

PATHOLOGICAL T HELPER POLARIZATION REQUIRES PRE-EXISTING CROSS-REACTIVE MEMORY T CELLS

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ABSTRACT

Naive CD4⁺ T cells engage cognate peptide MHC-II complexes (pMHC-II_s) to differentiate and acquire one of several T helper (Th) fates whose specific trajectories are guided by a dynamic cytokine milieu that develops in response to antigenic entity. This physiological process is often erroneously conflated with a pathological one termed Th polarization. Using the SPIRAL model, we argue here that unlike Th fate choice, innate signaling alone is insufficient to initiate Th polarization in naive CD4⁺ T cells, that it instead develops from pre-existing memory CD4⁺ T cells that express cross-reactive TCRs, and that it inevitably leads to immunopathology.

Keywords: T helper differentiation, T helper polarization, Cross-reactivity, Regulatory T cells, Microbiota, Original Antigenic Sin

INTRODUCTION

Upon recognition of pMHC-II_s, naive CD4⁺ T cells expressing cognate TCRs undergo a Th differentiation process primarily orchestrated by the cytokine milieu that develops during antigenic challenge. Most models to explain Th differentiation developed when only two types of T helpers, Th1 and Th2, were known to exist (Mosmann and Coffman, 1989), which may be why TCR affinity became the simplest way to explain their apparent dichotomy: high-affinity interaction with cognate pMHC-II_s would lead to Th1 and low-affinity to Th2 or vice versa (van Panhuys et al., 2014).

Though this simplistic model seemed plausible for a binary (Th1 vs. Th2) choice, it cannot explain actual T helper differentiation which we now know entails multiple choices (Th1, Th2, Th17, Tfh, Tregs, etc.). New experiments further complicate matters by revealing a vast heterogeneity of effector states with distinct cytokine footprints (Tortola et al., 2020). Moreover, recent single cell analyses combining TCR and cytokine profiles provide evidence that during immune response, naive CD4⁺ T cells expressing identical TCRs acquire several functional helper phenotypes such as T helper 1 (Th1), T follicular helper (Tfh), T helper 2 (Th2), or T helper 17 (Th17) (Becattini et al., 2015; Khatun et al., 2020).

Subsequently, in addition to TCR affinity (Tubo et al., 2013; van Panhuys et al., 2014; Tubo and Jenkins, 2014; Kotov et al., 2019), microbial (Iwasaki and Medzhitov, 2015) or tissue-tailored mechanisms (Matzinger and Kamala, 2011) also attempt to explain how T helper fate is determined.

The microbe-tailored is the most prevalent and widely accepted model (Iwasaki and Medzhitov, 2015). It is an ‘umbrella’ term under which several sub-models are interchangeably used to explain how different pathogen-associated molecular patterns (PAMPs) activate various types of dendritic cells or other accessory cells to create different cytokine milieu that in turn drive different types of Th programs (Yin et al., 2021).

The tissue-tailored model, on the other hand, argues that tissue and its components determine overall Th differentiation pathways, the primary rationale being that tissues ‘know’ best which type(s) of Th cells they could accommodate to achieve clearance of an invading pathogen with minimal damage to themselves (Matzinger, 2007; Matzinger and Kamala, 2011).

These models all share the characteristic that they describe fixed, stereotypical responses. Here we propose that to properly understand Th phenotype differentiation, we have to bifurcate two phenomena associated with this process that are frequently and erroneously conflated: instruction to develop a particular Th phenotype *per se*, i.e., normal Th differentiation (Th fate choice) versus Th

polarization. We argue that the former is a physiological stochastic process while the latter is a strictly pathological response requiring presence of pre-existing cross-reactive memory Th cells (Fig. 1).

Th POLARIZATION VS. Th FATE CHOICE

Though Th fate choice is a neutral sounding concept, the same *in vitro* studies that originally identified Th1 and Th2 helper cells in the 1980s also embedded the notion of Th polarization from the very beginning. Thus, different Th programs were deemed mutually exclusive and cross-inhibitory, and much research was dedicated to validate this idea. For example, the notion that IL-12 drove Th1 and inhibited Th2 while IL-4 drove Th2 and inhibited Th1 dominated the literature for several years. The exclusive nature of Th fate choice being taken for granted also prompted the construction of theoretical frameworks that relied on innate signals such as PAMPs or damage-associated molecular pattern (DAMPs) to explain such apparent exclusivity.

