Review

T cells in helminth infection: the regulators and the regulated

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Helminth parasites survive through a combination of parasite longevity, repeated re-infection and selective immune suppression to prevent protective Th2 responses. To counteract helminth-induced immunosuppression, and to induce long-term immunological memory, understanding of the multiple regulatory pathways within the T cell compartment is needed. Extrinsic inhibition by regulatory T cells is a key element of Th2 suppression. In addition, Th2 cells in chronic regulatory environments become functionally impaired, indicating cell-intrinsic regulation, which compromises protective Th2 memory. We discuss these pathways and consider the potential for reversing unresponsiveness through stimulatory signals or replacement by new responder populations. Future vaccine or therapeutic strategies should aim to minimize extrinsic regulatory effects and simultaneously negate Th2 anergy to drive effector responses into a long-term functionally competent state.

Helminths and the immune system

Helminth parasites establish and assimilate themselves for long periods in the host and immunoregulation plays a key role in their survival strategy [1]. Helminths are large, multicellular pathogens and the immune system has evolved a suite of specialized effector mechanisms centered around the Th2 pathway (Box 1) to degrade and eliminate them [2]. However, parasites have countered by engaging directly with host signals that regulate and tune effector pathways [3,4]. When the host response does overcome parasite resistance, it is often at the cost of incurring pathology [5]; perhaps for this reason immunity is restrained by parasite immunomodulators and by endogenous regulatory mechanisms. We review recent data showing how regulatory networks develop through a combination of extrinsic inhibition by $Foxp3^+$ regulatory T cells (Tregs) and intrinsic regulation of Th2 effector cell populations, through processes such as anergy, exhaustion or adaptive tolerance. The impact of these regulatory networks on the development of Th2 memory, and the potential for reversing unresponsiveness through stimulatory signals and/or replenishing effector populations, are discussed.

The regulators: regulatory T cells in helminth infection

Multiple types of immunosuppressive cells operate in the immune system, including $CD4^+Foxp3^+$ regulatory T cells (mostly expressing CD25), B cells and macrophages [6]. In addition, suppressive cytokines such as TGF-β and IL-10, produced by diverse hematopoietic and non-hematopoietic cells are integral to immunoregulatory pathways [7,8]. Although the complexity of regulatory cell types continues to be charted, $CD4^+CD25^+Foxp3^+$ Tregs remain the most prominent population of immunoregulatory cells operating during helminth infections described to date.

Immunoregulation in helminth infection was first recognized in early human studies, because peripheral T cells in infected patients were frequently unresponsive to parasite antigens and responses to bystander antigens (including allergens and vaccines) were also reduced [9]. In addition, antibody isotypes were distorted, with exceptionally high concentrations of IgG4 that is, in part, attributable to Treg activity [10]. TGF-β and IL-10-producing Tregs were then cloned from *Onchocerca volvulus*-infected patients [11], and higher Foxp3 expression was found in peripheral blood T cells from cases of active lymphatic filariasis and schistosomiasis [12–14]. This field research provides a firm basis for investigating the role of Tregs in experimental infection models.

Helminth infections drive $Foxp3^+$ Treg responses

In murine infection models, helminth infections elicit both 'natural' and 'adaptive' $Foxp3^+$ Treg cell responses, which dampen Th2 immunity. $Foxp3^+$ Treg numbers can expand rapidly following filarial and gastrointestinal nematode infections, with significant increases within 3–7 days [15–20]. Moreover, $CD4^+Foxp3^+$ cells respond to infection more rapidly than $CD4^+Foxp3^-$ effector T (Teff) cells, increasing their frequency and biasing the initial response towards a regulatory phenotype [16,17,21]. Early $Foxp3^+$ Treg responses are not merely homeostatic as induction requires live (rather than heat-killed) parasites [16], whereas other infections, such as *Toxoplasma gondii*, cause a precipitous loss of $Foxp3^+$ Tregs [22]. Conversely, drug-induced clearance of helminths reduces $Foxp3^+$ Treg numbers and improves responses to bystander antigens [23,24].

In parallel with quantitative expansion of Tregs, helminths can also induce elevated expression of CD103 and other activation markers on $Foxp3^+$ Tregs [15–17,25–27], which exert a more potent suppressive function [15,18]. Thus, the regulatory capacity of the Treg subset in vivo may be reflected not simply by the proportion of $Foxp3^+$ cells, but more accurately by expression of activation markers.

