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TCR signal quantity and quality in CD4+ T cell differentiation

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Abstract

The adaptive immune system protects its host from a myriad of pathogens. This capacity stems from a vast set of lymphocytes, each with a different antigen receptor, a small number of which will bind to antigens derived from a given pathogen. Although the cells within any antigen-specific population appear relatively homogenous before antigenic encounter, recent work on T cells indicates that individual cells within the population differentiate in very different ways after exposure to the antigen. Here we focus on studies of $CD4^+$ T cells, and review evidence indicating that variable differentiation of effector cells from single naïve cells is caused by both cell-extrinsic stochastic factors and cell-intrinsic factors related to T cell antigen receptor signal quantity and quality.

Keywords

T cell differentiation; TCR signal strength; polyclonal

Introduction

$CD4^+$ T cells use αβ T cell antigen receptors (TCRs) to detect short peptides bound to major histocompatibility complex class II molecules (MHCII) on antigen-presenting cells [1]. TCR signaling induced by binding of the relevant peptide:MHCII (p:MHCII) ligand causes $CD4^+$ T cells to proliferate and differentiate into specialized effector cells such Th1, Th2, Th17, and follicular helper cells (Tfh) that help other cells of the immune system control infections and cancer [2, 3]. A key goal in immunology has been to understand how effector $CD4^+$ T cell specialization occurs.

The conventional model of CD4+ T cell differentiation posits that TCR signaling triggers naïve $CD4^+$ T cells to proliferate and differentiate into specialized effector cells in a process that is guided by cytokines produced by other cells. TCR signaling along with IL-12 or type 1 interferon (IFN) [4], IL-4 [5, 6], IL-6 and TGF-β [7], or IL-2 and TGF-β [8] causes naïve $CD4^+$ T cells to differentiate into IFN-γ producing Th1 cells, IL-5- and IL-13-producing

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Th2 cells, IL-17-producing Th17 cells, or induced regulatory T cells, respectively. Similarly, naïve $CD4^+$ T cells differentiate into follicular helper cells (Tfh) in response to TCR signaling in the presence of IL-6, IL-21 and other less understood factors [2, 3]. In each case, the inducing cytokines are produced by certain cells of the innate immune system in response to pathogen associated molecular patterns (PAMPs), and act with TCR signaling to induce lineage-enforcing transcription factors such as T-bet for Th1 cells [2].

Although much evidence indicates that cytokines produced by cells of the innate immune system are critical for this process, emerging evidence suggests that the quantity or quality of the TCR signal itself plays a role. In this review we discuss recent evidence in support of this idea.

Differences in positive selection influence the naïve $CD4^+$ T cell response to foreign p:MHC

In the most extreme form of the conventional model, the TCR is simply a switch that turns on the expression of requisite cytokine receptors, which then transduce the signals that drive differentiation. In this model, all naïve $CD4^+$ T cells are expected to produce similar types of effector cells during a primary immune response to a foreign antigen because each cell would experience a similar cytokine environment.

Recent evidence, however, suggests that individual naïve T cells are already poised for different effector behaviors before encountering the relevant foreign p:MHCII ligand due to differences in TCR signals received during thymic positive selection. αβ TCRs are generated in developing thymocytes by random recombination of *Tcra* and *Tcrb* gene segments [9]. This gene shuffling mechanism creates a situation in which billions of T cells are produced in the thymus, each with an independently rearranged TCR with a unique specificity. The complementarity determining regions (CDR) 1 and 2 of the TCR V beta chains contain conserved amino acids that facilitate binding to MHC molecules [10], while the TCR alpha and beta chain CDR3 regions at the recombination joins confer an affinity for an MHC-bound peptide [11]. The specificity and affinity of the TCR expressed by a nascent thymocyte, which now expresses the MHCII-binding CD4 and MHCI-binding CD8 co-receptors, will determine whether it passes the positive and negative selection checkpoints [9]. $CD4^+$ $CD8^+$ thymocytes with TCRs with high affinity for a self p:MHC are deleted or become regulatory $CD4^+$ T cells [12]. In contrast, $CD4^+$ $CD8^+$ thymocytes with TCRs with low affinity for a self p:MHCI or self p:MHCII receive a positive selection signal that causes the loss of CD8 [13]. If the cell has a self p:MHCI-specific TCR, then the loss of CD8 causes the TCR signal to cease, causing the cell to lose CD4 and re-express CD8 to become a $CD4^-$ $CD8^+$ mature T cell. Conversely, if the cell has a self p:MHCII-specific TCR, then the loss of CD8 has no effect, allowing the TCR signal to persist, causing the cell to retain CD4 to become a $CD4^+$ $CD8^-$ mature T cell [13].

