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# An identical mechanism governs self-nonself discrimination and effector class regulation

Tirumalai Kamala[1], David Usharauli[1]

[1] Tregeutix Inc., Orlando, FL 32820, USA.

Correspondence: tkamala@tregeutix.com, dusharauli@tregeutix.com



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**Abstract**

Prevailing immunological dogma dictates self-nonself discrimination, meaning to respond or not, and effector class regulation, meaning choosing the most effective response, are two separate decisions the immune system makes when faced with a new antigen. Representing a cardinal departure from the past, our model instead predicts both self-nonself discrimination and effector class regulation are in fact one and the same process controlled by $Foxp3^+$ regulatory T cells (Tregs) whose antigen-specific repertoire is entirely maintained by commensal microbiota-derived cross-reactive antigens.

## Introduction

For the past sixty years, advances in immunology closely followed transformative concepts in the field. Several, such as clonal selection theory (Burnet, 1976), (Lederberg, 1959), two-signal model (Bretscher and Cohn, 1970), dominant tolerance model (Coutinho et al., 1993), (Coutinho et al., 2001), and pathogen-associated molecular patterns (PAMPs) (Janeway, 1989) and Danger models (Matzinger, 1994), formed the basis for much scientific progress observed to date in immunology.

Upon scrutiny, a common theme is these models work “in theory” either without Tregs altogether or suffice with presence of Tregs of a single or limited antigen specificity controlled by the innate immune system. Though compelled to acknowledge their existence, these models are instead “rescued” by assigning dubious functions to Tregs, such as regulation of anti-nonself T cell response magnitude or even actually representing as yet undefined $CD4^+$ T helper cells. Such inconsistencies and contradictions are to be expected as these concepts emerged long before the exact identity and nature of Tregs became known. However, more than fifteen years



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since their discovery, no model of predictive value has emerged to harmoniously incorporate their role in self-nonself discrimination (antigen-specific immunological tolerance) and effector class regulation. Therapeutic applications of Tregs to treat immune disorders have thus been exceedingly slow to materialize.

Here, we propose, for the first time, a novel harmonized model that predicts antigen-specific Tregs control both self-nonself discrimination and effector class regulation, which are in fact one and the same process. Our model provides guiding principles for Treg function and bridges it to therapeutic application.

## Self-Nonself Discrimination

Conceptually, to respond or not to a new antigen may be the immune system's most consequential decision. As a single, naive antigen-specific T cell clone can initiate a complete immune response, its activation must be tightly controlled (Stemberger et al., 2007), (Stemberger et al., 2014), though strikingly neither it nor antigen-presenting cells (APCs) can intrinsically determine if an antigen (epitope) is self or nonself.

Initially, self-nonself discrimination was thought to occur at the embryonic or immature T cell level. However, subsequent discovery of mature T cells fully capable of responding to self antigens necessitated introduction, first, of antigen-specific variants, and later, following the collapse of T “suppressors” in the 1980s, of antigen non-specific variants of the two-signal model, which still dominate the field.

Hypothetically, self-nonself discrimination could be accomplished by either selective amplification of anti-nonself or selective abrogation of anti-self responses. Selective amplification of anti-nonself response, as proposed in the Associative Recognition of Antigen



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(ARA) model, can essentially function without Tregs as it relies exclusively on an embryonic "primer" anti-nonself T helper to initiate immune responses, with antigen-specific tolerance as the default state (Cohn and Langman, 2002), (Cohn, 2004). Alternatively, selective abrogation of anti-self response, such as proposed by PAMP or dominant tolerance models, can essentially function with Tregs having single or limited antigen specificity that suppress anti-self T cell responses in an antigen-nonspecific manner following "innate instructions". However, these aforementioned models are incomplete since Tregs with a diverse T cell receptor repertoire do play a central role in maintaining tolerance (Levine et al., 2017), (Yu et al., 2017).