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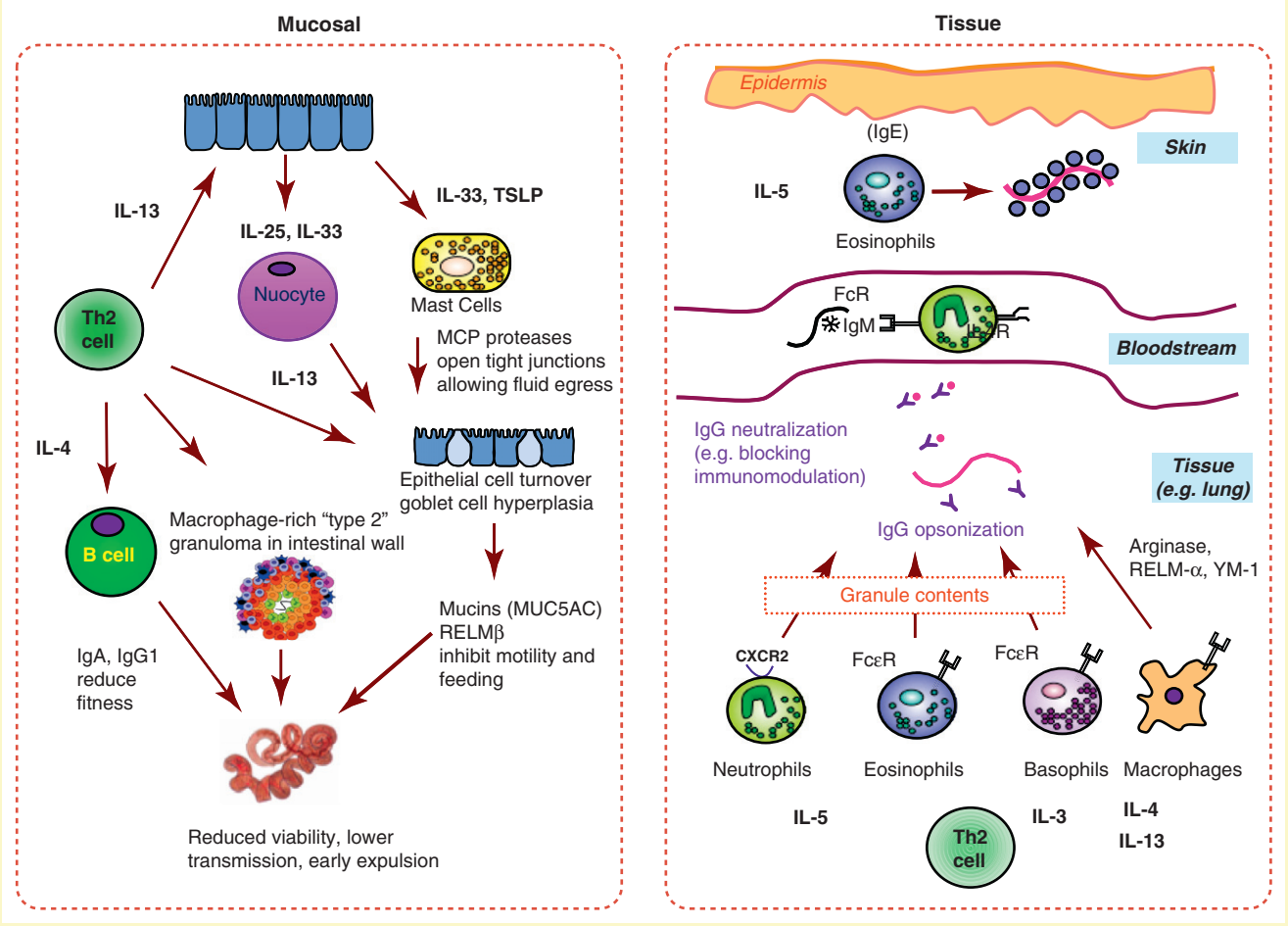
Box 1. Mechanisms of Th2 immunity to helminths

Two main arenas are considered: in the gastrointestinal tract, mucosal immunity involves both innate and adaptive populations. Innate effector mechanisms include goblet cells, which increase mucin production, switch mucin types (e.g. to MUC5AC) and release the effector protein resistin-like molecule-β (RELM-α); mast cells and alternatively activated macrophages, each driven by type 2 cytokines from Th2 and innate lymphoid ('nuocyte') cells. In the different setting of the tissues, skin-penetrating helminth larvae can be intercepted by IL-5-dependent eosinophils; bloodstream microfilariae are cleared by IgM-dependent antibody mechanisms; and in internal organs multiple (and potentially redundant) populations or granulocytes and macrophages are guided by chemokines, cytokines and antibodies to attack helminths through pre-formed mediators and reactive metabolites.



Foxp3$^+$ Tregs in resistance to helminth infections

Although the main role of Foxp3$^+$ Tregs is to maintain tolerance and control excessive inflammatory responses, the trade-off is inhibited protective immune responses, a property exploited by helminth parasites to immunosuppress their host. Experimentally, two imperfect approaches are available to test whether Foxp3$^+$ Tregs are required for parasite survival. Anti-CD25 antibodies can be used for long-term depletion of CD25$^+$Foxp3$^+$ Tregs, with the caveat that this also depletes activated Teffs while sparing CD25$^-$Foxp3$^+$ Tregs. Alternatively, a diphtheria toxin receptor (DTR) transgene expressed specifically by Foxp3$^+$ cells [28,29] permits short-term depletion of Foxp3$^+$ Tregs, but this approach is less applicable to chronic infection models.

The rapid rise in Foxp3$^+$ Treg numbers in infection suggests that a suppressive response pre-empts establishment of Th2 immunity. Accordingly, antibody-mediated depletion of CD25$^+$ Treg cells prior to infection increases Th2 responses to, and killing of, filarial parasites [17,30]. Likewise, depletion of Tregs in Foxp3-DTR *Strongyloides ratti*-infected mice increases host resistance if performed within the first 4 days [20]. In infections with both filarial *Litomosoides sigmodontis* and geohelminth *S. ratti* parasites, increased resistance appears to act on the adult, rather than larval, stage [17,20] and long-term Th2 enhancement outlasts the direct effects of depletion [17]. In these settings, Foxp3$^+$ Tregs inhibit the priming of Th2 responses and limit the quantity or functional quality of the effector T cell pool available at later times.

