

REVIEW

Atopy, asthma, and experimental approaches based on the linear model of T cell maturation

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Summary

The linear model of maturation of IFN- γ -producing cells from a proliferative pool of type 2 cytokine-producing T cells represents a fundamental shift in interpreting how changes in cytokine production by T cell populations are regulated. A major tenet of this model is antigen-independent, bystander proliferation of type 2 T cells and their maturation to IFN- γ^+ cells. Both clinical observations and prevailing theories of immune system development in asthma are consistent with this highly interpretative *in vitro* model, which allows unambiguous characterization of the modulation of the intrinsic features of T cell proliferation and differentiation by environmental and genetic factors. Hypotheses based on the linear model of T cell maturation are readily testable and should lead to a greater understanding of not only allergen-specific responses, but also the non-specific, bystander effects associated with specific responses to allergens or pathogens. Topics to be discussed in the context of the linear model of T cell maturation in this review include: (1) allergic responses to an inciting allergen that may enhance sensitivity to subsequent yet different allergens; (2) dampening the preferential accumulation of type 2 T cells during a typical immune response against viral and bacterial pathogens; (3) allergen-independent sensitization in asthmatics; (4) the 'hygiene hypothesis' for the reported increased allergy development in industrialized countries; (5) elevated IFN- γ levels in asthmatics, in addition to the expected high levels of type 2 cytokines; (6) testing the effects of inflammatory mediators, as well as various anti-inflammation therapies on T cell maturation; and (7) testing the influence of gene variation on T cell maturation.

Introduction

Many of the inflammatory events associated with atopic disorders such as allergic asthma are orchestrated by type 2 T lymphocytes, which secrete a variety of cytokines and chemokines that activate eosinophils (IL-3, IL-5, granulocyte macrophage-colony stimulating factor), mast cells (IL-9, IL-4, IL-13), and mucus-producing goblet cells (IL-13) and promote inflammatory cell influx into the airways [1–4]. Beyond the immediate site of inflammation, the type 2 cytokines IL-4 and IL-13 promote survival of and IgE synthesis by B cells and generation of basophils and eosinophils. Asthma and other allergic diseases are thought to be due in part to an increased ratio of type 2 T to type 1 (IFN- γ^+) T cells [2–4]. Dissecting the factors and pathways that modulate the accumulation of type 2 and IFN- γ^+ T cells is therefore important to our understanding of the development, and therapeutic management, of atopic diseases.

Although the innate and acquired immune systems are both believed to contribute to asthma pathogenesis, experimental investigation of these systems and their interactions relevant to asthma in human subjects has proven to be extremely difficult. This difficulty stems from the multifactorial nature

of both asthma and immune system regulation, and the seemingly infinite combination of interactive features that confound interpretation of the gestalt. One approach to this problem is to examine separate components of immune system regulation in order to generate hypotheses for testing in integrative models. This review discusses recent studies that argue a shift in the paradigm of T cell maturation, based on a highly interpretative *in vitro* model capable of characterizing intrinsic features of T cell proliferation and differentiation and their modulation by environmental and genetic factors. Importantly, findings from these studies interpreted within this new paradigm of T cell development are consistent with both clinical observations and prevailing theories of immune system development in asthma.

The linear model of T cell maturation

From studies of haematopoietic progenitor cells, rare peripheral immature lymphocytes, and mature lymphocytes, a common functional maturation pathway of natural killer (NK) and T cells was elucidated. Haematopoietic progenitor cells (CD34 $^+$, Lin $^-$) and immature NK cells (CD161 $^+$ CD56 $^-$) were characterized to be capable of IL-13 but not IFN- γ production (IL-13 $^+$ IFN- γ^-) upon maximal stimulation, while mature NK cells (CD161 $^+$ CD56 $^+$) produced no IL-13 and minimal to high amounts of IFN- γ (IFN- γ^+ IL-13 $^-$) [5]. A small proportion of CD3 $^-$ CD161 $^+$ CD56 $^-$

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lymphocytes ($\sim 1\%$ of $CD3^+ CD161^+$ cells) were found in peripheral blood and shown to also be predominantly $IL-13^+$ [6]. These cells were isolated, expanded *in vitro*, and shown to be equivalent to those immature NK cells obtained from *in vitro* progenitor cell differentiation cultures [7]. Immature NK cells isolated from peripheral blood or obtained after culture of haematopoietic progenitor cells could develop into $CD56^+ IFN-\gamma^+ IL-13^-$ mature NK cells in response to IL-12 and B-lymphoblastoid cells. This maturation is associated with expression of the triggering and inhibitory receptors possessed by most peripheral blood NK cells. These studies established that the accumulation of $IL-13^+$ NK cells in peripheral blood lymphocytes (PBLs) was dependent on the proliferation of the few $IL-13^+ CD56^-$ NK cells pre-existing in the starting PBL population, and led to a model of linear development of NK cells from immature, type 2 cytokine-producing to an intermediate $IL-13^{lo} IFN-\gamma^{lo}$ stage, to the mature, $IFN-\gamma$ -producing stage [7]. NK cells at this final stage are terminally differentiated cells that have diminished proliferative capacity and are destined to die upon engagement of their death receptors. This model is in contrast with the prevalent dogma for T-helper (Th) cells (summarized in Fig. 1) which proposes naïve T cells are capable of differentiating into Th type 1 (Th1) ($IFN-\gamma^+$) or Th type 2 (Th2) (type 2 cytokine $^+$) cells upon antigenic stimulation, dependent upon the cytokine environment (reviewed in [8–10]). Specifically, IL-12 would polarize the differentiation of naïve T cells into the $IFN-\gamma$ -producing Th1 cells [11] whereas IL-4 would polarize the differentiation into IL-4-producing Th2 cells [12].

