Notch signalling in the regulation of peripheral T-cell function

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Abstract

The Notch signalling pathway plays a highly-conserved role in regulating the cellular differentiation and proliferation events that characterise pattern formation in the embryo. As cells in the embryo respond to environmental signals, similarly T-cells in the peripheral immune system must monitor their environment for antigens and respond accordingly by entering one of several potential differentiation pathways. Recent studies have identified a role for the Notch pathway in regulating the responses of T-cells in the periphery. In this review, we discuss these findings in the context of the Notch signalling pathway’s role as an orchestrator of cellular differentiation, and propose a central role for Notch as a regulator of immune system function.

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1. Introduction

The Notch receptor and its associated ligands represent a family of highly-conserved transmembrane proteins which are expressed during embryonic development in both vertebrates and invertebrates [1]. Binding of a Notch ligand to a Notch receptor activates signalling cascades which alter the transcriptional programme of the target cell, ultimately determining its fate. Recent findings that Notch and its ligands are expressed in both central and peripheral compartments of the immune system have led researchers to investigate the role of these traditionally developmentally-restricted proteins in immune system function. Elsewhere in this issue, Hernández-Hoyos and Alberola-Ila discuss the established role of Notch signalling in the development of immune cells within the thymus before they leave for the periphery to fulfill their role in immunity. In this review, we will discuss peripheral immune cell studies and their implications on the regulation of in vivo immune responses, in the context of Notch’s well-defined role in cell fate determination and lineage commitment.

2. Notch receptors and ligands

The Notch gene encodes a cell surface transmembrane protein which was initially described as the gene product of a neurogenic locus in Drosophila [2]. Vertebrate homologues were subsequently identified, and there are currently four Notch proteins (Notch-1–4) recognised in the mammalian system [3]. These proteins are highly homologous and share a common architecture. They are translated as single polypeptide chains which are proteolytically processed within the endoplasmic reticulum to generate two polypeptides which remain associated with one another. The resulting Notch heterodimer is inserted into the plasma membrane via a single transmembrane pass to form the mature Notch protein, consisting of a wholly extracellular (NEc) domain and a domain containing the transmembrane and intracellular domain (NIC). The domain structure of Notch proteins is discussed elsewhere in this issue, but, briefly, the NIC domain is characterised by multiple Epidermal Growth Factor (EGF)-like repeats, whilst the NIC contains a range of sequence modules involved in protein–protein interactions, as well as two nuclear localisation sequences which are required for its role as a transcriptional regulator. These modules include a RAM domain and a set of six ankyrin repeats: both of these units are responsible for protein–protein interactions.
and receptor combinations. Some light has been cast on downstream signalling events triggered by different ligands in physiological conditions, and whether there are distinct systems, little is known about the extent to which differences which might suggest distinct biological functions (VWF) domain immediately proximal to the transmembrane pass. This feature is not present in the Deltas, a difference which might suggest distinct biological functions for the Jagged and Delta family. Indeed, in mammalian systems, little is known about the extent to which different Notch ligands activate different Notch receptors under physiological conditions, and whether there are distinct downstream signalling events triggered by different ligand/receptor combinations. Some light has been cast on this apparent redundancy of the system in *Drosophila*, where experiments on the wing margin have shown that Notch signalling alone is not responsible for establishing a cell’s dorsal or ventral status. The gene responsible for this process is the glycosyltransferase Fringe [4]. Glycosylation of Notch within the Golgi by Fringe modifies Notch’s ligand-binding capabilities such that it preferentially binds Delta, and not Serrate [5,6]. This establishes an asymmetry of Delta and Serrate signalling across the wing margin, which ultimately establishes the demarcation of dorsal and ventral characteristics. Importantly, the establishment of an asymmetric signalling system implies that Delta and Serrate signalling have distinct effects upon the differentiation status of a cell, in this case, defining the dorsal or ventral positional fate. This suggests that the two Notch ligands can evoke different downstream transcriptional programmes, although this has yet to be demonstrated experimentally.

