A (Fig. S10C), indicating that B cells are critically required for the optimal generation of B3K508 T-cell memory. However, upon secondary infection with Lm.3K, T-bet^{high} memory T cells could be recovered from all B-cell-deficient hosts, with the greatest number of secondary effector T cells generated by high dose 3K memory T cells (Fig. S10D and E). These data demonstrate that T cells activated by low affinity antigen are critically dependent on B cells for differentiation into both T_{FH} effector and memory T-cell compartments.

Discussion

The experiments presented here underscore the importance of TCR affinity in directing Th cell fate. We found that T cells activated by increasing affinity ligands undergo more T-cell expansion and increased Th1 differentiation. In contrast, T_{FH} differentiation and memory T-cell generation is not limited by antigen affinity and can occur in response to very weak, intermediate, and high affinity activation. Our results were unexpected in light of previous reports that increased TCR signal strength results in enhanced T_{FH} cell and memory T-cell generation (15, 17). However, the complex relationship between T-cell expansion and differentiation makes it difficult to discern the potentially distinct contributions of pMHC affinity and pMHC dose in these studies. By normalizing the degree of proliferation induced by high and low affinity ligands, we observed distinct influences on T-cell differentiation

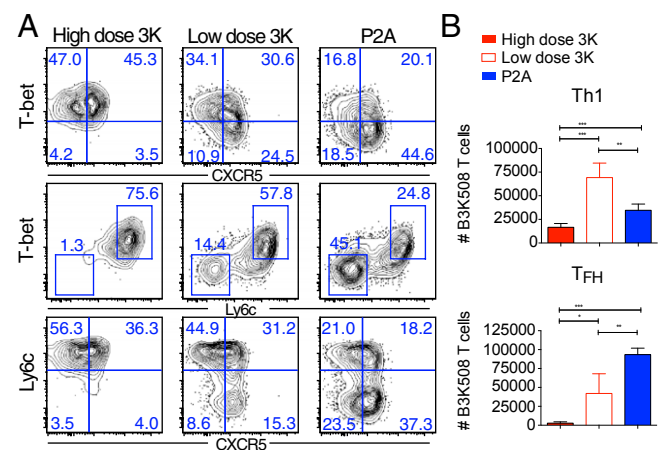


Fig. 5. Low affinity TCR-pMHC ligands support memory T-cell differentiation. Phenotype (A) and absolute cell number (B) of B3K508 T cells recovered at day 4 after recall infection with virulent Lm.3K. Data represent $n \geq 3$ and are representative of three independent experiments. (* $P < 0.05$, *** $P < 0.0001$).

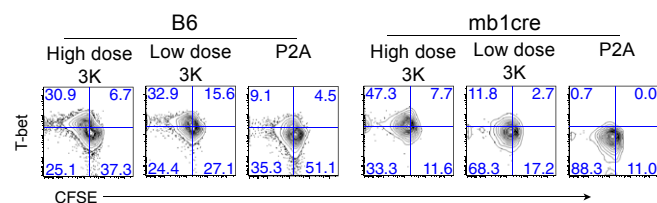


Fig. 6. Effector and memory T-cell differentiation in B-cell-deficient mice. Phenotype of B3K508 T cells recovered from spleen and lymph nodes of B6 or mb-1cre mice at day 6 after infection. Data representative of two independent experiments.

during an infection. Regardless of antigen dose, high affinity antigen induced efficient Th1 differentiation, whereas low affinity antigen did not. Despite impaired Th1 differentiation, however, low affinity activated T cells were able to up-regulate T_{FH} -associated surface markers, home to splenic B-cell areas, and were capable of providing help to germinal center B cells in an adoptive transfer model. Importantly, however, strong stimulation conditions, which induce the most T-cell expansion (i.e., high dose of high affinity antigen), result in the highest number of T_{FH} effectors that are likely to provide more help to B cells. Although our model allows us to track very low affinity antigen-specific T-cell responses, studying a monoclonal population has inherent limitations and cannot address the relative impact of low affinity T-cell responses in a polyclonal setting, where low affinity T cells might make a greater contribution to antigen-specific responses (discussed further below) (36).