In light of the contrast between the NK cell linear maturation model and the Th1/Th2 branching model, mechanisms of how different cytokine-producing subsets of T cells may accumulate were reconsidered. Basic scenarios by which accumulation of cytokine-producing cells ($IL-13^+$ or $IFN-\gamma^+$) might occur are depicted in Fig. 2. One possibility is that cytokine $^+$ cells exist in the starting population, and then proliferate at a rate faster than do cells in the cytokine $^-$ population to result in a preferential accumulation of cytokine $^+$ cells (Fig. 2a). The other possibility is that cells originally cytokine $^-$ gain the ability to produce the cytokine under specific conditions (Fig. 2b). As described above, the linear maturation pathway of NK cells is a combination of these two options (Fig. 2c), with $IL-13^+$ cells either: (1) preferentially proliferating to increase their number in response to IL-2 and IL-4; or (2) differentiating in response

to IL-12 to lose the ability to produce IL-13 as ability to produce $IFN-\gamma$ is acquired.

Whether this linear model of maturation applies to T cells as well as NK cells was tested, and was indeed found to be the case (detailed in [13, 14]). In this linear maturation model (summarized in Fig. 3), type 2 T cells in PBL accumulate by preferential proliferation in response to IL-2, relative to the predominant $IL-13^-$ ($IFN-\gamma^{+/+}$) population. Importantly, T-cell receptor (TCR)-mediated stimulation is not required

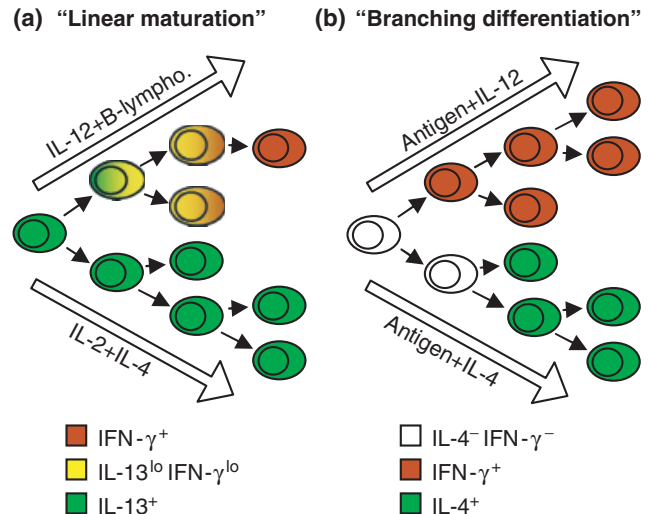


Fig. 1. The linear maturation model vs. the branching model of differentiation. (a) In the linear maturation model, $IL-13^+$ natural killer (NK) cells accumulate by proliferation of pre-existing, immature $IL-13^+$ cells in response to IL-2+IL-4, while $IFN-\gamma$ NK cells develop from the immature $IL-13^+$ cells in response to IL-12 and co-stimulation with B-lymphoblastoid cells. (b) In the branching model of T cell differentiation, non-committed, cytokine $^-$ T cells are induced to differentiate into either T-helper type 1 ($IFN-\gamma^+$) or T-helper type 2 ($IL-4^+$) cells in response to antigenic stimulation and IL-12 or IL-4, respectively.

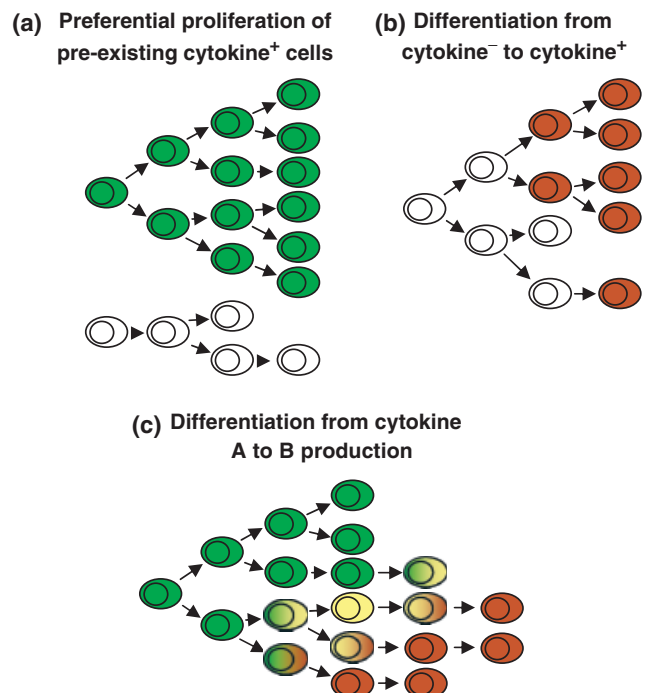


Fig. 2. Mechanisms for accumulation of cytokine $^+$ cells. Increases in the numbers and proportions of cytokine-producing (cytokine $^+$) cells can occur by two mechanisms. In this figure, accumulation of $IL-13^+$ and $IFN-\gamma^+$ cells will be considered, represented by green and red, respectively. (a) depicts the preferential accumulation of pre-existing cytokine $^+$ cells. Both $IL-13^+$ (green) and $IL-13^-$ (white) cells are present at the start of culture. During culture, $IL-13^+$ cells proliferate to increase their number over several divisions. The $IL-13^-$ cells may also divide, but do not contribute to the accumulation of $IL-13^+$ cells. In (b) accumulation of cytokine $^+$ may occur by differentiation from cytokine $^-$ to cytokine $^+$ cells. In this, $IFN-\gamma^+$ cells (red) derive from cells that were previously $IFN-\gamma^-$ (white). This change may be associated or not with proliferation. In (c) a combination of both mechanisms characterizes the accumulation of cytokine $^+$ cells, with cells producing a certain cytokine ($IL-13$, green) losing ability to produce that particular cytokine while gaining ability to produce another ($IFN-\gamma$, red). Intermediate cells producing null-to-low levels of both cytokines may be detectable.