Cells within the positively-selected population experience subtly different amounts of TCR signaling due to clonal differences in binding to the selecting self p:MHC ligands. The amount of TCR signaling received during positive selection sets the level of expression of CD5, which in turn can be associated with the activity of phosphatases that modulate TCR

signaling [14, 15], implying that T cells that received a strong selecting signal in the thymus would exhibit blunted signaling downstream of the TCR. However, recent work has shown that T cells that express high levels of CD5, and were therefore presumably at the high end of the positive selection spectrum, contain higher basal levels of phosphorylated Erk, a second messenger downstream of the TCR, perhaps due to peripheral TCR sensing of the self p:MHCII that caused positive selection [16]. This characteristic poises $CD5^{high}$ naïve T cells for higher IL-2 production and proliferation in response to foreign p:MHCII than $CD5^{low}$ cells [16, 17]. Thus, $CD5^{high}$ cells within a given foreign p:MHC-specific population are intrinsically capable of more proliferation when the host is exposed to the relevant foreign peptide than the $CD5^{low}$ cells. Whether $CD4^+$ T cells expressing different levels of CD5 differentiate into different types of effector cells remains to be determined.

Antigen dose influences effector cell differentiation

Studies on the effect of antigen dose on the immune response have lent support to the idea that the amount of TCR signaling can have qualitative effects on $CD4^+$ T cell differentiation. Experiments on the immune response to the intracellular pathogen *Leishmania major* have been particularly informative in this regard. Control of *L. major* infection is achieved by Th1 cell-mediated mechanisms [18]. This is highlighted by the fact that BALB/c mice, which generate robust Th2 and antibody responses but poor Th1 cell responses, are highly susceptible to *L. major* infection, while C57BL/6 mice generate a strong Th1 cell response and are resistant. Bretscher and colleagues, however, found that infection of BALB/c mice with very low numbers of parasites elicited a strong and protective Th1 cell response. When subsequently challenged with a larger number of parasites, the Th1 response was maintained and protected the mice from further pathology [19]. This is in agreement with more recent data showing that lower doses of vaccine confer more protection from *L. major* than high doses [20]. Together, these studies indicate that low, or at least intermediate antigen doses, favor Th1 cell-dominated responses while high doses favor Th2 cell-dominated responses.

Other evidence indicates that antigen dose influences Th1/Th2 differentiation. Bottomly and coworkers showed that naïve TCR transgenic T cells differentiated into Th2 cells when cultured with small amounts of antigen and Th1 cells when the antigen dose was increased [21]. O'Garra and colleagues found that naïve TCR transgenic T cells cultured within moderate amounts of antigenic peptide became Th1 cells, while Th2-like, IL-4-producing cells were generated in cultures with very low or very high amounts of peptide [22].

Recent experiments suggest that antigen dose may even be able to trump the effects of innate immune system cytokines on effector T cell differentiation. Using adjuvants that favor Th1 or Th2 polarization, Germain and colleagues demonstrated that antigen-presenting cells activated with a Th1-polarizing adjuvant formed more stable contacts with $CD4^+$ T cells than those activated with a Th2-polarizing adjuvant [23]. Importantly, Th1-polarizing adjuvants promoted greater TCR signaling, as measured by Ca^{2+} flux and increased immunological synapse size. Remarkably, increasing the antigen dose allowed the induction of Th1 cells even in the presence of a Th2-polarizing adjuvant. This study, therefore, blurs the line between the effects of TCR signal strength and innate immune system cytokine receptor signaling on effector cell differentiation.

Antigen dose also affects the differentiation of Th1 and Tfh cells [24]. Intravenous infection with attenuated *Listeria monocytogenes* bacteria generates 3 distinct effector $CD4^+$ T cell subsets: Th1 cells, $CXCR5^{intermediate}$ $PD\text{-}1^-$ Tfh cells, which help B cells at the T-B border and give rise to central memory cells, and $CXCR5^{high}$ $PD\text{-}1^+$ germinal center Tfh cells (GC-Tfh), which reside in the germinal centers and facilitate isotype switching and affinity maturation of germinal center B cells [3, 25, 26]. Low dose infection generated a mixed *L. monocytogenes* p:MHCII-specific effector cell population with mainly Th1 and Tfh cells and a few GC-Tfh cells [24]. As the number of bacteria was increased, the fraction of Th1 cells in the population progressively decreased as Tfh cells and especially GC-Tfh cells increased. This result is in agreement with another recent study that showed that increasing the dose of a model antigen facilitates GC-Tfh development, and correlates with the magnitude of the germinal center B cell response [27].