Reflecting the diversity of helminth life histories, it is not surprising that each species displays a unique interaction with Tregs. For example, in *Trichuris muris* infection, early intervention with anti-GITR, but not anti-CD25, antibodies is most effective at reducing worm loads [31]. Anti-CD25 depletion is reported to be ineffective at boosting immunity to other helminths, including *Schistosoma mansoni* [32,33] and *Trichinella spiralis* [34]. Experiments with Foxp3-DTR mice will be interesting in this regard and, although Foxp3-depleted mice are not more resistant

to the gastrointestinal nematode *Heligmosomoides polygyrus* in the first 14 days of infection [19], a longer time may be required for primary protective Th2 immunity to take effect in this infection.

Do Foxp3$^+$ Tregs also inhibit protective immunity during established infections? Anti-CD25 depletion, administered both prior to and during *S. mansoni* infection of C57BL/6 mice, significantly reduced egg numbers, although administration solely during the chronic phase had no effect on the BALB/c background [32,33]. Similarly, mid-term depletion in *L. sigmodontis* infection can enhance resistance but, unlike early depletions, only if combined with blocking CTLA-4 or providing co-stimulation through GITR [26,35]. Thus, at later stages Foxp3$^+$ Tregs act alongside other regulatory elements, rather than playing a dominant role, and hence regulation is more easily reversed early rather than late in infection.

The functional significance of Foxp3$^+$ Treg cells in susceptibility to human infections is more difficult to elucidate beyond the correlations between regulatory markers and suppressed effector T cell responses already discussed. Notably, co-infection with human T cell-lymphotropic virus 1 and *Strongyloides stercoralis* results in an intensified helminth infection and greatly increased Foxp3$^+$ Treg numbers [36]. However, in *O. volvulus* infection, Foxp3$^+$ Treg cells tend to be associated with dead rather than live parasites, indicating a role in resolving inflammation rather than in susceptibility [37].

Foxp3$^+$ Tregs in the control of immune pathology

Immune regulation is a beneficial and essential aspect of host immunity in dampening potentially pathogenic inflammatory responses and Foxp3$^+$ Tregs clearly control Th2-mediated immune pathology in helminth infections. For example, expanded and activated Foxp3$^+$ Treg populations down-regulate Th2 responses towards the *S. mansoni* eggs that engender pathogenic reactions when trapped in tissue vasculature [25,38] and the severity of egg-induced liver pathology is negatively correlated with Foxp3$^+$ Treg numbers [39]. The down-modulation of perioval granuloma 8 weeks post infection is associated with increased Foxp3$^+$ Treg activation [25] and pathology can be alleviated by retroviral transfection of mice with Foxp3 [40] or transfer of infection-associated CD4$^+$CD25$^+$ Tregs [41]. *Rag*$^{-/-}$ mice reconstituted with naïve CD4$^+$ T cells immediately prior to egg release develop worse immunopathology if CD25$^+$Foxp3$^+$ cells are absent [42]. Impaired Foxp3$^+$ Treg responses in C57BL/6 *Tlr2*$^{-/-}$ mice leads to augmented granuloma formation and pathology in the liver that can be recovered by transfer of CD4$^+$CD25$^+$ Tregs or mimicked by depletion of CD25$^+$ Treg cells [32]. Hence, Foxp3$^+$ Tregs are crucial moderators of both susceptibility to infection and the resultant immunopathology.

In the context of immune pathology in *S. mansoni* infection, inhibiting Foxp3 function can be less effective than blocking IL-10 and the full regulatory effects of Foxp3$^+$ Tregs are mainly apparent in the absence of IL-10 [42], indicating that IL-10 plays a more dominant role in regulating granuloma formation. This highlights that, despite the association of IL-10 with the down-regulation of helminth immunity in humans [43–45] and with Foxp3$^+$ Tregs in other settings, helminth-induced Foxp3$^+$ Tregs largely act independently of IL-10 [25,35,38,46]. In both human [13,47] and murine [25,34,38,46] infections, Foxp3$^-$ cells are the main source of CD4$^+$ T cell-derived IL-10, representing either Th2 cells [38] or the development of a distinct population of Foxp3$^-$IL-4$^-$ Tr1 cells [11,47,48].

Foxp3$^+$ Tregs also play a role in regulating intestinal inflammation during infections with *T. muris* and *H. polygyrus* [19,31]. Thus, ablation of Foxp3$^+$ Tregs during both infections leads to increased villous blunting and atrophy and crypt hyperplasia, although mucus production or epithelial cell turn-over are unaffected, potentially explaining unaltered susceptibility. In human helminth infections, patients with filarial lymphedema tend to have reduced expression of Foxp3, CTLA-4 and TGF-β concomitant with increased Th1 and Th17 responses, again associating an imbalance in Foxp3$^+$ Treg responses with pathology [49].

Natural versus adaptive Foxp3$^+$ Treg cells

The natural and adaptive Foxp3$^+$ Treg cells activated during helminth infections may have distinct or overlapping functions. The rapid expansion of total Foxp3$^+$ cell numbers following infection suggests the stimulation of natural Tregs, and the effects of Treg depletion immediately prior to infection demonstrate the functional importance of this cell type. A key question is whether natural pre-committed Foxp3$^+$ Tregs form the first line of regulation, while adaptive Foxp3$^+$ Treg cells appear later with similar kinetics to the Th2 response, perhaps because both adaptive Tregs and effector populations require time to be primed and differentiate.