We argue that the role of diverse antigen-specific Foxp3$^+$ Tregs that control self-nonself discrimination and effector class regulation is best described by a model wherein (a) Tregs are continually needed to prevent T cell-mediated inflammatory tissue damage since conventional T cells are constantly exposed to cognate antigens in an activation context, and (b) antigen-specific T cells, when activated, continue responding until their specific antigens are cleared as neither they nor any non-specific source can "tell" if this response is directed against self or nonself antigens.

To fully understand our model requires we rethink how central and peripheral tolerance are established. Prevailing concepts, largely variants of a two-signal model (Baxter and Hodgkin, 2002), argue peripheral tolerance to tissue-specific antigens is a "theoretical" necessity that must exist separately from central, thymic tolerance. Underlying presumptions are that:

(a) thymus cannot possibly tolerize against all peripheral tissue-specific antigens, and

(b) since naive T cells are intrinsically unable to distinguish self from nonself antigens in the periphery, there must be certain mechanism(s) that make that call for them.

Following these arguments, possible outcomes for a self-specific naive T cell leaving the thymus and seeing its antigen in the resting peripheral tissue for the first time include



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(a) deletion/inactivation.

(b) Treg conversion.

(c) activation and effector/memory differentiation.

Leaving “no trace” or “memory” of antigen encounter, option (a) is an unreliable peripheral tolerance mechanism as it risks autoimmunity with every single occurrence of infection since absent permanent “memory” of “what is what”, next encounter of the same specificity naive T cell clone with the same self antigen could occur in the context of chance inflammation leading to its activation. As activated anti-self T cells would invariably lead to non-productive response, it would seem relatively safe to conclude that deletion/inactivation of self-specific T cells by antigen (signal 1) in the periphery could not on its own be a reliable peripheral tolerance mechanism. In other words, to reliably and predictably avoid autoimmunity, the immune system must “know” where each of its T cell clones “stands”.

In contrast, models such as Danger allow a given antigen to be self one time and nonself at another as they unequivocally rely on the premise that activated, bystander anti-self T cells can be inactivated under steady state conditions once the “danger” is past. However, such models do not need Tregs to maintain antigen-specific tolerance.

Options (b) and (c) produce “trace” or “memory” of past self antigen encounter, either as benign Tregs or as potentially damaging conventional memory T cells, respectively. However, for the former to prevail the host immune system must ensure the initial wave of naive T cells that first migrates out of the thymus and encounters self antigens in the periphery become Tregs rather than effector/memory T cells. Problem is the immune system cannot anticipate that a self-specific naive T cell leaving the thymus wouldn't encounter its antigen in the periphery for the first time in the context of chance inflammation.

Why does this matter? Hypothetically, the response of activated T cells specific for a nominal, peripherally and transiently expressed nonself antigen would run its course, become



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naturally extinguished upon memory T cell formation following antigen clearance, and require no Tregs. However, response of activated T cells specific for a persistent self (or nonself) antigen would run perpetually to exhaustion as functional memory T cells cannot be formed during continual engagement of TCR signaling by such antigen. As such sustained T cell responses invariably lead to irreversible tissue damage, we refer to them as non-productive "spiral" responses.

Obviously only antigen clearance or memory T cell formation could end such a T cell "spiral" response. With neither option ordinarily available to T cells responding to persistent antigens such as self antigens, how could the immune system deal with inevitable "spiral" responses? We conclude that

(a) Foxp3 transcription factor and associated Treg signature prevents perpetual T cell exhaustion by stabilizing T cell memory program to persistent antigens, and

(b) Foxp3$^+$ regulatory T cells evolved to prevent non-productive T cell "spiral" response to such persistent antigens.

Essentially, we posit that Tregs represent "memory" T cells specific for persistent antigens (van der Veeken et al., 2016). Unlike conventional memory CD4$^+$ T cells, their stability and/or functional viability would strictly depend on continual engagement of their TCRs with corresponding persistent antigens (Levine et al., 2014), (Vahl et al., 2014). We predict that in health, antigen (epitope)-specificity of Tregs and conventional memory CD4$^+$ T cells would be non-overlapping and mutually exclusive (Golding et al., 2017), (Bacher et al., 2016).