Our data support a model in which CD4 T-cell fate diversification begins at the earliest stages of naïve T-cell priming with DCs. T cells activated by high affinity antigen at both high and low antigen doses differentiate into distinct $IL-2R\alpha^{high}$ and $IL-2R\alpha^{low}$ populations that are likely to be the precursors to Th1 and T_{FH} effectors, respectively (37). These data indicate that the early generation of Th1 and T_{FH} effectors is dose independent. In contrast, the early generation of Th1 effectors is strongly linked to antigen affinity. T cells activated by low affinity ligand demonstrate impaired Th1 differentiation as early as 3 days after infection, while the induction of early ($CXCR5^{+}IL-2R\alpha^{low}$) T_{FH} effectors occurs normally. T_{FH} generation is thought to be a two-step process with early differentiation initiated by T-cell–DC interactions followed by a maintenance phase that is dependent on T-cell–B-cell interactions. Although we observed early T_{FH} induction under all conditions, T cells activated by low antigen dose had a decreased proportion of T_{FH} effectors at later time points after infection. The $PD-1^{high}$ T_{FH} compartment, believed to contain germinal center T_{FH} cells, was particularly affected, indicating the importance of antigen dose for sustained T-cell help to B cells (13, 15). One possible explanation for this is that low antigen dose results in decreased antigen availability during the maintenance phase of T_{FH} differentiation. It is plausible that increased T-cell competition for limited amounts of antigen might inhibit late phase T_{FH} expansion, resulting in a higher proportion of Th1 effectors. Of note, ongoing interactions between T and B cells at the peak of infection with lymphocytic choriomeningitis virus were recently shown to result in multiple rounds of T_{FH} cell proliferation, while Th1 cells were largely refractory to stimulation at this later time point (37). These data suggest that temporal requirements for antigen may allow antigen dose and antigen affinity to set different thresholds for T_{FH} development. B cells have also been suggested to play an active role in suppression of the Th1 response, which may be an alternate way in which they promote T_{FH} generation (16). Our observation that T cells activated with high dose 3K in the absence of B cells express higher levels of T-bet support these findings.

In a study published last year, the aggregate dwell time (t_a) of an antigen was suggested to be a better predictor of Th differentiation than antigen affinity (15). The authors reported that antigens with increased t_a values (2.8 and 3.1 s) supported

greater accumulation of T_{FH} effectors, whereas an antigen with an intermediate t_a value (2.3 s) supported maximal Th1 differentiation. Similar to our study, the authors also observed T_{FH} generation in response to antigens with lower t_a values. Although our results are largely in agreement with this study, it is possible that we do not see maximal Th1 differentiation with intermediate strength ligand because the t_a value of the intermediate ligand in our study (0.9 s) is much lower than the intermediate ligand used in ref. 15 (2.3 s). Alternatively, small differences in the experimental systems, such as the number of transferred precursors or the time point of analysis might play a role. Tubo et al. (15) analyzed differentiation at day 7 after infection, which might not account for differences in the expansion and/or survival of Th1 and T_{FH} effectors. For example, high antigen doses have been shown to induce more activation-induced cell death, particularly in Th1 effectors, which might in turn elevate the proportion of Th1 effectors at later time points (38).

An important but unresolved issue is how T_{FH} differentiation can be supported by both strong and weak TCR signals. One possibility is that high and low affinity antigens are permissive for T_{FH} differentiation at the level of IL-2 signaling. Strong TCR signals have been shown to transiently block IL-2R signaling, whereas weak TCR signals result in less IL-2/IL-2R expression (39). Both of these conditions would be permissive for Bcl6 up-regulation and T_{FH} generation, which are negatively regulated by IL-2 (3, 32, 33). Of note, high affinity TCR signals have been shown to promote more stable T-cell contacts with antigen-presenting cells (APCs) compared with low affinity TCR signals (10). These data suggest that longer T-cell–APC contacts are required for the accumulation of signaling intermediates that facilitate IL-2 expression and Th1 differentiation. Long T-cell–APC contacts are also required for the induction of asymmetric cell division (28), which might lead to the generation of $IL-2R\alpha^{high}$ and $IL-2R\alpha^{low}$ daughter cells that subsequently differentiate into Th1 and T_{FH} effectors. In contrast, low affinity antigens that induce shorter T-cell–APC contacts might still support the accumulation of signaling intermediates required for T_{FH} differentiation.