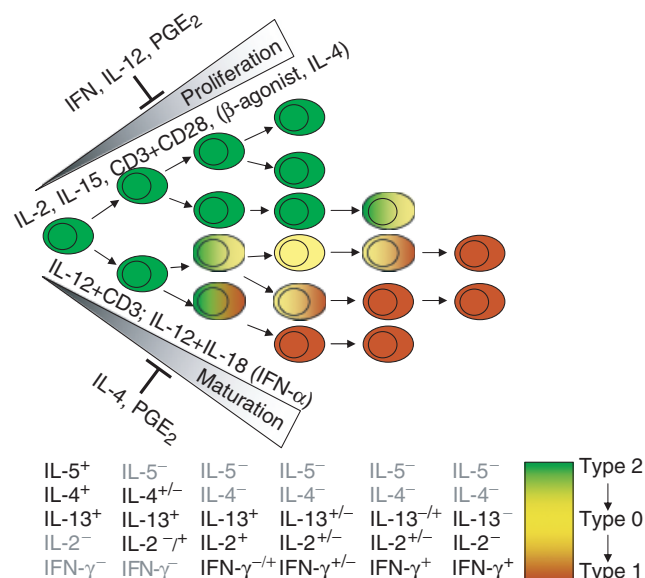


Fig. 3. Model of the linear maturation of T cells. Type 2 T cells (green) proliferate in response to the factors such as IL-2, IL-15, and CD3-mediated stimulation to increase in number. IL-4 and β-agonists (unpublished observation) may potentiate, whereas prostaglandin E₂ (PGE₂) and IFN (unpublished observations) may attenuate this proliferation and preferential accumulation. These cells may proceed through functional maturation in response to IL-12 with IL-18 and IFN-α or CD3-mediated stimulation, transiting through an intermediate IFN-γ^{+/lo} IL-13[±] stage before becoming IFN-γ^{hi} IL-13⁻. This maturation is facilitated by IFN-α and attenuated by agents such as IL-4 and PGE₂.

for this IL-2-induced accumulation, though the rate of proliferation of type 2 cells, as well as the total T cell population, is further increased upon TCR-mediated stimulation [14]. The type 2 T cells differentiate to type 1 (IFN-γ^{hi} IL-13⁻) cells, transiting through an intermediate IL-13^{+/lo} IFN-γ^{+/lo} stage, in response to IL-12- and TCR-mediated stimulation. This gain in ability to produce IFN-γ can also be induced by the cytokines IL-12, IL-18, and IFN-α, independent of TCR-mediated proliferation. Type 1 T cells may also accumulate directly from differentiation of the predominant IFN-γ^{-/lo} IL-13⁻ T cell population found in PBL. Because both the accumulation of type 2 cells and their differentiation to IFN-γ⁺ cells can occur independently of direct antigenic stimulation solely in response to soluble factors in the environment, these processes are referred to as 'bystander' proliferation and differentiation, respectively. IL-15, produced by monocytic cells, can substitute for IL-2, enabling these bystander effects to occur solely in response to factors produced by monocytic cells [13, 14].

In contrast to the branching model of T cell maturation, the linear model is supported by empirical evidence from a tightly controlled *in vitro* analysis of human T cells. Moreover, many observations that have been interpreted based on a branching model are nevertheless consistent with the linear model of maturation. To cite several examples: (1) Type 2 cytokine⁺ T cells are present among naïve T cells in mice – the source of T cells used in many *in vivo* and *in vitro* mouse studies [15]. These data complement the finding that a subset of naïve human T cells is indeed capable of producing type 2 cytokines [14]. Such pre-existing, naïve type 2 T cells are critical to the interpretations of the linear model. (2) The requirement for proliferation in the accumulation of IL-4⁺ T cells was

interpreted as a necessity to rearrange the chromatin containing the IL-4 locus [16] to stably commit to a particular 'Th1' or 'Th2' profile [17]. An alternative interpretation of these data not considered by the respective authors is that proliferation is required for the expansion of pre-existing, naïve T cells, directly supported by the data that form the bases of the linear model [14]. (3) IL-4Rα- and IL-4- deficient BALB/c mice have type 2 T cells (e.g. IL-5⁺ and IL-13⁺ T cells), supporting an *in vivo* lack of requirement for IL-4 in the accumulation of type 2 T cells [18, 19]. This lack of requirement for IL-4 may be strain-dependent, as IL-4^{-/-} BALB/c but not IL-4^{-/-} C57BL/6 mice produce significant levels of antigen-induced IL-13 after nematode infection [20]. It remains to be tested whether similar differences in requirement for IL-4 may exist in human populations with disease-associated phenotypes. (4) Ablation of IL-4-producing T cells *in vivo* in mice results in diminished type 1 T cell responses [21], consistent with evidence within that type 2 T cells are precursors to type 1 T cells. (5) Most CD4⁺ T cell clones expanded from human, postnatal thymocyte preparations express IL-4, IL-5, and IL-2, but not IFN-γ and IL-10, with the latter two expressed only after the addition of IL-12 [22].