Could peripheral tissues initiate Treg conversion pathway? Though it sounds quite intuitive, thymus-independent peripheral Treg conversion by resting tissue injects much uncertainty into immune system decision-making since it necessarily relies on antigen non-specific readout of tissue "health" status. For a tissue to instruct Treg conversion, it must "know" that it was "healthy" and did not contain any nonself antigen. However, tissues capable of such



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fine distinction between self and nonself antigens or between “health” and “non-health” status would not require Tregs to maintain antigen-specific tolerance to begin with. Thus, peripheral Treg conversion is only acceptable for T cells already committed in the thymus to Treg pathway or for such conversion to be directed by another Treg in an antigen-specific manner.

If peripheral Treg conversion is unlikely, how then is tolerance to tissue-specific self antigens in the periphery established? Since they dependably jump-start tolerance to such antigens (Legoux et al., 2015), (Malhotra et al., 2016), we hold that the only reliable mechanism for establishing peripheral tolerance is to export ready-made, committed thymic Foxp3$^+$ Tregs specific for self antigens that naive T cells are likely to see in the periphery after emigrating from the thymus. Such thymic Treg export makes requirement for a special developmental “tolerance window” in the periphery obsolete.

However, how to account for thymic Tregs specific for nonself microbial antigens such as those derived from commensal microbiota that the host hasn't yet encountered but must tolerate? We predict the host had to “adopt” and thymically express all those antigens (as epitopes), including those from pathogens, that applied evolutionary selection pressure on the immune system in the form of non-productive T cell “spiral” responses. Over evolutionary time, such antigens became part of the “cross-reactive” self epitope landscape leading to thymic generation of functional “cross-reactive” Foxp3$^+$ regulatory T cells. Though thymically derived, such antigen-specific Tregs would be peripherally maintained by microbiota-derived “cross-reactive” nonself epitopes. Epitopes expressed synchronously by thymus and periphery guarantee development and peripheral maintenance of Foxp3$^+$ regulatory T cells specific for those epitopes.

It must be emphasized here that maintenance of both anti-self- and anti-nonself-(pathogen) Tregs would depend on microbiota-derived “cross-reactive” antigens. If peripheral self antigens maintained self-specific Tregs, their loss would require loss of self antigens that



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maintained them. However, loss of such self antigens would preclude autoimmunity in the first place. Similarly, microbiota-derived “cross-reactive” antigens would maintain anti-pathogen Tregs since pathogens themselves, unlike commensal microbiota, cannot be constantly present in the host to maintain such Tregs. Here we only refer to pathogen-derived nonself antigens that applied evolutionary selection pressure on the immune system in the form of non-productive T cell “spiral” responses.

While prior models erroneously accept *a priori* that self-nonself discrimination is configured *de novo* ontogenetically each time within an individual's own lifetime, we posit it to be a phylogenetically powered process established, inherited and being perfected over the evolutionary history of a species.

Finally, if ordinarily thymus generates most Tregs, then default pathway for naive T cells that encounter their cognate antigen in the periphery would be activation and effector/memory differentiation (Bingaman et al., 2000), (Anderson et al., 2001).

We refer here to antigen-specific naive T cells newly emerged from the thymus that lack corresponding thymic Treg counterparts to buffer them. Normally, evolutionarily relevant self or nonself antigens (epitopes) would be “covered” by “cross-reactive” thymic Tregs that would prevent either by deletion, inactivation or conversion, naive T cells from responding to them (Kendal et al., 2011). Remaining “non-covered” antigens (epitopes) recognition would by default lead to cognate naive T cell activation and effector/memory differentiation.