However, mutually exclusive and cross-inhibitory models of Th fate programming are fundamentally flawed.

These models cannot explain how to prevent one type of Th phenotype from constantly ‘dominating’ over other types of Th cells in the course of an ordinary adaptive immune response. If high-affinity TCR interaction with cognate pMHC-II generated one type of Th cell, let's say Th1, considering total TCR and pMHC-II repertoire diversity, what would prevent such a system from constantly generating Th1 cells that could ‘dominate’ Th2, Tfh or other types of Th cells?

Similarly, if innate signals induced a particular Th phenotype that happened to be non-protective against a pathogen, how could it be stopped? The immune system couldn't just switch to another Th polarization as such models presume that first Th fate induced would cross-inhibit subsequent Th subtypes (Kalinski and Moser, 2005).

This problem with microbe-tailored models is best illustrated by the classical mouse model for leishmania infection. *Leishmania major*, a protozoan parasite, drives ‘appropriate’, protective Th responses in B6 mouse strain while it drives ‘inappropriate’, non-protective Th responses in Balb/c mouse strain (Sacks and Noben-Trauth, 2002). This example illustrates that failure to drive ‘appropriate’ Th cells can lead to immunopathology. Yet an innate immune system that could effectively deal with a pathogen would make the adaptive immune system superfluous and one that couldn't wouldn't be able to instruct the adaptive immune system anyway.

Our solution for this dilemma is to separate Th fate choice (or Th differentiation) from Th polarization. The adaptive immune response to an antigen is polyclonal by default. Even if unique factors play some deterministic role in programming a particular Th fate at the T cell clonal level, the overall composition of different Th fates within the responding antigen-specific CD4⁺ T cell pool would still represent a wholly unpredictable stochastic process determined by the summary of various signaling in a given milieu. Individual naive T cells would acquire one or several Th fates in a non cross-inhibitory process. Indeed the immune system generates all sorts of Th cells during an immune response. From an evolutionary perspective, it does not matter for the adaptive immune system how many different Th types are generated during an immune response, one or a dozen, as long as they are not anti-self and are ultimately protective against a given pathogen.

PROTECTIVE AND EFFECTIVE Th CELL RESPONSES REQUIRE SELF-NON-SELF DISCRIMINATION

The central conceptual flaw in prior models of Th fate determination is they focus on explaining how to achieve protective and effective Th cell responses (Kalinski and Moser, 2005; Cohn, 2008). However, this goal is not achievable unless and until the mechanism that drove Th programs also ‘knew’ how to make *self-nonself discrimination* at the same time. This is because there is no ‘best’ protective and ‘success-driven’ effective Th response against self (Kalinski and Moser, 2005). Any immune response against self is non-protective and ineffective by definition.

This means that when responding to a pathogen, the immune system should ‘know’ it responds to pathogen (nonself) and pathogen alone, and not to self. However, as we explain in detail in our previous papers (Usharauli and Kamala, 2018, 2021), innate-based mechanisms cannot be a part of self-nonself discrimination process as such a system would require neither thymic negative selection of auto-reactive T cell clones nor presence of thymus-derived Foxp3⁺ regulatory T cells (Tregs) to maintain tolerance.

Likewise, while some aspect of Th program could be influenced by tissue-derived components or DAMPs, any tissue needs to differentiate between ‘good’ and ‘bad’ immune responses to know which immune response is best for it. However, any anti-self immune response is bad by default and the tissue must be able to stop it. Simply changing one type of Th response to another wouldn't suffice since all types of anti-self Th responses are bad by default. This means that for a tissue to stop anti-self immune responses, it should make self-nonself discrimination. If tissues themselves could do that, they

would require neither thymic negative selection of auto-reactive T cell clones nor presence of thymus-derived Foxp3⁺ regulatory T cells (Tregs) to maintain tolerance. For the same reason, as discussed in our previous papers, tissues cannot induce *de novo* Tregs from naive T cell clones but only support Treg maturation from precursors already epigenetically poised for Treg pathway in the thymus before being seeded in the periphery (Usharauli and Kamala, 2018, 2021).