Application of bystander effects to allergic processes

Based on the principles described above for bystander proliferation, hypotheses to explain certain features of the development of atopy can be formulated. One such hypothesis is that allergic responses to an inciting allergen may enhance sensitivity to subsequent yet different allergens via bystander proliferation of T cells. Repeated exposure to non-pathogenic particles (i.e. particles that would cause no pathology in the absence of an immune response) that are nonetheless immunogenic (allergens) would produce accumulation of: (1) T cells specifically reactive to a particular allergen (allergen-specific) and (2) type 2 T and NK cells not specifically reactive to the particular allergen (allergen-non-specific). The allergen-specific T cell population activated by allergen would initially include type 2 cytokine- and IFN-γ-producing cells. Because type 2 cells are the most proliferative, they would preferentially accumulate in the absence of an inflammatory environment capable of inducing their maturation. Type 2 cells not specific for that particular allergen would proliferate in response to IL-2 produced by the allergen-specific T cells. In this manner, a diverse pool of T cells reactive to a number of different allergens would be induced to expand specifically or non-specifically in response to different allergens. A second hypothesis is that viral or bacterial infections can exacerbate this response by inducing non-specific proliferation of resident, allergen-specific T cells. Bystander proliferation could be driven directly by IL-15 produced by monocytic cells in response to pathogens such as viruses or by IL-2 produced by T cells specific for pathogen-derived antigens. This bystander increase in resident type 2 T cell number could cooperate with the selective recruitment of Th2 cells into the lungs, as described in murine models of allergic inflammation [23, 24].

Alternatively, bystander maturation may play a part in dampening the preferential accumulation of type 2 T cells. During a typical immune response against viral and bacterial

pathogens, monocytic cells (i.e. monocytes, macrophages, and dendritic cells) and plasmacytoid dendritic cells release inflammatory factors including the cytokines IL-12, IL-18, and IFN- α (reviewed in [25–27]). The presence of these cytokines at sites of inflammation, as well as in peripheral, secondary lymphoid tissue, could induce the maturation of T cells from type 2 cytokine producers to IFN- γ ⁺ cells, regardless of the antigen specificity. Allergen-reactive T cells could mature to lose type 2 cytokine production even prior to initial sensitization by allergen, as well as upon their allergen-dependent activation. As stated earlier, such cytokine-induced, bystander maturation would not occur in the initial responses to allergen because monocytic cells would not be activated by viral or bacterial pathogens to release their cytokines. Interestingly, it has recently been reported in a murine model that depletion of plasmacytoid, but not myeloid, dendritic cells from lungs prior to inhalation of otherwise inert antigen leads to allergic sensitization to the antigen and an asthma-like phenotype. Tolerance to the antigen is induced to antigen when plasmacytoid dendritic cells are present, regardless of depletion of myeloid dendritic cells [28]. Being that plasmacytoid dendritic cells are the major IFN- α producers of the immune system, it is interesting to speculate that their absence during sensitization results in a diminished level of antigen-independent maturation of T cells from IL-13 to IFN- γ production. IFN- α can also inhibit CD3-stimulated proliferation and accumulation of human type 2 T cells *in vitro* (unpublished observations). It has yet to be tested whether such a phenomenon also occurs in humans during the early stages of allergic sensitization for asthma. From unpublished observations in humans, T cells obtained from bronchoalveolar lavage of allergic subjects after segmental allergen challenge could be expanded extensively in cultures with IL-2 for several weeks. Most of these cells continue to produce IL-13 in culture (Fig. 4), even in the prolonged absence of antigenic stimulation. As with cells derived from peripheral blood, these IL-13⁺ cells can be induced *in vitro* to differentiate and produce IFN- γ . These results demonstrate the capacity of bronchoalveolar, type 2 T cells to undergo cytokine-induced, bystander maturation. Whether differences between allergic and non-allergic subjects exist in the ability of bronchoalveolar T cells to mature, and whether regulatory properties of bronchoalveolar T cells differ from those of peripheral blood T cells, are interesting yet unexplored questions.

Unlike most studies based on the branching 'Th1/Th2' paradigm, a central part of the linear maturation model is the potential contribution of antigen-independent (bystander) effects to the balance of type 2 (IL-2-induced preferential proliferation) and IFN- γ ⁺ T cells (cytokine-induced differentiation). Bystander proliferation may potentially explain allergen-independent sensitization in asthmatics, e.g. respiratory infection leading to an IL-2- or IL-15-induced proliferation of resident memory, viral antigen-non-specific, allergen-specific type 2 T cells. Defects in cytokine-induced, allergen-independent maturation of type 2 to IFN- γ ⁺ T cells, which would otherwise diminish the type 2 T cell pool, would allow for the maintenance of a greater pool of type 2 T cells to expand in allergen-dependent and -independent (bystander) processes. Other cell types such as macrophages (which would both influence T cells via IL-12+IL-18+IFN- α production

and IL-15 and respond to IFN- γ and IL-4/IL-13 produced by T cells), endothelial cells, or airway smooth muscle [29] could influence these processes.

The hygiene hypothesis and interferon- γ

Bystander proliferation and maturation that occur within a linear model of T cell development are consistent with the 'hygiene hypothesis' for the reported increased allergy development in industrialized countries in the past several decades [2, 4, 30–33]. The major tenet of this hypothesis is that individuals have been exposed to fewer and less severe viral and bacterial pathogens in industrialized countries, as a result of cleaner living conditions, improved sanitation and water treatment, and vaccination against childhood diseases. This better hygiene results in a lower incidence of microbial and viral infections during childhood. A result of this lowering of infections is fewer exposures of immune cells to inflammatory environments elicited by protective type 1, IFN- γ -dependent immune responses. Hence, there is less opportunity for T cells to be driven to the type 1 phenotype. It has been suggested that microbial infections can contribute to the development of allergic asthma [2–4, 33, 34]. However, the role played by viral infections in asthmatics is complex. Some viruses (rhinovirus) appear to be important in asthma exacerbations in patients with already existing asthma. Others (respiratory syncytial virus, paramyxovirus) could play a role in the development of asthma, and different viruses produce relatively different effects on Th1 vs. Th2 inflammatory parameters [2, 30, 34].