A model of successive and complementary waves of Tregs during infection is consistent with available data on *in vivo* depletion (Figure 1). Mice depleted of CD25$^+$ Treg cells prior to infection show increased Th2-mediated resistance to filarial parasites, indicating a role for natural Foxp3$^+$ Tregs in early phases [17,30]; subsequently, adaptive Foxp3$^+$ Treg cells are generated [16]. Similarly, whilst natural CD25$^+$Foxp3$^+$ Treg cells are able to control *S. mansoni* egg-induced Th2 responses [25,38,42], naïve CD4$^+$ T cells from NOD (but not C57BL/6) mice convert towards an adaptive Foxp3$^+$ Treg cell phenotype upon exposure to *S. mansoni* egg antigens (SEA) *in vitro* [50,51]. Evidence for a delayed adaptive Treg response is also seen in *H. polygyrus* infection as early ablation of Foxp3$^+$ Tregs (days 0–14) fails to impact protection, but TGF-βR signaling blockade post day 28 to inhibit conversion of adaptive Foxp3$^+$ Tregs increases parasite killing [3].

The conversion of naïve NOD CD4$^+$ T cells into adaptive Foxp3$^+$ Tregs can occur upon exposure to SEA (or a key component, ω-1), and is dependent upon host TGF-β; SEA is able to up-regulate directly both secreted TGF-β and its chaperone, membrane latency-associated peptide LAP, in CD4$^+$ T cells [4,50,51]. Congruent with impaired Foxp3$^+$ Treg responses and increased egg-induced pathology in *Tlr2*$^{-/-}$ mice [32], Foxp3 conversion is partially TLR2-dependent with TLR2 signals enhancing TGF-β production. This suggests that synergistically induced adaptive Tregs, together with natural Tregs, curb infection-associated inflammation and pathology.

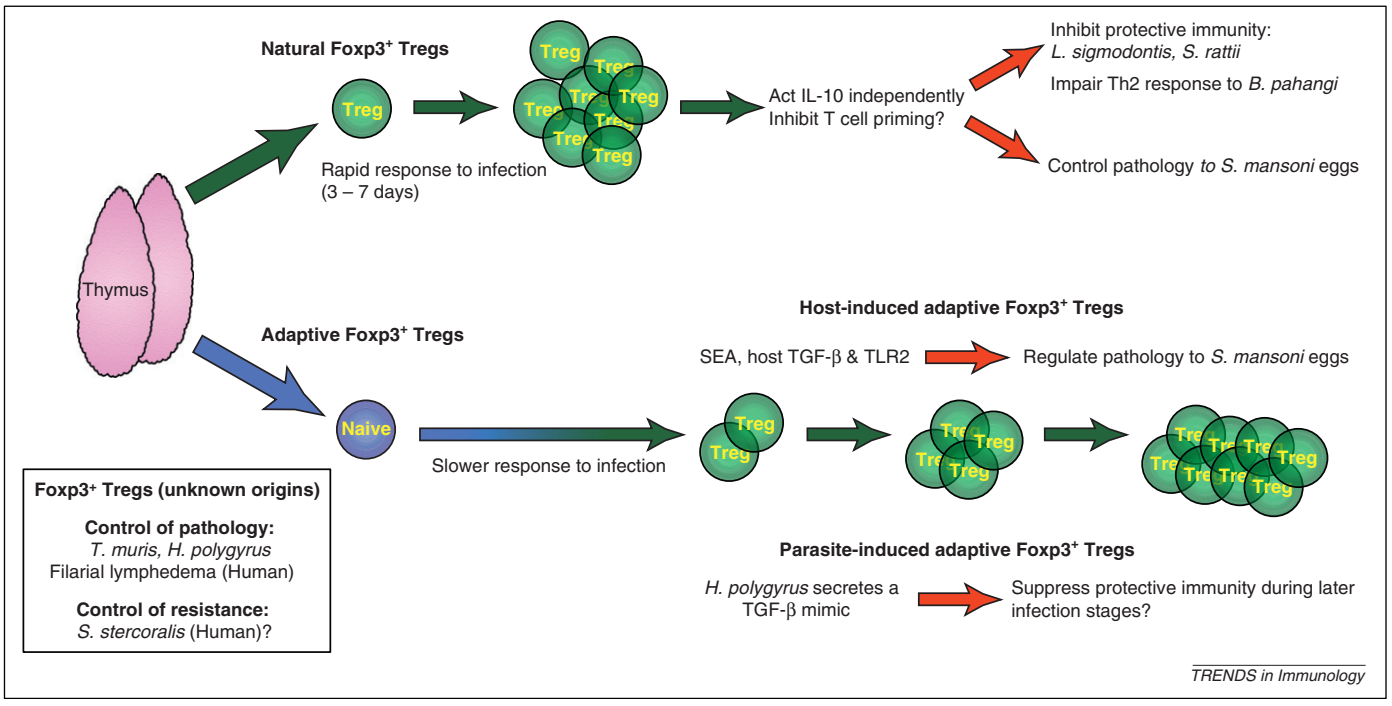


Figure 1. Successive waves of Foxp3$^+$ Tregs regulate immunity to helminths. Foxp3$^+$ Treg responses to helminths encompass cells of both natural and adaptive origins. Evidence indicates that natural Foxp3$^+$ Tregs respond rapidly to infection, forming the first line of regulation, whereas adaptive Foxp3$^+$ Treg responses develop with slower kinetics. To limit immune pathology, Tregs must dampen infection-induced inflammation; however, activated Tregs can inhibit protective immunity, opening an exploitable avenue for parasites. Reflecting this, both host and helminth can create adaptive Foxp3$^+$ Tregs, because helminths secrete a TGF-β mimic to hijack the TGF-β pathway used to convert naïve T cells towards a regulatory phenotype.