**Effector class regulation**

Having provided a foundation for self-nonself discrimination based on thymic Foxp3$^+$ Tregs' ability to specifically prevent non-productive “spiral” T cell responses directed to



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183 evolutionarily relevant “persistent” antigens, both genuine self and “cross-reactive” microbiota-
184 derived nonself (extended self), we now expand this novel model to argue self-nonself
185 discrimination and effector class regulation are one and the same process mediated by antigen-
186 specific Foxp3$^+$ Tregs.

187 Avoiding a non-productive anti-self “spiral” response and selecting the most effective
188 anti-nonself effector class are assumed goals of self-nonself discrimination and effector class
189 regulation, respectively. At first glance these two goals seem unrelated, requiring at least two
190 different sets of mechanisms. While self-nonself discrimination is essentially a “binary” choice,
191 effector class regulation presents a unique “multiple choice” dilemma given the immune
192 system's ability to deploy multiple response classes such as $Th_1$, $Th_2$, $Th_{17}$, etc (Becattini et al.,
193 2015). What mechanism(s) could govern “multiple choice” decisions that yield appropriate
194 effector class?

195 No viable model for effector class regulation exists at present. Most if not all of several
196 proposals put forward over the past forty years exclusively focus on trying to explain how the
197 most effective anti-pathogen or tissue-compatible effector class could be selected. These
198 models could be broadly divided into two major categories:

199 (a) those with innate signals that specifically instruct stereotypical effector classes to
200 antigens, and

201 (b) those with adaptive feedback-loop learning processes.

202 While models based on adaptive feedback-loop learning processes gained little traction
203 due to their complicated mechanisms of action (Langman, 1984), (Kalinski and Moser, 2005),
204 models based on innate signals are simple, intuitive, somewhat predictive and widely referred to
205 in the scientific literature. Two such “instructional” models worth noting here argue either
206 PAMPs (Iwasaki and Medzhitov, 2015) or tissues (Matzinger, 2007) direct effective class(es) of
207 immune response.



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However, we posit neither proposal makes much evolutionary sense. On the one hand, it seems unlikely for a pathogen to direct the most effective immune response best able to clear it from the host. Rather, pathogens are more likely to evolve to instruct types of immune response less able to clear them efficiently.

On the other hand, for tissues to direct the most effective or tissue-compatible effector class requires they develop mechanism(s) to distinguish between effective (productive) and ineffective (non-productive) effector classes. However, anti-self immune response of any class being by default non-productive and thus non-selectable implies tissues must distinguish between anti-self (non-productive) and anti-nonself (productive) responses by selectively inhibiting the former at the very least. In essence, such effector class control would entail tissue-based self-nonself discrimination, which could function without Foxp3$^+$ Treg involvement, an untenable proposal.

Here we extend our new model and propose that contrary to such long-held views, self-nonself discrimination and effector class control are one and the same process mediated by antigen-specific Foxp3$^+$ regulatory T cells.

Earlier we concluded that self-nonself discrimination is essentially inhibition of ineffective (non-productive) immune responses by antigen-specific Foxp3$^+$ Tregs, allowing effective (productive) ones to develop naturally. Ineffective T cell response could be anti-self, leading to *bona fide* autoimmunity, or anti-nonself, leading to inflammatory tissue damage. Indistinguishable outcomes for the host, both would diminish host fitness and apply evolutionary selection pressure that directed development of novel specificity of Foxp3$^+$ regulatory T cells.

We can now seamlessly incorporate Foxp3$^+$ Tregs into effector class control. Rather than having some mechanisms supposedly select the most effective immune effector class, the reverse approach falls into place naturally: antigen-specific Foxp3$^+$ Tregs simply inhibit T cell specificities that “historically” led to non-productive (ineffective) T cell responses. In essence, if



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a system could prevent initiation of ineffective (non-productive) T cell responses then those that remain are by default effective (productive).

How could Foxp3$^+$ Tregs control effector class in an antigen-specific manner? For an answer we need to rethink how various effector classes are established.