3. Notch signalling

Interaction of the DSL domain of a Notch ligand with the EGF repeats of Notch induces conformational changes in the two polypeptide chains of the receptor which, in turn, lead to the proteolytic cleavage events responsible for the generation of NIC (reviewed elsewhere in this issue by Baron). This liberation of NIC allows it to move to the nucleus via its nuclear localisation sequences, where it interacts with downstream target molecules, affecting numerous signalling pathways. A detailed discussion of the biological outcomes of these signalling events is beyond the scope of this article, but they can be divided into three basic processes referred to as lateral inhibition, inductive signalling and cell-autonomous signalling [1]. Briefly, in lateral inhibition, neighbouring cells of equal developmental potential use Notch signalling to permit the differentiation of a single cell from the group while simultaneously restricting the fate choices of their neighbours by forcing them to remain in an undifferentiated state, where they await further signals before adopting a secondary cell fate. By contrast, in inductive signalling, a cell which has already adopted the primary fate delivers a Notch signal to a second cell type; this directly modifies the second cell’s ability to respond to the signals specifying the primary fate, forcing it into a non-primary differentiation programme. In the third, cell-autonomous mode of Notch signalling, a cell regulates its own fate choices through Notch signalling, and is independent of influences from neighbouring cells. In general, receiving a signal through the Notch receptor modulates the response of the cell to co-incident environmental stimuli rather than directly specifying a particular cell fate [7]. It is this ability of the Notch pathway to modify a cell’s response to co-incident signals that makes it potentially an important regulator of the peripheral immune system, where cells are consistently required to respond to environmental stimuli to generate an appropriate immune response. For the remainder of this review, we will concentrate on how Notch signalling may play a role in manipulating the function of T-cells in the peripheral immune system.

4. Peripheral T-cell function

Whilst the roles played by Notch signalling are well characterised in systems such as *Drosophila* or *Caenorhabditis elegans*, where the fates of individual cells within a developing organ (e.g. the wing or the eye) are relatively easy to define, little is yet known about its effects in the peripheral immune system. As discussed by Hernández-Hoyos and Alberola-Ila elsewhere in this issue, Notch’s role in early lymphocyte development has been the main focus of immunologists, principally through the use of transgenic animals either ectopically expressing a constitutively-active NIC [8], or null for the Notch receptors [9]. The peripheral immune system is, by definition, a spatially disparate “organ,” where the acquisition of cell fates by individual cells is difficult to follow in situ. Aside from the lymph nodes, spleen and other secondary lymphoid organs where antigen and immune cells make each others’ acquaintance, peripheral T-cells must find their way to their site of action in peripheral tissues in isolation and within a diverse cellular population. Furthermore, the peripheral immune system is responding to multiple challenges at any one time, and thus is rarely, if ever, entirely polarised in its differentiative status. For these and other reasons, the peripheral immune system does not lend itself well to typical studies of developmental cell biology. Consequently, much of the data regarding T-cell differentiation in general, and Notch signalling in particular, discussed here results from experiments involving in vitro manipulation of primary immune cells or cell lines.

Whereas in *Drosophila* there are only two Notch ligands, Delta and Serrate, studies have so far identified five distinct mammalian ligands, termed Delta or Delta-like-1, -3 and -4, and Jagged-1 and -2 [3]. Like Notch itself, the Notch ligand proteins possess extracellular domains containing multiple EGF-repeats, but they also contain a characteristic cysteine-rich region referred to as the Delta-Serrate-Lag-2 (DSL) domain. Whilst the DSL domain and the EGF repeats are highly conserved between all the Notch ligands, the Jagged proteins contain a distinct cysteine-rich region with structural homology to the Von Willebrand Factor (VWF) domain immediately proximal to the transmembrane pass. This feature is not present in the Deltas, a difference which might suggest distinct biological functions for the Jagged and Delta family. Indeed, in mammalian systems, little is known about the extent to which different Notch ligands activate different Notch receptors under physiological conditions, and whether there are distinct downstream signalling events triggered by different ligand/receptor combinations. Some light has been cast on this apparent redundancy of the system in *Drosophila*, where experiments on the wing margin have shown that Notch signalling alone is not responsible for establishing a cell’s dorsal or ventral status. The gene responsible for this process is the glycosyltransferase Fringe [4]. Glycosylation of Notch within the Golgi by Fringe modifies Notch’s ligand-binding capabilities such that it preferentially binds Delta, and not Serrate [5,6]. This establishes an asymmetry of Delta and Serrate signalling across the wing margin, which ultimately establishes the demarcation of dorsal and ventral characteristics. Importantly, the establishment of an asymmetric signalling system implies that Delta and Serrate signalling have distinct effects upon the differentiation status of a cell, in this case, defining the dorsal or ventral positional fate. This suggests that the two Notch ligands can evoke different downstream transcriptional programmes, although this has yet to be demonstrated experimentally.