The conversion of naïve T cells into Foxp3$^+$ Tregs reveals an opportunity for helminths to manipulate their host. For example, *H. polygyrus* excretory/secretory (HES) molecules are able to convert naïve T cells from C57BL/6 mice towards an adaptive Foxp3$^+$ Treg cell phenotype *in vitro* [3] and infected BALB/c mice have a greater propensity towards *in vivo* Foxp3 conversion following exposure to oral antigen. Foxp3 induction depends on the host TGF-βR signaling pathway, but not host TGF-β. Thus, conversion is directed by a parasite-derived TGF-β mimic rather than arising from the intrinsic host response to inflammation.

The regulated: Th2 effector responses in helminth infection

The long-term persistence of helminths presents the host with the challenge of maintaining CD4$^+$ T cell responses for decades. Although Type 2 immunity is, in general, down-regulated in established helminth infections, the fate of the underlying Th2 effector cells in an environment of persistent infection and dominant immune-regulation is not known (Figure 2).

One possibility is that Th2 effector cells remain fully functional but are held in check by different extrinsic regulatory cells and their mediators. This would require constant regulation to maintain immune down-modulation so that removal of regulation completely restores Th2 cell function. This is consistent with human studies, as drug clearance of helminth infection [52] or neutralization of TGF-β and IL-10 *in vitro* [53] recovers aspects of Th2 cell function. However, curative drug treatment does not reset immunity to a protective state, as most patients remain susceptible to re-infection [54].

Alternatively, persistent exposure to antigen and immune suppression may alter the phenotype of the responding Th2 cells, resulting in a temporary or permanent change in function. This could extend to conversion of Th2 into a Treg subset but it is more likely to result in an attenuated or non-responsive state. In this scenario, neutralizing extrinsic immune regulation may not be sufficient to restore immunity because the Th2 cells are intrinsically sub-functional. This could lead to a form of regulatory memory and/or imprinting of unresponsiveness in the Th2 effector population. These outcomes on the underlying Th2 cells have quite different implications for designing strategies or vaccines to counteract parasite-induced immune suppression and for therapeutically harnessing helminth-induced immune regulation for the control of allergies or autoimmune diseases.

Not all Type 2 responses are equal

Chronic helminth infections are associated with a shift in the overall Type 2 phenotype of the host. In filariasis and schistosomiasis, IL-5 is often down-regulated more than IL-4 [55,56], indicating modulation of selective constituents of Type 2 immunity rather than a global inhibition. In general, the chronic Type 2 profile can be seen as retaining the 'inducer' cytokine IL-4 while diminishing 'effector' cytokines, such as IL-5. A major conceptual advance was the description of a 'modified' or 'tolerant' Th2 phenotype in patients with attenuated allergic symptoms [57], having switched from inflammatory IgE production towards non-inflammatory IgG4 driven by increased IL-10. Similarly, modified Th2 phenotypes have been described in chronic helminth infections [9]. However, the