Unlike T cell response to self antigen where any response is non-productive “spiral” and thus detrimental by default, response to pathogen antigen could be divided into either productive or non-productive. Were every effector class able to run simultaneously and independently then immune response to any pathogen antigen would always end up productive as at least one of them would be expected to be effective. The fact that non-productive T cell responses to pathogen-derived antigens do occur suggests that at least in certain conditions multiple effector classes cannot co-exist, and in fact in such conditions, a non-productive effector class abnormally dominates by inhibiting other potentially effective classes. In essence, it is in the pathogen's interest to promote a non-productive T cell response to its antigens, which invariably ends up as a highly polarized dominant but ineffective effector class.

Reasons for such dominance of a particular effector class could be several. For one, immunodominant microbial antigens could inherently promote a highly polarized non-productive effector class that constrained other effector classes to other antigens. For another, the host could harbor genetic polymorphism(s) in signaling pathways that favored excessive generation of a particular effector class that also happened to be non-productive against a given microbial challenge.

Essentially, we conclude that nonself antigens being “neutral” in nature and host genetic mutations not favoring any particular effector class would allow host's immune response to such nonself antigens to be diverse, balanced and always effective, making presence of anti-nonself Foxp3$^+$ regulatory T cells obsolete. However, if either a host mutation or the inherent nature of nonself antigens favored one particular effector class, such a polarized effector class would



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inevitably undermine other effector classes and become non-productive by default. As such, highly polarized non-productive T cell response to nonself antigens would be subject to negative evolutionary selection.

In our opinion, effector class control is in fact control of non-productive T cell responses by $Foxp3^+$ Tregs in an antigen (epitope)-specific manner same as self-nonself discrimination. The host does not care whether effective (productive) T cell response to microbe is $Th_1$, $Th_2$ or $Th_{17}$ (Becattini et al., 2015). It could be either one or all of them, as long as they aren't non-productive. In evolutionary terms, rather than investing in selecting an effector class optimal to a particular microbe, we propose the immune system invested in preventing, in an antigen-specific manner, those types of T cell responses that were “historically” non-productive “spiral” and would have invariably diminished host fitness.

A nonself antigen that inherently promotes one particular effector class, for example, $Th_1$ response, would do it to each of its linked epitopes, a concept referred to as “coherence” (Bretscher, 2014). Were such a $Th_1$ response non-productive, then each of those epitopes would require a separate $Foxp3^+$ Treg to oversee them. Consequently, such nonself antigens would be off-limits to any effector class as typical self antigens (and their epitopes) are. How then, one may ask, are such nonself antigens eliminated? Since such nonself antigens are ordinarily themselves part of larger biological life forms such as pathogens, rather than being directly targeted, other nonself antigens and their epitopes from the same pathogen would be targeted by other effector classes that could now operate without constraint from one dominant overzealous effector class. Surely one of them would be productive and eliminate the pathogen.

If, on the other hand, a nonself antigen promotes a productive immune response while having one of its epitopes cross-reactive to a self epitope then only that particular cross-reactive “epitope” would be covered by existing epitope-specific $Foxp3^+$ Tregs while other epitopes would be targeted by conventional, productive T cell responses.



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We conclude that microbial antigens evolutionarily associated with non-productive T cell responses would have been "adopted" by the successful host into "cross-reactive" self epitope map in the thymus to generate antigen (epitope)-specific Foxp3$^+$ Tregs, which would be maintained in the periphery via acquisition of commensals providing such "cross-reactive" antigenic epitopes. When a host encountered the same antigens expressed by a pathogen, existing antigen-specific Foxp3$^+$ Tregs would prevent activation of those naive T cells that could lead to non-productive response classes. Consequently T cell responses to remaining pathogen antigens would be productive (effective) by default.

By explaining how the immune system dealt, or rather avoided, the hard to solve "multiple choice" dilemma unique to effector class control, our model reveals it to be not a unique, separate concept but rather an integral part of self-nonself discrimination. Each principle that applies to the latter applies to the former. These two decisions entail one and the same biological process based on the Foxp3$^+$ regulatory T cell's ability to prevent non-productive T cell "spiral" responses in an antigen-specific manner.