Importantly, our data also reveal that T cells activated by low affinity ligand can give rise to long-lived memory T cells capable of undergoing robust recall responses. Several studies demonstrated a relationship between $CXCR5^{+}$ T cells present at early time points after infection and memory T-cell generation (3, 19, 20, 37). Consistent with this, low affinity activation supports both predominant differentiation of $CXCR5^{+}$ T-cell effectors as well as memory T-cell development. Surprisingly, memory T cells generated by low affinity activation demonstrate a bias toward T_{FH} phenotype upon recall, whereas memory T cells generated by high affinity activation are biased toward a Th1 recall response. By comparing memory T cells generated after priming with either high dose/low affinity or low dose/high affinity antigen, our data additionally show that T cells with a similar history of primary expansion have a similar capacity to expand during secondary infection, independent of effector lineage. These data are consistent with a recent report showing an inverse correlation between primary T-cell expansion and memory T-cell development (40).

To date, efforts to understand the contribution of low affinity antigens to polyclonal CD4 T-cell responses have been limited by the use of tetramers for the identification of antigen-reactive T cells. In addition to the observation that various peptides can bind MHC-II molecules in different registers, a recent study using a 2D binding assay showed that low affinity T cells, below detection with MHC-II tetramer, are present at a greater frequency and comprise approximately half of the effector cytokine response compared with tetramer positive cells (36). Thus, the contribution of low affinity CD4 T cells to immune responses is likely to be underestimated. Although our model reveals efficient T_{FH} generation in response to high, intermediate, and low affinity ligand, there is a clear impact of antigen affinity on the number of effectors that can be generated. These data raise the question: To what end is the propagation of a low avidity T-cell

response useful? Our data suggest the possibility that low affinity T cells might be particularly relevant to host protection against chronic pathogens or pathogens that are able to vary their antigenic motifs. In the case of chronic infection, resistance to exhaustion (41) coupled with a bias toward T_{FH} differentiation might position low affinity T cells to provide help to B cells producing protective neutralizing antibody responses. Alternatively, the preservation of low affinity CD4 T cells in the memory T-cell compartment might confer broader host protection to pathogens such as influenza, which undergo frequent antigenic drift. Thus, understanding the cellular and molecular mechanisms by which low affinity antigens contribute to and shape immune responses has important implications for enhancing T-cell clonal diversity, boosting B-cell responses, and improving long-term protection against reinfection.

Materials and Methods

Mice. All animal work was approved by the Cantonal Veterinary Office of the City of Basel. See *SI Materials and Methods*.

Infections. Recombinant ActA-deficient *L. monocytogenes* [Lm] stably expressing 3K or variant ligands were generated following established

protocols. For detailed *L. monocytogenes* preparation, see *SI Materials and Methods*.

Immunofluorescent Microscopy. See *SI Materials and Methods*.

Purification of T Cells, Adoptive Cell Transfer, and Cell Labeling. See *SI Materials and Methods*.

Flow Cytometry. All samples were analyzed on BD FACSCalibur or BD Fortessa cytometers. See *SI Materials and Methods*.

Analysis of T_{FH} Function. See *SI Materials and Methods*.

Data Analyses. Flow cytometry data were analyzed with FlowJo software (Tree Star). Graphs were prepared with Prism 6 (GraphPad Software) using the unpaired two-tailed Student *t* test.