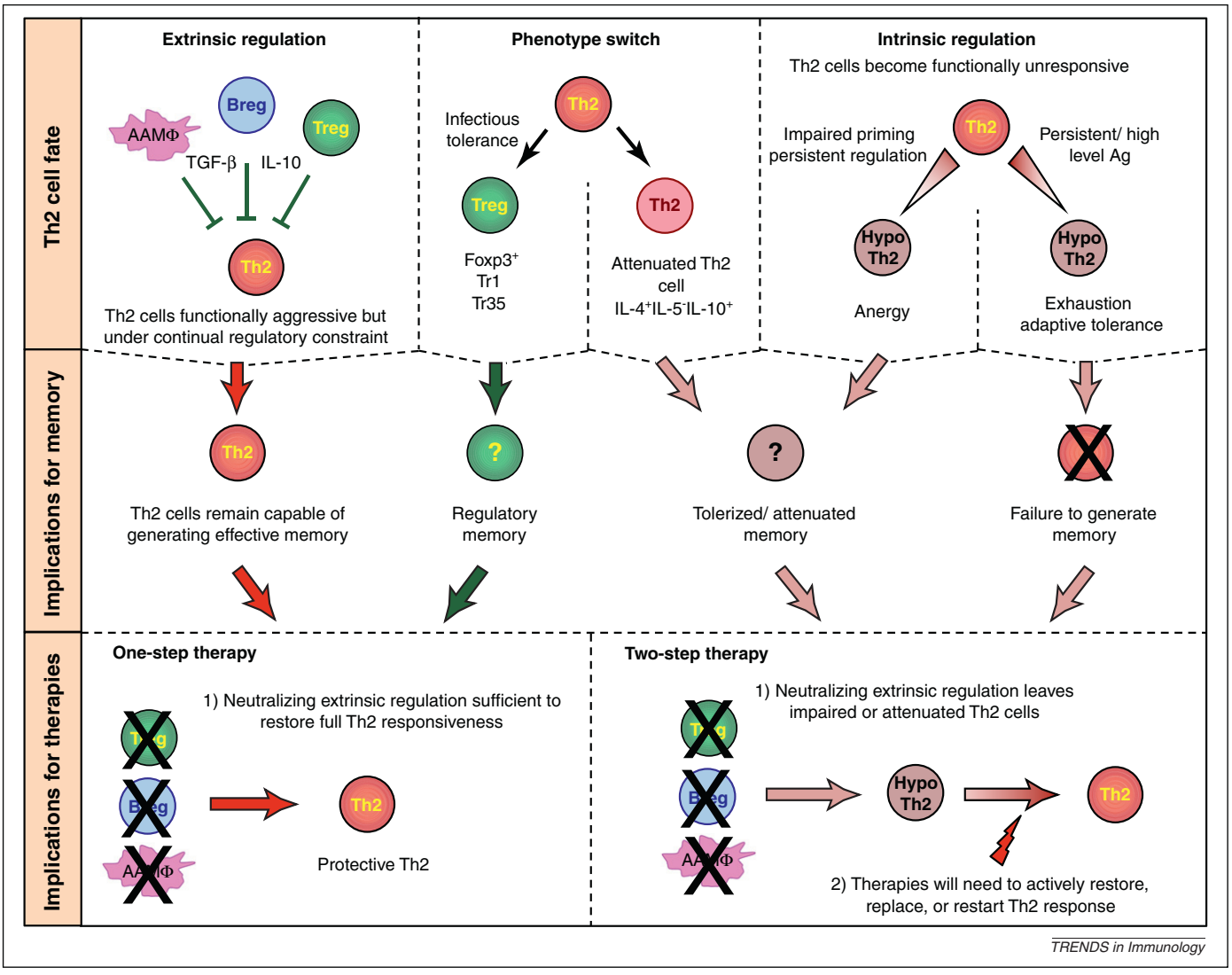


Figure 2. Th2 cell fate underlying chronic down-modulated/modified Type 2 immunity. Th2 cells exposed to a chronic regulatory environment may undergo a variety of different fates, resulting in a down-regulated Type 2 phenotype. These diverse fates have different implications for the ability of an infected host to develop protective Th2 memory responses and will require different types of therapeutic strategies to restore protective immunity. If there is an intrinsic impairment in the functional ability of the Th2 cells, or a switch towards a regulatory phenotype, then this may have long-term consequences for the generation of Th2 memory. The effects may be to prevent the development of Th2 memory cells and to encumber the host with a tolerized or regulatory memory response. This will require the design of therapies or vaccines that can counter or prevent the intrinsic loss of Th2 cell effector functions.

mechanisms underlying this change are unknown, not least because mice do not have an IgG4 equivalent and so do not present a corresponding modified Th2 phenotype.

Competition between different T cell subsets could explain the alteration of Type 2 immunity; for example, through expansion of regulatory Tr1 populations [58] or differential susceptibility of Th2 cells to apoptosis [59]. However, it is becoming clear that the 'classical' Th2 cell is an oversimplification because individual Th2 cells do not express all Type 2-associated cytokines but can, for example, produce IL-9 independently of IL-4 (Th9 cells) [60], IL-5 without IL-4 under the influence of IL-33 [61] or be Tfh cells with Th2 characteristics [62]. A Th2 response clearly comprises a spectrum of subtypes producing different cytokine combinations, with shifts in phenotype reflecting changes in the dominance of different Th2 subtypes through competition within existing populations or recruitment of new subtypes from the naïve T cell pool. Alternatively, Th2 cells may convert between subtypes or subsets. Thus, a modified Type 2 response may represent inflammatory Th2 cells switching towards an attenuated phenotype, shutting down IL-5 and reinforcing IL-10. In extremis, this scenario could involve conversion of Th2 towards a regulatory T cell phenotype, such as Tr1, Tr35 [63] or Foxp3$^+$, resulting in the development of infectious tolerance.

Intrinsic regulation: anergy versus exhaustion?

A global change in the Type 2 phenotype towards a modified form may also reflect the differentiation of Th2 cells into an intrinsically unresponsive or hyporesponsive state. Initial evidence for T cell anergy was provided in human filariasis, where *in vitro* immune responsiveness could be restored by the addition of IL-2 [64]. The characterization of an anergic molecular signature within the PBL of filariasis patients, comprising c-cbl, cbl-b, Nedd4 and Itch, provides further evidence for the development of T cell anergy [12]. In experimental chronic filarial infection with

L. sigmodontis, $CD4^+$ T cells purified from the infection site lose the ability to proliferate and produce Th2 cytokines in response to parasite antigen [35]. This defect is intrinsic within the effector $CD4^+$ T cell population as neutralization of IL-10 or removal of $Foxp3^+CD25^+$ Treg cells fails to restore responsiveness [26,35]. Blockade of CTLA-4 in PBMC cultures increases Th2 cytokine responses and reduces expression of c-cbl, cbl-b and Itch in filarial patients [12], and promotes resistance to filarial and gastrointestinal nematodes in murine models [26,65]. Thus, during chronic helminth infection, $CD4^+$ Th2 cells develop an intrinsically unresponsive functional state in both mice and humans that potentially represents T cell anergy and is, at least partially, dependent upon CTLA-4.

Murine studies investigating Th2 cell fate in the down-modulation of *S. mansoni*-mediated inflammation confirmed that an intrinsic loss of Th2 cell function occurred *in vivo* [66]. Overt Type 2 responses decreased over time and tracking Th2 cells using IL-4gfp reporter mice showed that the numbers of IL-$4gfp^+$ Th2 cells remained constant. Instead, the ability of GFP-labeled Th2 cells to proliferate and produce Th2 cytokines was impaired through the anergy factor GRAIL. This phenotype was independent of the extrinsic environment because it was maintained upon transfer to a new host. Interestingly, GRAIL is one of the anergy factors not up-regulated in human filarial patients [12], indicating that mechanisms of hyporesponsiveness differ between infections.