The 'branching' model of Th1/Th2 cell development predicts that allergen-reactive T cells would be responding to cognate allergen during the times of type 1 immune responses against microbes and be polarized to Th1 cells at these specific times. But only naïve, non-committed T cells initially responding to allergen would be driven to IFN- γ production in the branching model, because allergen-specific memory T cells would already be committed to the Th2 lineage if previously activated in an IFN- γ -deficient environment. Alternatively, the linear model of T cell maturation allows for not only initially allergen-responding T cells, but also established memory type 2 T cells, to be driven to IFN- γ production. Also, established memory and naïve T cells can be driven to lose the ability to produce atopy-driving type 2 cytokines and gain that of IFN- γ production. Under this scenario it is more likely that non-specific microbial infections that normally induce an IFN- γ -driven immune response contribute to minimize the incidence of aberrant accumulation of type 2 T cells that is associated with the development of atopy. It is likely that the loss of type 2 cytokine production is more important than the gain in IFN- γ production. Although sustained IFN- γ -driven immune responses may dampen sensitivity to allergic inflammation in the long term by diminishing the type 2 T cell pool, an acute increase in IFN- γ production during an allergic reaction might actually exacerbate inflammation by increasing the recruitment and activation of immune cells [35].

In asthmatics, IFN- γ levels are often elevated in addition to the expected high levels of type 2 cytokines (as discussed in [36, 37]). The studies establishing the linear maturation of

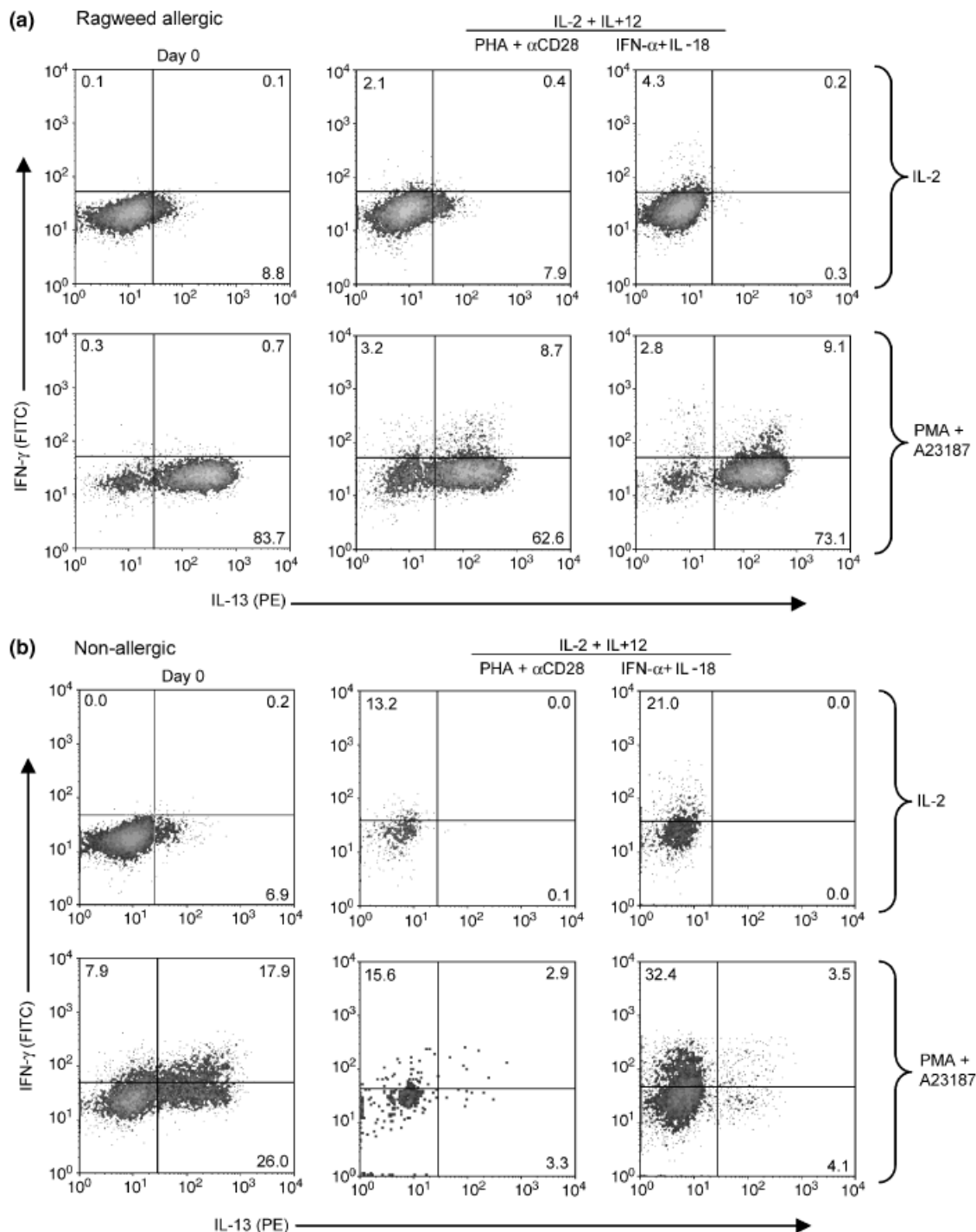


Fig. 4. Maturation of bronchoalveolar T cells. One week after segmental allergen challenge with ragweed antigen E, lymphocytes were isolated from bronchoalveolar lavage fluid of ragweed-allergic (a) and non-allergic (b) subjects. These lymphocytes were cultured 3 weeks with IL-2- and IL-12-neutralizing monoclonal antibody, then aliquoted into 3-day secondary cultures with the stimuli indicated at top. Cells were subsequently stimulated for 5 h with IL-2 or phorbol myristate acetate + Ca^{2+} ionophore + IL-2, as indicated. Expressed cytokines were retained in cells by treatment with Brefeldin A for the final 3 h of stimulation, stained for surface markers and cytokines, then analysed by flow cytometry as described in [59].