Immune responses do occasionally end up being anti-self and/or non-protective (ineffective) against a pathogen (nonself). We explain below how TCR cross-reactivity could be the basis for both anti-self immune response and non-protective Th polarization phenomena, both leading to immunopathologies. Specifically, using the SPIRAL (Specific ImmunoRegulatory Algorithm) model, we show that while individual Th fate choices at the T cell clonal level are unpredictable stochastic processes and a summation of variety of signals from both microbes and tissues, Th polarization is a predictable, pathological T cell response that strictly requires the presence of pre-existing memory CD4⁺ T cells expressing cross-reactive TCRs that ‘dominate’ other naive CD4⁺ T cells trying to acquire other Th fates (Fig. 1).

WHY Th POLARIZATION REQUIRES CROSS-REACTIVE MEMORY CD4⁺ T CELLS

As discussed in our previous papers introducing the SPIRAL model (Usharauli and Kamala, 2018, 2021), an ineffective anti-pathogen Th response is polarized by default and dominantly cross-inhibits other Th subtypes. Normally, Th fate choice is a non-zero-sum process inducing multiple Th subtypes that do not inhibit each other and the overall immune response is protective against the pathogen as at least one of them would be effective against it. Such a system however fails if one Th subtype dominates others but does not provide any protection. Thus any non-protective T cell response against any pathogen is polarized. Here we explain how such polarization is not by default but rather requires the presence of pre-existing memory T cells expressing cross-reactive TCRs.

Initially we considered that certain types of antigens in conjunction with certain types of innate signaling would suffice to drive Th polarization from naive CD4⁺ T cells (Usharauli and Kamala, 2018). However, further development of the SPIRAL model suggests that unlike Th fate choice where any random naive CD4⁺ T cell can acquire any Th fate, a pathological Th polarization would strictly require the presence of pre-existing cross-reactive memory CD4⁺ T cells. The SPIRAL model further predicts that Tregs can prevent such Th polarization by relying on shared TCR cross-reactivity.

Why must Th polarization ensue from pre-existing, cross-reactive memory T cells? According to the SPIRAL model, thymus-derived Foxp3⁺ T cells use cross-reactivity to control T cells ‘destined’ to cause immunopathologies such as auto-reactive T cells or T cells involved in allergies or non-protective responses to pathogens. Previously, we proposed that these T cells are so called natural IL-2 producing T cells (IL-2p T cells). The SPIRAL model predicts that this control by Tregs occurs because both Tregs and those ‘faulty’ T cells. i.e. IL-2p T cells, recognize mutually cross-reactive epitopes (Fig. 2). In other words the SPIRAL model predicts Tregs control both self-nonsel self discrimination and Th polarization through mutual TCR cross-reactivity while host microbiota provide the pMHC-II necessary for Treg functionality (Al Nabhani et al., 2019; Usharauli and Kamala, 2018, 2021).

Ordinarily, innate signaling cumulatively determines overall Th fate trajectories of naive T cells. However, if innate signaling could induce Th polarization from any random naive CD4⁺ T cell, it would also allow any pathogen-derived pMHC-II to induce Th polarization from any cognate naive T cell. Such ‘total’ [every T cell epitope] polarization cannot be rescued by antigen-specific Tregs but must be shut down completely, rendering it evolutionarily non-selectable. On the other hand, a ‘partial’ Th polarization when one or few pathogen-derived pMHC-II selectively induce it from specific naive T cells suggest there's something ‘special’ about such naive T cells (which we think are instead pre-existing memory T cells).

That the immune system occasionally ends up with ‘unbalanced’, polarized immune responses brings up the role of pre-existing memory T cells. During immune response, if innate signaling (lets say type II cytokines) and cross-reactive pMHC-II were able to engage pre-existing memory T cells then such combination would be able to induce type II dominated Th polarization that could ‘dominate’ other naive CD4⁺ T cells trying to acquire other Th fates.

Thus, a pathological T helper polarization controlled by antigen-specific Tregs requires it to be ‘partial’ at the pMHC-II level but still act as ‘total’ from the T cell side. This is only possible if

- (a) pre-existing memory T cells engaged pathogen-derived pMHC-II that were cross-reactive by chance,
- (b) these pre-existing memory CD4⁺ T cells ‘dominated’ other naive CD4⁺ T cells trying to acquire other Th fates,
- (c) and these pre-existing memory CD4⁺ T cells could in turn be shut down by Tregs that recognized mutually cross-reactive epitopes.

Such a process would depend on the combination of antigen-specific Treg repertoire, the microbiota that maintained them and the prior immunological experience of the host.