Anergy is associated with impaired T cell priming [67] and helminth parasites are adept at impairing DC function [68] or promoting regulatory DC populations [69]. Treg activity has also been linked to the development of T cell anergy [67]. Thus, a strong early Treg response combined with down-modulated APC capability could favor development of hyporesponsiveness. However, in *S. mansoni* infection, hyporesponsiveness follows early strong Th2 priming, potentially indicating the development of exhaustion [70]. Exhaustion is well defined in $CD8^+$ T cell immunity to viral infections and tumors and is associated with strong immune activation induced by high antigen load or persistent challenge. The loss of effector functions is hierarchical depending on the level of exhaustion. It is possible, therefore, that the differential loss of Th2 effector cytokines seen in human helminth infections could reflect different stages of exhaustion. Interestingly, in helminth-infected Ethiopian migrants in Israel, individuals with a highly activated peripheral $CD4^+$ T cell phenotype indicated exhaustion through reduced proliferative responses, impaired TCR signaling with reduced ERK phosphorylation and increased expression of cbl-b, CTLA-4 and TGF-β [71,72].

Murine studies show exhaustion can be mediated through a range of inhibitory receptors, including PD-1 and TIM-1 [70], and macrophage expression of the PD-1 ligands, PD-L1 and PD-L2, inhibits T cell immunity in *S. mansoni* and *Nippostrongylus brasiliensis* infections, respectively [73,74]. Yet, the involvement of GRAIL during *S. mansoni*-induced $CD4^+$ T cell hyporesponsiveness [66] indicates that either $CD4^+$ Th2 cell hyporesponsiveness is a form of exhaustion different from that observed in $CD8^+$ T cells, or that it is a form of adaptive tolerance that develops in situations of persistent antigen [75]. Although anergic and exhausted T cells may appear functionally similar at a superficial level, gene expression studies indicate they represent distinct differentiation states [70], with very different potential for recovery.

Implications for therapeutic and protective memory responses

The development of an anergic (or possibly, exhausted) $CD4^+$ Th2 cell phenotype has important implications for the treatment of infections and the development of memory. Even if extrinsic immune-regulation is ablated, Th2 effector cells will remain functionally impaired and unable to kill the parasite (Figure 2). In agreement with this, depletion of $CD25^+$ Tregs during established *L. sigmodontis* infection is successful only if combined with blocking CTLA-4 or providing co-stimulation through GITR [26,35], both of which promote Th2 effector cell function. However, there remains no clear characterization of T cell memory in the regulated helminth-infected host, in contrast to the strongly protective memory observed in immunization models [2].

$CD8^+$ T cell exhaustion is associated with impaired memory cell development and survival in the absence of antigen [70]. If the majority of Th2 cells are exhausted, then drug-induced clearance of infection would result in the collapse of the Th2 effector population and failure to generate Th2 memory. This could be beneficial for avoiding an overzealous memory response and associated pathology on re-infection (e.g. to *S. mansoni* eggs). However, to develop protective immunity, Th2 responses would have to be re-initiated on each subsequent exposure.

In contrast, anergic $CD4^+$ T cells can survive long-term, offering potential for infected individuals to develop a tolerized memory state [76]. It is not known to what extent regulatory T cells develop a memory function, but if either Tregs or anergic effector cells persist, the host would remain immunosuppressed even after infection is cleared. In these circumstances, once an exhausted or regulated T cell response has established, immunity to all subsequent exposures will be historically constrained towards hyporesponsiveness.

Historical constraints and Th2 flexibility

In the setting of long-term regulated T cell responses, how much flexibility is there for the Type 2 response to overcome the constraint of a historically unresponsive status (Figure 3)? Does natural resistance take decades to develop because the host has to first rewrite or replace their initial regulated response, and how can this process be accelerated therapeutically? Interestingly, beekeepers that develop tolerance to bee stings via a modified Th2 response lose their unresponsive phenotype if they remain unexposed for several months [77], and anergic T cells can regain effector function over time in the absence of antigen. Thus, there is certainly flexibility within the immune system to reverse a tolerized immune response.

Vaccination prior to infection is the obvious route for pre-empting regulation, particularly if it induces a rapid memory Th2 response that prevents the early Treg dominance. In this situation it may be necessary to design