T cells demonstrate that both bystander and TCR-dependent proliferation occur primarily in IL-13^+ cells, whereas proliferation of $\text{IL-13}^{\text{lo}}/\text{IFN}^{\text{lo}}$ cells is less and that of $\text{IFN-}\gamma^+$ cells is minimal [14]. Increased proliferation of IL-13^+ cells could possibly account for increases in type 2 cytokines observed in asthmatics and atopic subjects. Similarly, this increased proliferation could account for elevated $\text{IFN-}\gamma$

levels and increased type 1 cell number, with such levels being modulated by mechanisms of differentiation of this increased precursor pool (summarized in Fig. 5). The stimuli that drive the maturation of the type 2 precursor pool need not be dependent on allergens. Rather, non-specific stimulation of monocytic cells by pathogens could provide the cytokines required for maturation independent of simultaneous stimu-

lation of T cells by allergen. Thus, a linear model of T cell maturation is consistent with the observed elevation of both type 2 cytokines and IFN- γ in allergic asthmatic subjects, whereas the branching model is not. According to the branching model, allergen-reactive IFN- γ^+ T cells could only be generated from naïve precursors upon stimulation by allergen, in the presence of IL-12. In the absence of allergen-reactive IFN- γ^+ T cells, allergen challenge in asthmatic individuals would be expected to result in the predominant release of type 2 cytokines, preventing the differentiation of naïve, allergen-reactive T cells into IFN- γ^+ cells.

Modulatory influences on T cell proliferation and maturation

The approach used for establishing the linear model of T cell development can be readily applied to test the effects

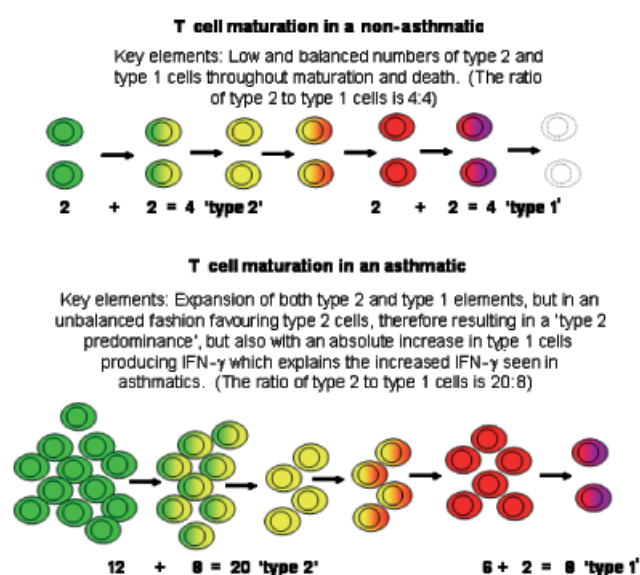


Fig. 5. Proposed models for T cell maturation.

Table 1. Parameters for screening pharmacological agents

Accumulation of type 2 T cells	
Possibility #1: Increased proliferation of IL-13 ⁺ T cells	
Experiment: CFSE analysis of IL-13 ⁺ T cells upon culture of fresh PBL*	
Possibility #2: Lack of maturation of IL-13 ⁺ T cells	
Experiment: Number of IL-13 ⁺ T cells after culture of CD2 ^{-/-} T cells with maturation stimuli†	
Accumulation of IFN-γ^+ cells	
Possibility #1: Enhanced survival of IFN- γ^+ T cells	
Experiment: CFSE analysis of IFN- γ^+ T cells upon culture of fresh PBL*	
Possibility #2: Accelerated maturation of IL-13 ⁺ T cells into IFN- γ^+ T cells	
Experiment: Number of IFN- γ^+ T cells after culture of CD2 ^{-/-} T cells with maturation stimuli†	

CFSE, carboxyfluorescein diacetate succinimidyl ester; mAb, monoclonal antibody; PBL, peripheral blood lymphocyte.

*Include conditions with IL-2 $\pm$ agent and IL-2 + α CD3 + α CD28 mAb $\pm$ agent to screen for effects on bystander- and antigen-dependent accumulation, respectively.

†Include conditions with IL-12 + IL-15 + IL-18 + IFN- α $\pm$ agent and IL-2 + IL-12 + α CD3 + α CD28 mAb $\pm$ agent to screen for effects on bystander- and antigen-dependent maturation, respectively.

of inflammatory mediators, as well as various anti-inflammation therapies on T cell maturation (summarized in Table 1). Prostaglandins are a family of arachidonic acid-derived molecules produced by myeloid cells (e.g. mast cells, macrophages) and non-immune cells (epithelium, airway smooth muscle) during immune responses, and have diverse effects on different cell types. The literature describing the effects of prostaglandin E₂ (PGE₂) on type 2 T cells and allergic inflammation appears contradictory, with some studies demonstrating that PGE₂ attenuates eosinophilic inflammation in murine models of ovalbumin-induced allergic inflammation by favouring a type 1 cytokine profile *in vivo* [38], while others suggest PGE₂ promotes generation of type 2 cytokines *in vitro* [39]. Obviously, the effects of PGE₂ likely depend on the system used and how results are interpreted. For example, effects of PGE₂ *in vitro* may depend on the starting population of T cells. IL-4 production by T-helper type 0 clones (IL-4⁺IFN- γ^+) is inhibited by PGE₂ while that by established Th2 clones (IL-4⁺IFN- γ^-) is not [39]. Preliminary studies from our lab suggest the effects of PGE₂ on accumulation of type 2 T cells and IFN- γ^+ T cells depend on whether they are being modulated by antigen (CD3)-dependent or IL-2-induced bystander stimulation. It has been reported that β -agonists and PGE₂ inhibit proliferation of T cells, dependent on cyclic adenosine monophosphate generation and coincident with protein kinase A activation [40]. Such studies only considered proliferation induced by CD3-mediated stimulation and did not simultaneously analyse proliferation and cytokine production. Moreover, interpretation of data from these studies assumed a 'branching' model of T cell development. The effects of PGE₂ on T cell development are more readily interpretable in a model system in which the starting population is well defined and environmental influences are tightly controlled. Current work testing how PGE₂ affects the linear pathway of T cell maturation (Fig. 6) indicates that PGE₂ significantly inhibits the IL-2-induced accumulation of IL-13⁺ T cells and the maintenance of IFN- γ^+ cells. There was a slightly greater effect of PGE₂ on IL-2- vs. CD3-mediated stimulation in the decrease in IL-13⁺ T cells, in part because of greater variability with CD3-mediated stimulation. Preliminary experiments tracking proliferation by carboxyfluorescein diacetate succinimidyl ester (CFSE) analysis indicate that this attenuation is associated with a significant decrease in proliferation of IL-13⁺ T cells. There was a more prominent difference in the effects of PGE₂ on the proportion of IFN- γ^+ T cells: the proportion was decreased significantly in cultures with IL-2 but not those with CD3-mediated stimulation. These results underscore the importance of considering potential differences that may occur between effects of agents on CD3-mediated and bystander-induced changes in cytokine⁺ T cells. Moreover, the effects of PGE₂ appear system-dependent and highlight the difficulties in reconciling data from *in vitro* and integrative model systems.