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4.1. T-cell differentiation in the peripheral immune system

CD4+ and CD8+ T-cells emerging from the central immune system have a fundamental protective role to play in the life of an organism: to generate antigen-specific responses which are long-lived and can be retrieved upon antigen re-challenge. In order to perform this role, T-cells require a variety of developmental cues from their microenvironment. Activation of naïve T-cells requires two distinct types of signal. The first of these, so-called Signal 1, is an antigen-driven signal generated by the interaction of a specific T-cell receptor (TCR) with an antigen complexed to a major histocompatibility (MHC) molecule presented upon the surface of an antigen-presenting cell (APC). This signal defines the antigen-specificity, activating only specific T-cells with a particular TCR rearrangement. Signal 2, on the other hand, is not dependent on a particular antigen, being delivered through a set of co-stimulatory molecules presented on the APC surface. For example, ligation of APC-expressed co-stimulatory molecules such as CD80 or CD86 to T-cell CD28 at the same time as TCR antigen/MHC engagement initiates signalling events that drive the T-cell down one of a number of differentiation pathways.

Which of these pathways is followed depends on a variety of factors, including the strength of the TCR signal transduced, the specific co-stimulatory molecules expressed upon the APC, and the cytokines present in the immediate microenvironment [10]. In the case of cytokines, interleukin (IL)-12 derived from APCs in response to infectious organisms such as intracellular bacteria drives recently-activated T-cells towards a T-helper 1 (Th1) phenotype, characterised by interferon-gamma (IFN-γ) production and a strong cellular immune response. In contrast, immune challenge from parasitic nematodes generates IL-4, which promotes the formation of Th2 cells producing IL-4, IL-5, IL-9 and IL-13, cytokines which favour a humoral immune response. Following antigen recognition, commitment to either Th1 or Th2 lineages and proliferative expansion of cell numbers, CD4+ T-cells then organise and orchestrate the protective immune response appropriate for the particular immune challenge.

Although the Th1/Th2 differentiation pathway can explain certain patterns of immunological responses observed in vivo and in vitro, it is important to note that these represent extreme phenotypes, and that most T-cell responses likely fall somewhere within the spectrum between Th1 and Th2. Indeed, alternative outcomes of CD4+ T-cell differentiation have been identified, such as the T regulator 1 (Tr1) phenotype (reviewed in [11]). Generated in vitro by repeated activation of T-cells in the presence of IL-10, Tr1 cells generate high levels of the immunoregulatory cytokine IL-10 [12]. Adoptive transfer of these cells alleviates the disease state in a mouse model of colitis, suggesting that these cells have a regulatory or suppressor phenotype [12]. Phenotypically distinct cell populations with similar regulatory activities have also been described, such as CD4+CD25+ T-cells [13, 14], Th3 cells [15] and Natural Killer T-cells [16]. Collectively, these cells possessing regulatory activities will be referred to hereafter as regulatory T-cells (Tregs). Tregs are presumed to be the in vitro equivalent of an in vivo population of T-cells which can negatively influence immune responses. These cells are thought to play a critical role in establishing and maintaining an immunological state referred to as peripheral tolerance, where reactivity to a specific antigen is actively downregulated in order to prevent inappropriate immune responses [17]. Thus, tolerance is critical in maintaining homeostasis within the immune system: certain immune pathologies are thought to arise in the absence of naturally occurring Treg populations [18]. The ability to therapeutically generate Tregs and, presumably, tolerance has therefore been the focus of much attention [19]. Although a number of cytokotyks and cell surface molecules have been variously implicated in driving tolerance [20-24], no candidates have fulfilled all the necessary requirements. Recent studies showing that Notch receptors, Notch ligands and the associated signalling pathway molecules are expressed in peripheral T-cells and APCs have raised the intriguing possibility that Notch’s role in determining cell fate in embryonic development might also be mirrored in the lineage commitment decisions made by cells of the peripheral immune system.

5. Notch signalling and T-cell tolerance

Numerous studies have demonstrated expression of Notch receptors and ligands in cells of the haematopoietic system (reviewed by Ohishi and colleagues elsewhere in this issue). RT-PCR studies have demonstrated that populations of primary murine CD4+ T-cells express all four Notch receptors and, in addition, all five Notch ligands (GM, unpublished data). Furthermore, Notch ligands have been detected in dendritic cells, B-cells and macrophages [25] and GM, unpublished data), cells which have known APC functions. These observations raised an obvious question: what were these molecules with a demonstrated function in embryonic development doing in peripheral immune cells?