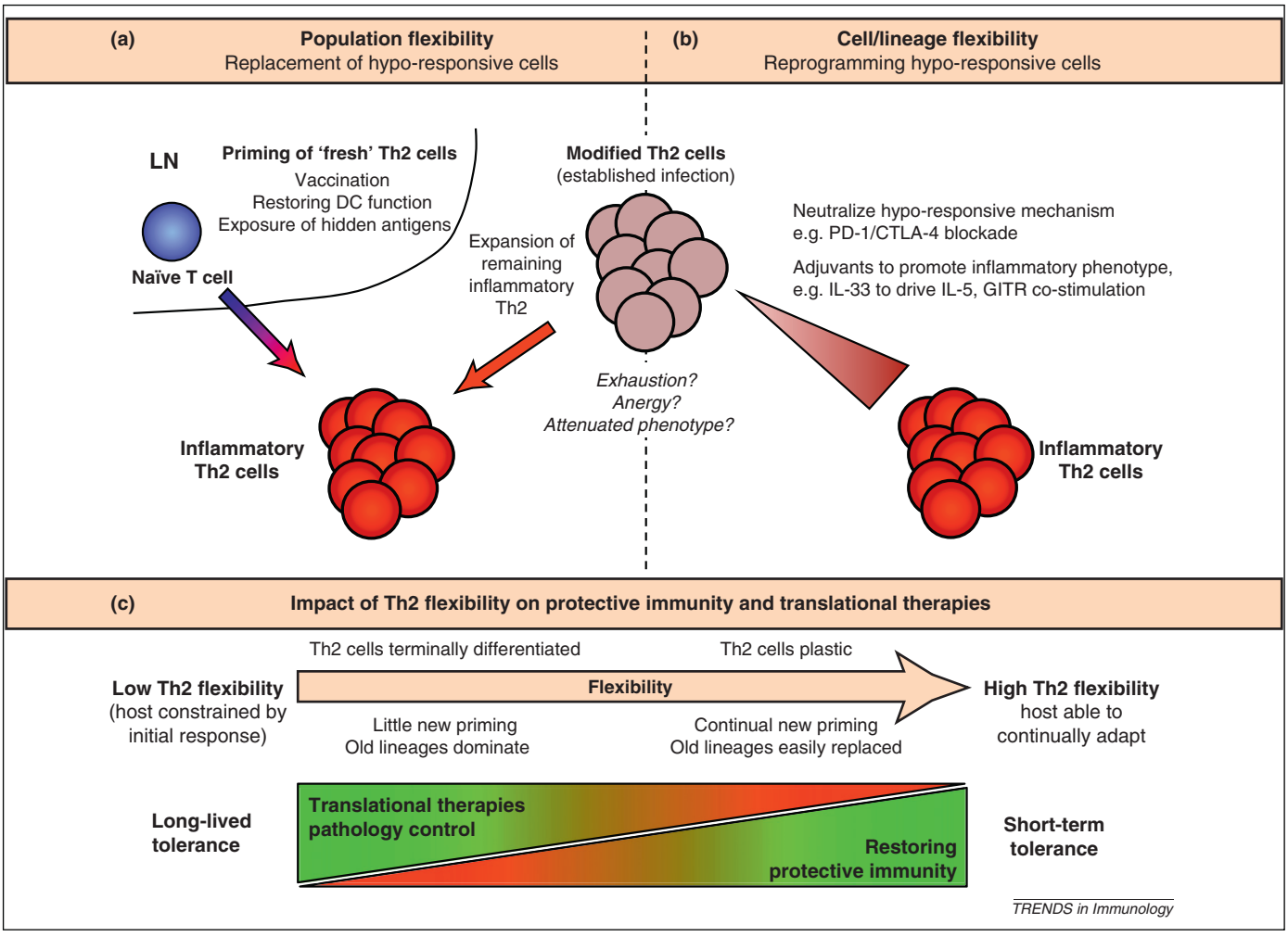


Figure 3. How can Th2 hyporesponsiveness be reversed? There are two potential routes for reversing Th2 hyporesponsiveness. **(a)** Replacement of hyporesponsive Th2 cells by expansion of the remaining inflammatory Th2 cells or by priming of new Th2 cells. **(b)** Reprogramming hyporesponsive Th2 cells by blocking down-regulatory mechanisms or by providing activation signals, such as cytokines or co-stimulation. **(c)** The flexibility of a Th2 response, both at the population and the cellular level, will determine whether Th2 hyporesponsiveness results in short- or long-term tolerance and will affect the ease by which Th2 hyporesponsiveness can be reversed to induce protective immunity. Translational therapies harnessing helminth-induced immune suppression to treat allergies or autoimmune diseases will be most effective if they can elicit long-term stable tolerance.

vaccines that omit any epitopes preferentially recognized by Tregs, or incorporate adjuvants that neutralize Treg activity. One consideration is that partially successful vaccines that still permit low-level chronic infections may lose efficacy over time due to exhaustion or anergy of the vaccine-primed Th2 cells in the face of persistent infection.

Once a down-modulated response to infection has established, strategies will be required to rescue the ineffective Th2 cells. One possibility would be to reactivate them to restore effector and memory capability, as seen with PD-1 blockade of exhausted virus-specific $CD8^+$ T cells [78]. The alternative is regeneration, either by recruitment of newly developed Th2 cells from the naïve pool, or expansion of the remaining inflammatory Th2 cells. Precedents for both are seen during viral exhaustion, with new cell priming helping to preserve CD8 responses [79] and Tfh cells maintaining a reservoir of responsive $CD4^+$ T cells [80].

Although it is likely that new T cell priming will continue throughout infection, its importance for maintenance of chronic Th2 responses is unknown. The rate of priming and extent to which pre-existing (and unresponsive) Th2 lineages are replaced may define the flexibility of an established Type 2 response (Figure 3). A low priming rate would allow established but ineffective Th2 lineages to dominate, requiring therapies to redirect these unresponsive Teff cells. In contrast, a high priming rate, or therapeutically boosting new priming, would allow the rapid replacement of down-modulated Th2 cells with freshly primed 'responsive' Th2 cells. An intriguing indication that new epitopes prime protective responses following chemotherapy has emerged from studies of *S. mansoni* infections, in which immunity correlates with IgE responses towards specificities exposed as parasites die [81]. Thus, it may be possible both to rescue anergized Th2 cells, and reinvigorate the Th2 pool with fresh recruits free of regulatory influences. In all likelihood, these approaches can be combined to provide the optimal restoration of anti-helminth immunity.

Concluding remarks

By analyzing helminth infections, it is clear that regulatory and effector T cells form a long-term interrelationship that compromises immunity and promotes parasite survival.

Recent findings on the generation, interactions and fates of both the regulators and the regulated during infection, provide a framework for applying the fundamental principles of T cell regulation to these highly prevalent diseases. Many key questions now require testing in experimental models to refine this framework, including the roles of natural and adaptive Tregs, the relative importance of the different co-inhibitory signals and the longer term fate of the hyporesponsive Th2 population. Answering these questions will open new avenues to promote long-term protective memory to helminth infection and by identifying the regulatory mechanisms responsible for long-term tolerance suggest new therapeutic strategies for controlling allergies or autoimmune diseases.

Acknowledgments

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