These differences in PGE₂ effects we have observed may shed some light onto the dual-character of PGE₂ in inflammation. During the early stages of an immune response, the predominant effect of PGE₂ may be the inhibition of IFN- γ . However, type 2 T cells may also be prevented from accumulating. If local PGE₂ levels are maintained, the overall effect would be a down-regulation of T cell responses.

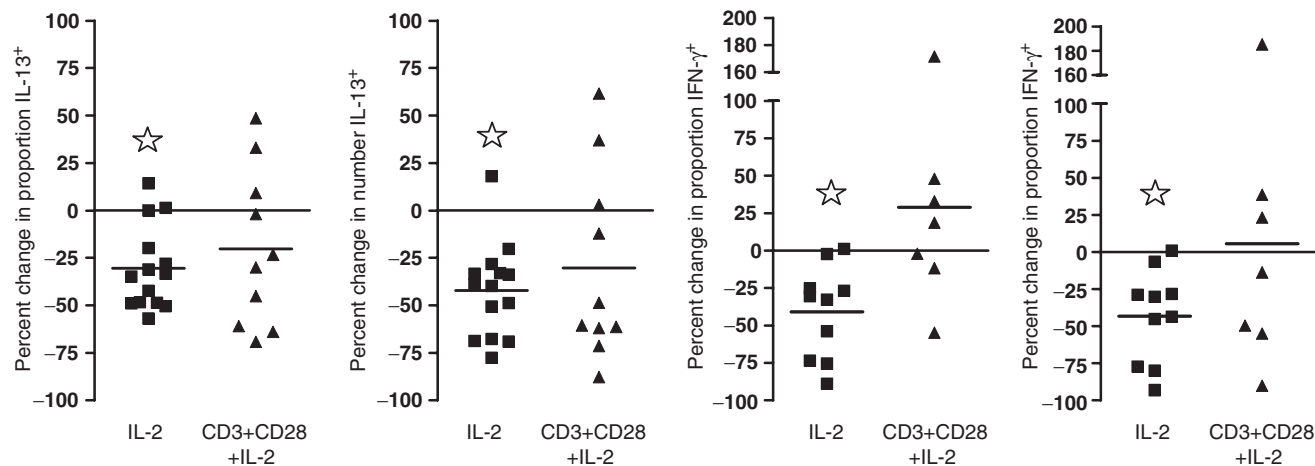


Fig. 6. Effects of prostaglandin E_2 (PGE_2) on T cells. Fresh peripheral blood lymphocytes were cultured with IL-2 for 6 days or IL-2 and α CD3+ α CD28 monoclonal antibodies for 5 days, without (control) or with PGE_2 , then stimulated and analysed for cytokine production. The percent changes in the proportion and number of IL-13⁺ and IFN- γ ⁺ T cells in cultures with PGE_2 are plotted relative to the respective control condition (= 0 percent change) indicated at the bottom. Each point represents results from one individual donor. * $P < 0.05$ vs. control condition.

However, if PGE_2 levels wane, then type 2 T cells may again be able to accumulate in a relatively IFN- γ -free environment. In this manner, PGE_2 would initially have anti-inflammatory properties via its inhibition of cytokine production by T cells, followed by preferential accumulation of type 2 T cells as PGE_2 levels decrease.

A controlled system for testing effects of pharmaceuticals

The treatment of allergic asthma and other atopic disorders relies heavily on pharmaceutical agents. Such agents can be systematically screened for how they modulate the accumulation of type 2 vs. IFN- γ ⁺ T cells *in vitro*. New insights might be obtained by planning experiments and considering the results in the context of the linear maturation pathway of T cells (summarized in Table 1). The first parameter to consider is the accumulation of type 2 T cells. IL-13 is the prototypical type 2 cytokine described in the linear maturation model because it is the most widely expressed type 2 cytokine and is readily detectable in T cells. As discussed above, IL-13⁺ T cells may accumulate via proliferation of a minor, relatively immature population of peripheral blood T cells. This proliferation occurs in cultures of PBL with IL-2- or CD3-mediated stimulation and can be tracked by CFSE dilution, as described in [14]. From such results it can be determined whether the changes in IL-13⁺ T cell numbers are a result of respective changes in proliferation of IL-13⁺ T cells by agents to result in higher or lower numbers of IL-13⁺ T cells. IL-13⁺ T cell numbers can also be increased or decreased if the rate of maturation to the IL-13⁺ IFN- γ ⁺ stage is reduced or enhanced, respectively. This is especially relevant when maturation-inducing stimuli (e.g. IL-12) are included.