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- Crotty S (2011) Follicular helper CD4 T cells (TFH). *Annu Rev Immunol* 29:621–663.
- Choi YS, et al. (2011) ICOS receptor instructs T follicular helper cell versus effector cell differentiation via induction of the transcriptional repressor Bcl6. *Immunity* 34(6):932–946.
- Pepper M, Pagán AJ, Igyártó BZ, Taylor JJ, Jenkins MK (2011) Opposing signals from the Bcl6 transcription factor and the interleukin-2 receptor generate T helper 1 central and effector memory cells. *Immunity* 35(4):583–595.
- Whitmire JK, et al. (2009) Requirement of B cells for generating CD4+ T cell memory. *J Immunol* 182(4):1868–1876.
- Corbin GA, Harty JT (2004) Duration of infection and antigen display have minimal influence on the kinetics of the CD4+ T cell response to *Listeria monocytogenes* infection. *J Immunol* 173(9):5679–5687.
- Bird JJ, et al. (1998) Helper T cell differentiation is controlled by the cell cycle. *Immunity* 9(2):229–237.
- Obst R, van Santen HM, Mathis D, Benoist C (2005) Antigen persistence is required throughout the expansion phase of a CD4(+) T cell response. *J Exp Med* 201(10):1555–1565.
- Gett AV, Sallusto F, Lanzavecchia A, Geginat J (2003) T cell fitness determined by signal strength. *Nat Immunol* 4(4):355–360.
- Malherbe L, Hausl C, Teyton L, McHeyzer-Williams MG (2004) Clonal selection of helper T cells is determined by an affinity threshold with no further skewing of TCR binding properties. *Immunity* 21(5):669–679.
- Gottschalk RA, et al. (2012) Distinct influences of peptide-MHC quality and quantity on in vivo T-cell responses. *Proc Natl Acad Sci USA* 109(3):881–886.
- Hosken NA, Shibuya K, Heath AW, Murphy KM, O'Garra A (1995) The effect of antigen dose on CD4+ T helper cell phenotype development in a T cell receptor- α beta-transgenic model. *J Exp Med* 182(5):1579–1584.
- Tao X, Grant C, Constant S, Bottomly K (1997) Induction of IL-4-producing CD4+ T cells by antigenic peptides altered for TCR binding. *J Immunol* 158(9):4237–4244.
- Baumjohann D, et al. (2013) Persistent antigen and germinal center B cells sustain T follicular helper cell responses and phenotype. *Immunity* 38(3):596–605.
- Fazilleau N, McHeyzer-Williams LJ, Rosen H, McHeyzer-Williams MG (2009) The function of follicular helper T cells is regulated by the strength of T cell antigen receptor binding. *Nat Immunol* 10(4):375–384.
- Tubo NJ, et al. (2013) Single naive CD4+ T cells from a diverse repertoire produce different effector cell types during infection. *Cell* 153(4):785–796.
- Ploquin MJ, Eksmond U, Kassiotis G (2011) B cells and TCR avidity determine distinct functions of CD4+ T cells in retroviral infection. *J Immunol* 187(6):3321–3330.
- Williams MA, Ravkov EV, Bevan MJ (2008) Rapid culling of the CD4+ T cell repertoire in the transition from effector to memory. *Immunity* 28(4):533–545.
- Kim C, Wilson T, Fischer KF, Williams MA (2013) Sustained interactions between T cell receptors and antigens promote the differentiation of CD4+ memory T cells. *Immunity* 39(3):508–520.
- Weber JP, Fuhrmann F, Hutloff A (2012) T-follicular helper cells survive as long-term memory cells. *Eur J Immunol* 42(8):1981–1988.
- Hale JS, et al. (2013) Distinct memory CD4+ T cells with commitment to T follicular helper- and T helper 1-cell lineages are generated after acute viral infection. *Immunity* 38(4):805–817.
- Aleksic M, et al. (2010) Dependence of T cell antigen recognition on T cell receptor-peptide MHC confinement time. *Immunity* 32(2):163–174.
- van der Merwe PA, Dushek O (2011) Mechanisms for T cell receptor triggering. *Nat Rev Immunol* 11(1):47–55.