Furthermore, for innate signaling alone to drive pathological T helper polarization from naive CD4⁺ T cells would require changes in it that only selectively affected responses to non-self. However, changes in innate signaling cannot serve to ‘perfect’ the adaptive immune response as no response to self can be ‘perfected’. Moreover, innate signaling could not ‘shut down’ T helper responses, even an anti-self one, as that would require it to ‘know’ the difference between T helper responses to self and nonself. However, this is the sole prerogative of antigen-specific thymic Tregs. On the other hand, control of pathological Th polarization using adaptive Treg/pMHC-IIs/microbiota axis is a more versatile system ‘perfectible’ at the species level in evolutionary terms (Fig. 2).

Further, if Tregs controlled T helper responses in an antigen non-specific manner, for example, by silencing T cells only in absence of innate signaling such as IL-6 (Pasare and Medzhitov, 2003), then a Treg clone with a single TCR specificity would suffice to control all other T cells. This is however squarely incompatible with the presence of diverse repertoire of antigen-specific thymic Tregs. On the other hand, SPIRAL predicts that Tregs do not control the magnitude of T helper response *per se* as some models argue (Cohn, 2008) but simply control self-nonself discrimination, and that suppression of antigen-specific T helper polarization is an integral part of this process.

These analyses lead us to conclude that Th polarization is a T cell-intrinsic feature that requires the presence of pre-existing memory T cells that can engage certain pMHC-IIs and, in combination with cytokine milieu, polarize overall T cell response. Such cross-reactivity for pMHC-IIs allows Th polarization and is likely also the basis for ‘original antigenic sin’.

An ideal immune system would have a T cell pool devoid of ‘original antigenic sin’ that could interfere with subsequent T cell responses to pathogens through chance cross-reactivity. However, the immune system cannot ‘know’ a priori which pre-existing memory T cells to nonself would cross-react with subsequent nonself cross-reactive epitopes and negatively influence the outcome of ongoing immune response. The only way to ensure a ‘balanced’ immune response to any pathogen or allergen is to delete those ‘faulty’ IL-2p T cells in the thymus from the get-go or to generate Tregs via thymus/microbiota axis to keep them under their control via shared TCR cross-reactivity as discussed above and in our previous papers.

In this light, specific allergies denote nothing special about any allergen but are a consequence of having pre-existing memory-like T cells cross-reactive to epitopes derived from that antigen. Likewise, non-protective anti-pathogen T cell responses denote nothing special about any pathogen but ensue from having pre-existing memory-like T cells cross-reactive to epitopes derived from that pathogen. Cross-reactivity at the level of pMHC-II/HLA-IIs (Selin et al., 2006; Welsh et al., 2006;

Bacher et al., 2019)) would dictate such processes and the SPIRAL model predicts lacuna in a host's microbiota and consequent lacuna in their Treg repertoire are the basis for these immunopathologies.

SUMMARY

Here we present a novel mechanism derived from the SPIRAL model to explain that Th fate choice and Th polarization are two different processes; the former a physiological one initiated from naive CD4⁺ T cells in response to innate signals alone and the latter a pathological one that in addition strictly requires the presence of pre-existing memory Th cells responding to cross-reactive pMHC-II_s. The SPIRAL model predicts that Th polarization is host-intrinsic and leads to immunopathologies such as allergy or ineffective immune response to pathogens and that cross-reactivity at the level of TCR/pMHC-II ultimately determine a given host's susceptibility to such immunopathologies.

Conflict of interest statement: David Usharauli and Tirumalai Kamala are shareholders of Tregutix Inc., a biotech company that focuses on developing microbiota guided antigen-specific immunotherapies.