To directly investigate the functional outcome of Notch signalling on a peripheral immune response, Hoyne et al. undertook a series of experiments using splenic APCs transduced with a retroviral construct over-expressing the Notch ligand Jagged-1 [26]. Mice treated with Jagged-1+ APCs pulsed with the immunodominant p1100–1127 peptide from the house dust mite antigen Der p1 showed a profoundly reduced response to subsequent immunisation with Der p1, as measured by in vitro T-cell responses. Importantly, adoptively-transferred CD4+ T-cells (but not CD8+ T-cells) from treated mice conferred a state of tolerance to recipient mice, and this tolerance was shown to be antigen-specific in that immune challenge with an unrelated antigen elicited a normal proliferative immune response. This data therefore supports the concept that peripheral T-cells are responsive...
to Notch signals in vivo, and strongly suggests that delivery of a Notch signal to a CD4+ T-cell via Jagged-1 at the same time as antigen/TCR ligation induces CD4+ T-cells to differentiate not into Th1 or Th2 effector cells, but into antigen-specific regulatory cells capable of regulating an in vivo immune response. This hypothesis is consistent with Notch’s classical function as a determinant of cell fate decisions.

The ability of Notch to induce antigen-specific tolerance at the time of T-cell activation has also been supported by recent studies using an alloantigen transplantation system [28]. Treating mice with APCs expressing MHC class I and II alloantigens and the Notch ligand Delta-1 prior to transplanting either allogeneic cells or hearts induced a state of tolerance specific for the alloantigen used. Interestingly, although this experimental approach was only configured to induce tolerance to the direct pathway of alloantigen presentation (i.e. those antigens expressed on donor cells), the heart transplant data indicated that antigenic spreading may occur, leading to some inhibition of responses to indirectly-presented alloantigens, which presumably were processed and presented by host APCs. In this model system, antibody depletion studies highlighted a role for CD8+ cells in establishing the state of tolerance.

Further evidence linking Notch signalling with regulatory functions arose from a study using humanCD4+CD25+ T-cells, a population with proven regulatory function. Comparing these cells with their non-regulatoryCD4+CD25- counterparts showed that Delux, a putative regulator of Notch signalling, was greatly upregulated in the CD4+CD25+ population [27]. These authors also described changes in Notch-4 and Delta-1 message levels upon stimulation ofCD4+CD25+ cells with anti-CD3 and anti-CD28.

If Notch signalling is indeed responsible for driving peripheral T-cells towards a regulatory phenotype, what effects does Notch signalling actually have upon the immunological characteristics of T-cells? We have addressed this recently in a series of studies using a recombinant Fc fusion protein of the Notch ligand Delta-1 to modulate the responses of anti-CD3/anti-CD28-activated murine T-cells in vitro (LY et al., manuscript in preparation). Using this system, CD4+ or CD8+ T-cells activated in the presence of Notch ligand secreted greatly increased quantities of the immunoregulatory cytokine IL-10 compared to those activated in the absence of Delta-1, whilst concurrently generating decreased quantities of effector cytokines such as IFN-γ and IL-13, both by cytokine protein production and mRNA levels. Furthermore, Delta-1 was able to enhance IL-10 secretion and suppress effector cytokine production even under culture conditions promoting the differentiation of either Th1 or Th2 effector cells. This suggests that the Notch signal acts at least in part by promoting an alternative differentiation programme in the T-cell (i.e. towards a regulatory rather than an effector cell). This fits well with observations from transplantation studies [28] which show that tolerance is induced even when the Notch ligand and alloantigen bearing cells are administered via a normally pro-inflammatory route (i.e. i.p. or s.c.). Additionally, we have recently shown that these Delta-induced changes in effector cytokines are unaffected by the addition of neutralising anti-IL-10 receptor antibodies, demonstrating that the reduction in cytokine is not an IL-10-dependent secondary effect (LY et al., manuscript in preparation).