- Zehn D, King C, Bevan MJ, Palmer E (2012) TCR signaling requirements for activating T cells and for generating memory. *Cell Mol Life Sci* 69(10):1565–1575.
- Govern CC, Paczosa MK, Chakraborty AK, Huseby ES (2010) Fast on-rates allow short dwell time ligands to activate T cells. *Proc Natl Acad Sci USA* 107(19):8724–8729.
- Huseby ES, Crawford F, White J, Marrack P, Kappler JW (2006) Interface-disrupting amino acids establish specificity between T cell receptors and complexes of major histocompatibility complex and peptide. *Nat Immunol* 7(11):1191–1199.
- Vanguri V, Govern CC, Smith R, Huseby ES (2013) Viral antigen density and confinement time regulate the reactivity pattern of CD4 T-cell responses to vaccinia virus infection. *Proc Natl Acad Sci USA* 110(1):288–293.
- Stepanek O, et al. (2014) Co-receptor scanning by the T-cell receptor provides a kinetic mechanism for T-cell tolerance. *Cell*, in press.
- King CG, et al. (2012) T cell affinity regulates asymmetric division, effector cell differentiation, and tissue pathology. *Immunity* 37(4):709–720.
- Enouz S, Carrié L, Merkler D, Bevan MJ, Zehn D (2012) Autoreactive T cells bypass negative selection and respond to self-antigen stimulation during infection. *J Exp Med* 209(10):1769–1779.
- Hemmer B, Stefanova I, Vergelli M, Germain RN, Martin R (1998) Relationships among TCR ligand potency, thresholds for effector function elicitation, and the quality of early signaling events in human T cells. *J Immunol* 160(12):5807–5814.
- Ansel KM, McHeyzer-Williams LJ, Ngo VN, McHeyzer-Williams MG, Cyster JG (1999) In vivo-activated CD4 T cells upregulate CXC chemokine receptor 5 and reprogram their response to lymphoid chemokines. *J Exp Med* 190(8):1123–1134.
- Johnston RJ, Choi YS, Diamond JA, Yang JA, Crotty S (2012) STAT5 is a potent negative regulator of TFH cell differentiation. *J Exp Med* 209(2):243–250.
- Oestreich KJ, Mohn SE, Weinmann AS (2012) Molecular mechanisms that control the expression and activity of Bcl-6 in TH1 cells to regulate flexibility with a TFH-like gene profile. *Nat Immunol* 13(4):405–411.
- Marshall HD, et al. (2011) Differential expression of Ly6C and T-bet distinguish effector and memory Th1 CD4(+) cell properties during viral infection. *Immunity* 35(4):633–646.
- Goenka R, et al. (2011) Cutting edge: dendritic cell-restricted antigen presentation initiates the follicular helper T cell program but cannot complete ultimate effector differentiation. *J Immunol* 187(3):1091–1095.
- Sabatino JJ, Jr, Huang J, Zhu C, Evavold BD (2011) High prevalence of low affinity peptide-MHC II tetramer-negative effectors during polyclonal CD4+ T cell responses. *J Exp Med* 208(1):81–90.
- Choi YS, et al. (2013) Bcl6 expressing follicular helper CD4 T cells are fate committed early and have the capacity to form memory. *J Immunol* 190(8):4014–4026.
- Zhang X, et al. (1997) Unequal death in T helper cell (Th)1 and Th2 effectors: Th1, but not Th2, effectors undergo rapid Fas/FasL-mediated apoptosis. *J Exp Med* 185(10):1837–1849.
- Yamane H, Zhu J, Paul WE (2005) Independent roles for IL-2 and GATA-3 in stimulating naive CD4+ T cells to generate a Th2-inducing cytokine environment. *J Exp Med* 202(6):793–804.
- Weber KS, et al. (2012) Distinct CD4+ helper T cells involved in primary and secondary responses to infection. *Proc Natl Acad Sci USA* 109(24):9511–9516.
- Caserta S, Kleczkowska J, Mondino A, Zamojska R (2010) Reduced functional avidity promotes central and effector memory CD4 T cell responses to tumor-associated antigens. *J Immunol* 185(11):6545–6554.