Together, the literature suggests that small amounts of antigen induce Th2 or Tfh cells, intermediate amounts induce Th1 cells, and large amounts induce Th2 cells or GC-Tfh cells, depending on the model system.

Antigen dose effects and TCR signal strength

Antigen dose could have influenced effector cell differentiation in the aforementioned studies by affecting TCR signal strength or duration. High antigen dose increases the number of antigen-derived p:MHCII molecules displayed by antigen-presenting cells [28], thereby increasing the amount of TCR signaling received by specific T cells at a key point in time. High antigen dose also is likely to increase the amount of time that antigen-derived p:MHCII molecules are displayed by antigen-presenting cells thereby prolonging TCR signaling. Thus, the observations mentioned in the preceding section could be explained by low amounts of TCR signaling inducing Th2 or Tfh cells, intermediate amounts inducing Th1 cells, and large amounts inducing GC-Tfh cells.

Evidence in the literature provides clues as to how TCR signal strength could influence $CD4^+$ T cell differentiation. Increasing the antigen dose used to pulse dendritic cells increased T cell-dendritic cell interaction time, expression of the Th1-polarizing IL-12Rβ2 in the T cells, and Th1 differentiation [23]. Other work indicates that very large amounts of TCR signaling inhibit IL-12Rβ2 expression [29, 30]. GC-Tfh cell differentiation is favored by prolonged p:MHCII presentation by germinal center B cells [27, 31], or for some [32] but not other [33] p:MHCII ligands, recognition by high affinity TCRs. Studies on $CD8^+$ T cells have shown that the transcription factor interferon regulatory factor 4 (IRF4) is induced in proportion to TCR signal strength [34, 35] and controls the expression of Blimp-1 [36], a transcription factor that is required for Th1 differentiation and suppresses the expression of the transcriptional regulator Bcl-6, which is needed for Tfh differentiation [37]. Thus, small amounts of TCR signaling could induce Bcl-6 but not Blimp-1, leading to Tfh differentiation. This is consistent with the fact that TCR signaling can induce Bcl-6 expression under Th1 polarizing conditions [38]. As the amount of TCR signaling increases, IRF4, Blimp-1, and IL-12Rβ2 could increase, leading to more Th1 differentiation. At the largest amount of TCR signaling, IL-12Rβ2 could be inhibited, suppressing formation of Th1 cells.

TCR signal quality influences effector cell differentiation

Variation in the amount of p:MHCII is only one way that $CD4^+$ T cells could experience different TCR signals. T cells in a polyclonal p:MHCII-specific population could also receive different signals in response to the same amount of p:MHCII due to differences in TCR affinity. The frequencies of T cells specific for different foreign p:MHC in immunologically naïve individuals ranges from ~1:30,000 to ~1:10,000,000 [39–42]. In an adult C57BL/6 mouse there are 1–300 $CD4^+$ naïve T cells that recognize a given p:MHCII [43, 44]. TCR sequencing experiments indicate that most of the cells in a given foreign p:MHC-specific naïve T cell population express different TCRs [24], and are thus uniquely generated clones. Although all the TCRs expressed by these clones bind the same foreign p:MHC, they could bind in different ways. Some may bind with a higher affinity [32], dwell time [45], or a different docking angle [46] compared to others, and consequently transmit different signals [47, 48] that could influence effector cell differentiation [24, 32, 49, 50].

Evidence that clonal differences in TCR signaling can influence effector differentiation comes from *in vivo* T cell limiting dilution experiments. A population of about 50 polyclonal listeriolysin O peptide (LLOp):MHCII-specific naïve T cells consistently produced an effector cell population of 50% Th1 cells, 25% Tfh cells, and 25% GC-Tfh cells in each mouse one week after *L. monocytogenes* infection [24, 25]. In contrast, single LLOp:MHCII-specific naïve T cells generated effector cells with diverse Th1/Tfh/GC-Tfh differentiation patterns, ranging from 100% Th1 to 100% Tfh [24]. The average pattern, however, for multiple clones was the 50% Th1, 25% Tfh, 25% GC-Tfh pattern observed for the entire LLOp:MHCII-specific population in intact mice. Thus, individual naïve $CD4^+$ T cells from a polyclonal repertoire produced widely different effector cell patterns, which when averaged together yielded the consistent pattern of the intact repertoire. The TCR played a role in the variability since effector cell populations derived from single TCR transgenic T cells that expressed the same TCR were much more similar to one another than the clonal populations derived from the polyclonal repertoire. In addition, different TCR transgenic cells with different TCRs, exhibited different effector cell differentiation patterns.