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SPIRAL model

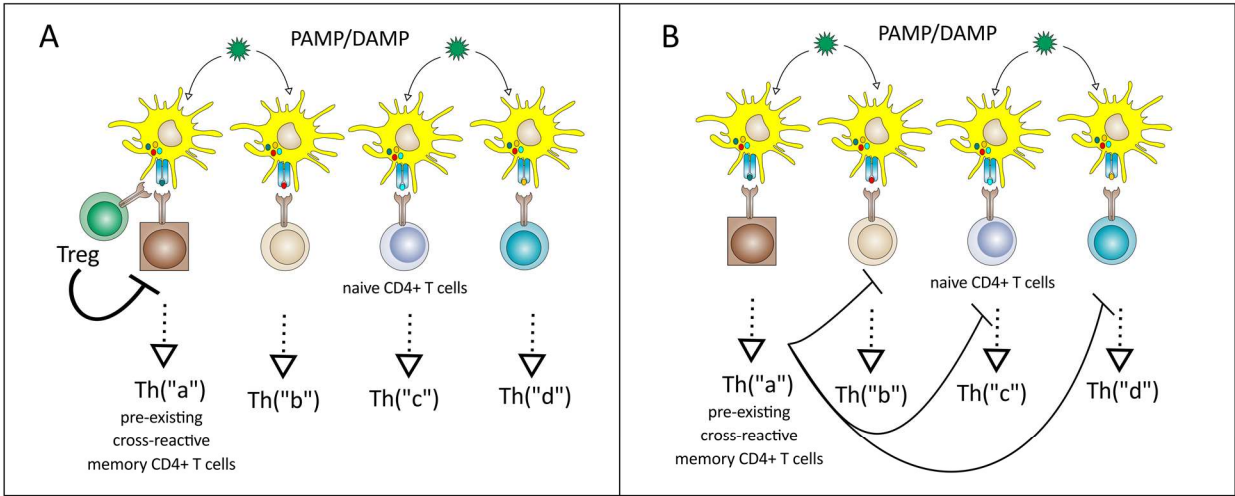


Figure 1. (A) Cross-reactivity provides the basis for control of pathological T helper polarization of pre-existing memory Th cells in the presence of the 'partner' Tregs. (B) In the absence of 'partner' Tregs, such memory Th cells would become polarized upon encounter with cross-reactive epitopes and would 'dominate' other naive CD4+ T cells trying to acquire other Th fates.

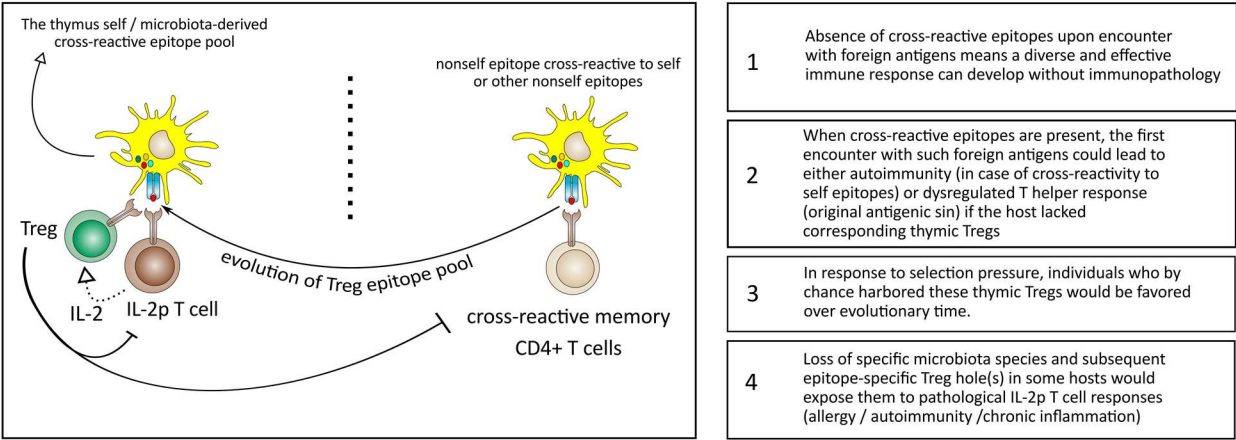


Figure 2. The SPIRAL model. An evolutionary selection cycle enriches for cross-reactive Treg pool that controls self-reactive or pathologically polarized foreign-specific T helper responses. Under evolutionary selection pressure, the thymus and microbiota would 'adopt' and present cross-reactive epitopes that happened to initiate immunopathology in order to generate and maintain specific Tregs, respectively. These Tregs control both autoimmunity and pathological T helper polarization in an epitope-specific manner by relying on cross-reactive epitopes generated from a select set of microbiota species. IL-2 essential for these Tregs is produced by 'partner' cross-reactive IL-2p T cells in dyads. When microbiota are lost, so too are corresponding epitope-specific Tregs and this loss enables their 'partner' IL-2p T cells to initiate immunopathology.