The accumulation of IFN- γ ⁺ T cells is a function of the size of the (IFN- γ ⁻) precursor pool and the maturation of this pool into IFN- γ ⁺ cells. A convenient tool for examining maturation of IL-13⁺ IFN- γ ⁻ T cells into IFN- γ ⁺ T cells is CD2⁻/lo T cells. This population of peripheral, immature T cells can be isolated and readily expanded into a large number

of cells that can be used in maturation assays, as detailed in [13]. Because no IFN- γ ⁺ cells exist in the starting population of CD2⁻/lo cells, any IFN- γ ⁺ cell that accumulates represents maturation from an IFN- γ ⁻ precursor, and interpretation is unequivocal. Thus, experimental designs examining proliferation (via CFSE analysis) and maturation of CD2⁻/lo cells are powerful in assessing the effects of therapeutic agents on T cell development.

In addition, numbers of IFN- γ ⁺ T cells may be modulated by changes in IFN- γ ⁺-T cell survival. IFN- γ ⁺ T cells are sensitive to death-inducing ligands such as TNF- α and FasL. Therefore, agents that enhance or attenuate the sensitivity of IFN- γ ⁺ T cells to death-inducing ligands would be expected to elicit lower and higher numbers of IFN- γ ⁺ T cells, respectively. Pharmacological agents can be added to cytokine- (IL-12+IL-15+IL-18+IFN- α) or IL-12+ α CD3+ α CD28 monoclonal antibody-stimulated T cells to determine the effects of the agents on bystander or antigen-dependent maturation. The effects do not necessarily have to be the same for bystander and antigen-dependent processes.

The two most common treatments for allergic asthma are inhaled glucocorticoids and β -agonists [41]. Glucocorticoids are general inhibitors of inflammation. They broadly suppress many cells of the immune system. One mechanism of suppression is inhibition of signalling via nuclear factor- κ B, which would inhibit CD3-dependent activation of T cells to suppress inflammatory responses. However, long-term use of steroids is associated with several adverse reactions [42]. β -agonists provide immediate benefits by inducing relaxation of airway smooth muscle cells to counter the constriction of airways induced by histamines, leukotrienes, and other pro-contractile agents released during allergic inflammation associated with asthma. Whether β -agonists alter airway inflammation in asthma is unclear; although cell-specific effects suggesting anti-inflammatory properties have been described [41], findings from epidemiologic and clinical studies have raised concerns over inflammation control in patients managed on β -agonists alone [16, 43–46]. The effect of steroids and β -agonists on bystander proliferation and maturation of type 2 T cells has yet to be tested, and may

provide insight into the long-term effects of mono- or combination therapy with these agents.

Testing the influence of gene variation on T cell maturation

A number of genes have been shown to be linked to atopic disorders, including allergic asthma. Variation in several of these genes may contribute directly to modifications in the functions of T cells. Such genes include *CTLA4* [13, 47], *IL-4RA* [13, 48–53], *IL-13* [54], and *CCR5* [55]. For example, single nucleotide polymorphisms in *CTLA4* have been shown to modulate the expression of CTLA-4, a negative signalling element that is important for the down-regulation of T cell responses. The functional consequence of lowered CTLA-4 expression in humans is not clear, but could result in an up-regulation of type 2 T cell responses. Blockade of [56] or genetic deficiency [57] in CTLA-4 in mice results in a preferential accumulation of type 2 T cells. The mechanisms for the predominance of type 2 cells are not established; possible mechanisms include the ability of CTLA-4 to attenuate TCR-mediated signalling or competitive inhibition of CD28:B7 interactions. In some murine models, deficiency/blockage of CTLA-4 also results in attenuated development of IFN- γ^+ T cells (e.g. susceptibility to *Leishmania major* [58]). Both results are consistent with the linear maturation pathway of human T cells from type 2 cytokine to IFN- γ -producing cells, whereby lack of accumulation of type 2 T cells would diminish the pool of cells from which IFN- γ^+ T cells could develop. In allergy, the accumulated type 2 T cells would not be driven to differentiate to IFN- γ^+ T cells, given the lack of appropriate stimuli to induce production of IL-12 – an important cytokine driving this maturation pathway. Because type 2 T cells proliferate to a much greater extent than do IFN- γ^+ T cells [14], mutations in genes encoding molecules that modulate proliferation of T cells would be expected to preferentially affect type 2 T cell number. In the case of allergy, genetic variations that promote proliferation of T cells might skew the type 2 cytokine:IFN- γ distribution associated with disease development, whereas those that attenuate proliferation would not. Even in the case of CTLA-4, which is involved in down-regulating CD3-initiated responses, bystander proliferation of type 2 T cells may still be augmented because IL-2 production would be more sustained in the absence of the negative contribution of CTLA-4, thus allowing a greater pool of type 2 T cells, including those not responding to antigen, to accumulate.

Conclusions

The linear model of maturation of IFN- γ -producing cells from a proliferative pool of type 2 cytokine-producing T cells demonstrates a fundamental shift in interpreting how changes in cytokine production by T cell populations are regulated. As discussed above, both clinical observations and prevailing theories of immune system development in asthma are consistent with this highly interpretative *in vitro* model, which allows unambiguous characterization of the modulation of

the intrinsic features of T cell proliferation and differentiation by environmental and genetic factors. A protocol based on the linear model of T cell maturation can be readily applied to test how variation in genes linked to atopy affect T cells. How a variety of pharmacological agents modulates the linear maturation pathway of T cells could also be screened to predict outcomes that may not otherwise be anticipated when interpretation is based on the branched model of T-helper type 1/T-helper type 2 differentiation. Because the development of atopic disorders involves the participation of many different cell types, as well as various genetic and environmental factors that render interpretation of *in vivo* models problematic, a well-controlled and highly interpretive *in vitro* model appears to be required to understand the fundamental characteristics of T cell developmental regulation that will ultimately enable the successful design and interpretation of useful integrative model systems.

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