These data raise the possibility that clones produce different effector cell differentiation patterns because of intrinsic differences in TCR-p:MHCII binding and signaling. This hypothesis is supported by the finding that transgenic T cells expressing the same TCR produce different effector cell subsets when confronted with variant p:MHCII that bind to the TCR for varying amounts of time [45]. A TCR that bound to a p:MHCII for one second induced only Tfh cells, while a TCR that bound a p:MHCII for two seconds induced a population of Th1 and Tfh cells [24]. Interestingly, a TCR that bound a p:MHCII for three seconds induced a population of effector cells dominated by Tfh with a few GC-Tfh cells. Together, these results indicate that the shortest TCR-p:MHCII interactions support only Tfh differentiation. As TCR-p:MHCII interaction time increases, a greater fraction of the population becomes Th1 cells until a point at which Th1 differentiation is inhibited leading to preferential production of Tfh and GC-Tfh cells.

The data on antigen dose and TCR-p:MHCII dwell time can be synthesized into a coherent model up to a point. Both types of study fit a model in which small amounts of TCR

signaling induce Bcl-6 but not Blimp-1, leading to Tfh differentiation. As the amount of TCR signaling increases, IRF4, Blimp-1, and IL-12Rβ2 increase, leading to more Th1 differentiation. The data, however, showing that very high antigen dose favors GC-Tfh formation [24] are harder to fit into a model based only on TCR signal strength because very strong TCR signaling would be expected to induce very high levels of Blimp-1, a suppressive factor for GC-Tfh. In other words, it is difficult to understand how Tfh and GC-Tfh, which are related Bcl-6-dependent cell types [25, 51], can be induced by TCR signal strengths at opposite ends of the spectrum.

We propose a more comprehensive model based on the assumption that TCR signals are not just weak or strong, but can vary in qualitative way (Figure 1). The model is based on the following propositions. The TCR has a basal apparatus that produces Signal A when engaged by any p:MHCII ligand, even ones with very short dwell times. Signal A induces Bcl-6. Another component can be brought into the TCR signaling apparatus in proportion to the p:MHCII dwell time, and this component produces Signal B, which induces Blimp-1, perhaps via IRF4. Each clone within a polyclonal p:MHCII-specific population generates a characteristic amount of Blimp-1-inducing Signal B when confronted with a fixed number of p:MHCII. Clones with TCRs with short p:MHCII dwell times have a high probability of producing Bcl-6 without Blimp-1 and committing to the Tfh path, while clones with long p:MHCII dwell times have a high probability of inducing Blimp-1 and committing to the Th1 path. Clones with TCRs with intermediate p:MHCII dwell times, like the one represented in Figure 1 have an equal probability of committing to either path. Progeny of the same clone that bifurcate and commit to the Tfh or Th1 path adhere to that path. Commitment behavior is solely a function of the TCR-p:MHCII dwell time controlling the Bcl-6/Blimp-1 ratio and occurs reproducibly for a given clone at any amount of p:MHCII. For clonal progeny that commit to the Tfh path, increasing the amount of p:MHCII causes more Tfh proliferation and conversion to GC-Tfh cells because the p:MHCII persists [27, 31]. For clonal progeny that commit to the Th1 path, increasing the amount of p:MHCII causes more Th1 proliferation but eventually induces apoptosis as Blimp-1 becomes very high [52]. An attractive feature of this model is that it can explain how GC-Tfh cells are enhanced and Th1 cells suppressed by large antigen doses and account for the odd low-high-low behavior of Th1 cell differentiation as a function of antigen dose observed by Hosken et al. [22]. The model is supported by the observation that a bifurcation of Th1- or Tfh-destined cells occurs several days after acute bacterial or viral infections that stimulate IL-12 or type 1 IFN production by the innate immune system [25, 51].