Finally, we hold that our model does not necessarily suggest that PAMPs or tissues play no role in T cell effector differentiation. In fact, both PAMPs and tissue signals are involved in initiation of different effector classes. We just predict that for a given anti-microbial response, effectiveness of those innate signals or dominance of any particular T cell response class would be hard to predict. PAMPs and tissues can initiate selective or diverse sets of effector classes but the "veto" power to selectively modify effector classes to improve effectiveness rests with antigen-specific Foxp3$^+$ regulatory T cells.

In summary we have put forward a new model to explain how thymic antigen-specific Foxp3$^+$ Tregs maintained by commensal microbiota-derived cross-reactive antigens control both self-nonself discrimination and immune effector class by preventing non-productive T cell "spiral" responses to evolutionarily relevant antigens, both self and nonself. This model



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harmoniously incorporates the role Tregs and microbiota play in self-nonself discrimination and effector class regulation, and provides a roadmap for microbiota guided antigen-specific immunotherapies for diseases such as allergies, autoimmune diseases and others.

The essential highlights of this novel "SPIRAL" model (see figure below) are the following:

1. Treg signature (Foxp3, etc) maintains functionality of regulatory T cell memory specific for persistent antigens.
2. Maintenance of $Foxp3^+$ regulatory T cells is antigen-specific and antigen-dependent.
3. Most, if not all, $Foxp3^+$ regulatory T cells are thymus-generated and peripherally-maintained by microbiota.
4. T cell response to self and abnormally polarized T cell response to non-self antigens are by default non-productive "spiral" responses that invariably lead to inflammatory tissue damage and diminish host fitness.
5. $Foxp3^+$ regulatory T cell repertoire is phylogenetically configured by host species' evolutionary experience with non-productive T cell "spiral" responses.
6. Anti-self and non-productive anti-nonself responses impose the same evolutionary burden.
7. Nonself antigens that historically caused non-productive "spiral" responses became evolutionarily "adopted" as self antigens (epitopes) expressed in thymus and maintained in periphery by "cross-reactive" microbiota.
8. Maintained by commensal microbiota-derived cross-reactive antigens, $Foxp3^+$ regulatory T cells control non-productive responses to self and pathogen-derived nonself antigens in an antigen-specific manner to prevent non-productive self-



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perpetuating T cell “spiral” responses.

9. A given antigen-specific Foxp3$^+$ regulatory T cell controls corresponding naive CD4$^+$ T cells with the same or similar specificity.
10. Self-nonself discrimination and immune effector class regulation is one and the same process.
11. Healthy antigen-specific TCR repertoires of conventional memory and Foxp3$^+$ regulatory T cells are mutually exclusive.
12. Activated conventional T cells autonomously self-perpetuate their own response as long as antigen is present.

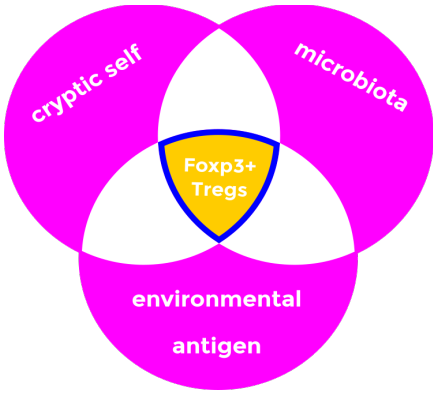


**Figure legend:** Thymic FoxP3$^+$ regulatory T cells (Tregs) control self-nonself discrimination and effector class regulation, and are peripherally maintained by cross-reactive commensal microbiota-derived antigens.

**Conflict of interest statement:** Tirumalai Kamala and David Usharauli are founders of Tregeutix Inc., a biotech startup that focuses on developing microbiota guided antigen-specific immunotherapies.



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