Extrinsic factors influence the clonal variability in effector cell differentiation

It is important to note that extrinsic factors also contribute to clonal heterogeneity in effector T cell differentiation. Although single $CD4^+$ T cells from the same TCR transgenic mouse tended to produce similar effector cell patterns in different mice, some variability still existed [24]. Since these T cells expressed the same TCR, this variability must have been due to other factors. The role of extrinsic factors in clonal heterogeneity has been very clearly demonstrated for naïve $CD8^+$ T cells. TCR stimulation by p:MHCI during acute

infection causes naïve $CD8^+$ T cells to differentiate into short-lived effector cells or memory progenitor effector cells, which can be distinguished based on their expression of IL-7Rα, CCR7, and L-selectin [53]. Similar to $CD4^+$ T cells, studies have shown that single naïve $CD8^+$ T cells can give rise to both subsets of effector cells [54, 55]. In these studies, the TCR does not appear to be a major determinant of the differentiation pattern because different single $CD8^+$ TCR transgenic T cells expressing the same TCR exhibited a wide range of clonal burst sizes and produced vastly different effector cell ratios [56–58]. These patterns were as variable as clonal populations derived from single naïve $CD8^+$ T cells from the polyclonal repertoire [58]. This is consistent with the findings that both strong and weak TCR stimulation is able to generate $CD8^+$ T cell memory [59], but contrasts the important role for the TCR found in $CD4^+$ T cell memory generation [50, 60]. The patterns of differentiation varied between infection types, however, demonstrating that the infectious environment is the primary controller of $CD8^+$ T cell effector differentiation [58]. This is consistent with data demonstrating that exposure to inflammatory cytokines can dynamically tune TCR signaling in $CD8^+$ T cells [61, 62].

The TCR did, however, play small role in clonal expansion of naïve $CD8^+$ T cells. After infection with vesicular stomatitis virus expressing ovalbumin, proliferation was weakly linked to the TCR, as OT-I TCR transgenic cells produced on average 7-fold more progeny than endogenous $CD8^+$ T cells specific for the same ovalbumin:K^b complex [58]. A role for the TCR in $CD8^+$ T cell differentiation is further supported by recent studies by Nayar et al. [34, 35]. These investigators found that IRF4 is induced in $CD8^+$ T cells in proportion to the strength of TCR signaling and that the amount of IRF4 controls the expression of the Eomesodermin transcription factor and the number of short-lived effector cells. Furthermore, it has been shown that TCR affinity for p:MHCI can instruct effector cell differentiation via asymmetric cell division in a model of autoimmune diabetes [49]. Therefore, although extrinsic factors appear to the main drivers of clonal heterogeneity in $CD8^+$ T cells, intrinsic factors related to the TCR probably play some role in certain cases, perhaps those cases in which the extrinsic factors are very uniform.

Concluding remarks

T cells differentiate into specialized effector cells with different functions during infection. As discussed above, while the cytokine milieu has been shown to play in important role in determining the types of effector cells generated, recent evidence has demonstrated that signals received through the TCR are also important for effector cell differentiation. Differences in TCR signaling itself have been shown to play an important role in the differentiation of Th1, Th2, and Tfh effector cells.

But many unanswered questions remain. The molecular mechanisms underlying the effects of TCR signal quantity and quality on effector cell differentiation remain to be worked out. The makeup of the TCR signaling apparatus, how this apparatus induces different transcription factors, and how those factors regulate cytokines and cytokine receptors to influence effector cell differentiation need to be understood. Different amounts of TCR signal appear to be able to differentially induce IRF4, Bcl-6, or Blimp-1, but the TCR-associated kinases, phosphatases, and second messengers that cause these changes have yet

to be defined. Ultimate resolution of these issues will likely require an understanding of how the TCR works as a molecular machine, and whether the machine can produce qualitatively different signals depending on the binding characteristics of the ligand. Assessment of the binding parameters of TCRs specific for the same p:MHCII but induce different effector cell differentiation patterns will probably be helpful in this regard.

Another challenging issue relates to definition of the effector cell subsets that are influenced by TCR signal quality and quantity. Antigen dose, and by inference TCR signal strength, has been reported to have effects on Th2 and Tfh cell differentiation [21–24, 31]. Since Tfh cells can make IL-4 [63], future studies should rely on lineage-defining transcription factors such as Bcl-6 and GATA-3 rather than cytokine production to distinguish Tfh and Th2 cells. Similarly, it would be helpful to clarify the relationship between Tfh and GC-Tfh cells, which are both Bcl-6-dependent populations [25, 51] but can behave differently in response to TCR signal strength changes [24]. Determining whether Tfh cells defined by the $CXCR5^{intermediate}$ $PD\text{-}1^{-}$ phenotype are the precursors of $CXCR5^{high}$ $PD\text{-}1^{+}$ GC-Tfh cells would set expectations about whether these cells types should behave similarly in response to TCR signal strength. It would also be of interest to determine if Th17 and Tfh cells are generated simultaneously during mucosal infections, and if so whether TCR signal strength plays a role in the bifurcation. If so, then it may be possible to build a consensus pathway in which TCR signal quality determines the Tfh/non-Tfh split, and innate immune system cytokines determine the type of effector cell that will emerge from the non-Tfh subset.

Finally, a better understanding of the effects the TCR signal quality and quantity could be instrumental to improving vaccine design and effectiveness. However, the road to get there is hardly clear. One suggestion that has emerged from the studies to date is that vaccines based on antibodies should involve administration of large amounts of antigen in a form that persists to facilitate the production of GC-Tfh cells.

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Highlights

- Single naïve $CD4^+$ T cells produce different effector cell patterns.
- TCR signal strength and quality are determinants of the effector cell pattern.
- TCR-independent factors also influence the effector cell pattern.
- A model of TCR-mediated Th1/Tfh/GC-Tfh differentiation is proposed.

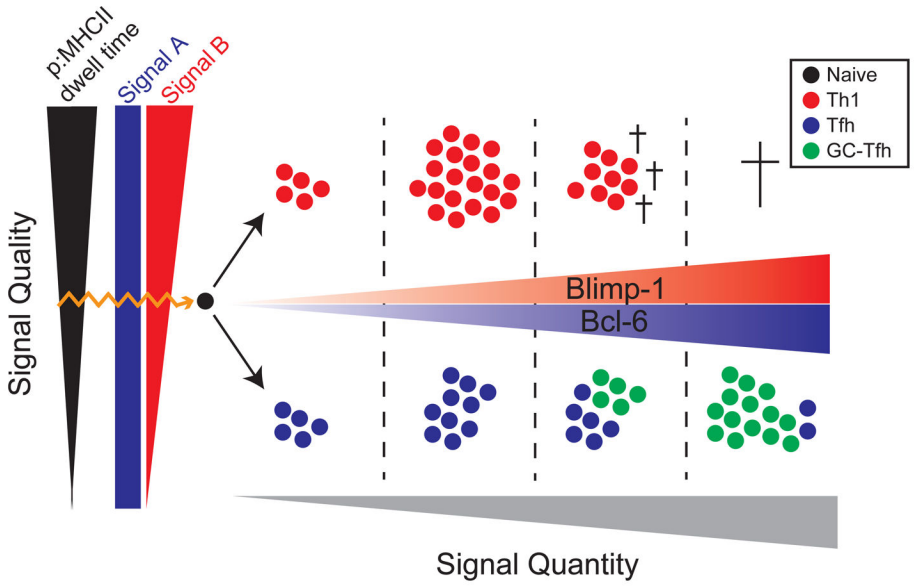

Figure 1. Proposed model of TCR-mediated Th1/Tfh/GC-Tfh differentiation

The black Y-axis depicts the range of possible p:MHCII dwell times that the TCRs on cells in a p:MHCII-specific population could have. The blue Y-axis depicts a signal designated Signal A from the basal TCR apparatus, whereas the red Y-axis depicts a signal designated Signal B from a component that is recruited to the TCR apparatus in proportion to the TCR-p:MHCII dwell time. Signal A is sufficient for induction of Bcl-6, while Signal B drives Blimp-1 expression. The Signal A:Signal B ratio determines the Bcl-6:Blimp-1 ratio, which determines the probability that early progeny of a clone will commit to the Tfh or Th1 paths. The gray X-axis displays increasing TCR signal quantity as would occur as the amount of p:MHCII ligand increases. The diagram shows the behavior of a naïve $CD4^+$ T cell clone with an intermediate TCR-p:MHCII dwell time that early on generates Signal A and B (indicated by the jagged orange line), equal amounts of Bcl-6 and Blimp-1, and produces equal numbers of Tfh- and Th1-committed progeny. Small numbers of p:MHCII generate equal numbers of Tfh and Th1 cells. Increasing the number of p:MHCII thereby increasing TCR signal strength causes more proliferation of each progeny type until very high numbers of p:MHCII cause a reduction in Th1 cells due to apoptosis and in increase in GC-Tfh cells as Tfh cells convert to this phenotype.