The significance of this observation lies in the fact that IL-10 is a key immunosuppressive cytokine which plays a central role in the homeostasis of the immune response [29]. IL-10-deficient mice are relatively healthy and free of immunological disorders whilst they are housed in a pathogen-free environment, but if housed in the presence of normally innocuous pathogens they develop chronic intestinal inflammation characterised by aberrant immune responses to enteric antigens [30]. In a related study, transfer of a CD45RBhi T-cell population (previously identified as possessing regulatory function) from IL-10-deficient mice failed to protect recipient mice from an induced colitis model, further demonstrating the importance of IL-10 in the regulation of immune responses [24]. Although IL-10 appears to be important for regulatory T-cell activity when assessed in vivo, in vitro studies of the role of IL-10 have generated somewhat conflicting results. Clearly, IL-10 can be used to promote the growth and differentiation of cells with regulatory activity [12], but most groups find that IL-10 plays little or no role in the suppressive activity of Treg cells in vitro [31–34]. Rather, suppression appears to require cell-to-cell contact. This data may be reconciled by hypothesising that IL-10 is necessary in vivo to ensure Treg cells differentiate, expand and/or survive to produce the number of cells necessary to provide measurable suppression rather than being critical for the actual regulatory activity of the cells. It is tempting to speculate that the requirement for cell-cell contact in Treg-mediated suppression might be due to Notch signalling effects, although this has not yet been directly tested. Cell surface TGF-β has been suggested as an alternative candidate molecule for mediating contact-dependent suppression, but the current data is controversial [35,36]. In addition to enhancing the production of IL-10, the ability of Notch signalling to downregulate the expression of effector cytokines such as IFN-γ and IL-13 will most likely affect the status of an immune response given their roles as central players in the Th1 and Th2 responses respectively.

In order to generate an effective immune system, antigen-driven phenotypes must be stored so that upon antigen re-challenge, appropriate effector or regulatory responses can be recalled. This is referred to as immunological memory. In order to clarify whether Notch signalling in CD4+ T-cells alters their differentiation status or merely modulates cytokine expression in a transient manner, cells originally activated in the presence of recombinant Delta-1 were reactivated in the absence of the ligand. Interestingly, the high IL-10 secretion was maintained, indicating that at least a subset of cells in the CD4+ population had differentiated towards a regulatory phenotype, and that this...
The obvious way to approach this problem is through the use of CD4\(^+\) T-cells, which has not yet been clearly defined. The transcription factor nuclear factor kappa B (NF-\(\kappa\)B) participates in the transcriptional control of numerous cytokine genes, including tumour necrosis factor-alpha (TNF-\(\alpha\)), IFN-\(\gamma\) and IL-2 [37]. Notch signalling has been shown to interact directly with the NF-\(\kappa\)B pathway: Notch-1 IC physically interacts with the p50 sub-unit of NF-\(\kappa\)B, preventing it from binding to its DNA target sites [38]. It is therefore possible that signals delivered through the Notch pathway will have a direct effect upon NF-\(\kappa\)B-mediated transcription of cytokine target genes.

Unfortunately, though not surprisingly, such animals are invariably non-viable. However, the generation of mice in which the Notch-1 gene is conditionally inactivated only in the CD4\(^+\) T-cells derived from Notch-deficient mice. Although recombinant ligands allow the experimental delivery of a Notch signal to cells in vitro, it should be noted here that little is known about which of the Notch receptors might modulate cytokine production in either human or murine CD4\(^+\) T-cells. The molecular mechanisms by which Notch signalling might modulate cytokine production in either human or murine CD4\(^+\) T-cells has not yet been clearly defined.

The molecular mechanisms by which Notch signalling might modulate cytokine production in either human or murine CD4\(^+\) T-cells generated similar increases in IL-10 production, along with decreases in effector cytokine production (EB, manuscript in preparation). This data indicates that in peripheral T-cells, as for many other cell types in a wide variety of organisms, the Notch signalling pathway drives an evolutionarily-conserved response.

The regulatory and not effector phenotype persisted upon re-stimulation (LY, manuscript in preparation). Repeating these experiments using human CD4\(^+\) T-cells generated similar increases in IL-10 production, along with decreases in effector cytokine production. This has far reaching implications for the understanding of cytokine production in vivo.

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Although recombinant ligands allow the experimental delivery of a Notch signal to cells in vitro, it should be noted here that little is known about which of the Notch receptors are responsible for transducing the signal in this system. The obvious way to approach this problem is through the use of CD4\(^+\) T-cells derived from Notch-deficient mice. Unfortunately, though not surprisingly, such animals are invariably non-viable. However, the generation of mice in which the Notch-1 gene is conditionally inactivated only in the CD4\(^+\) T-cell population has facilitated the investigation of T-cell responses [39]. In preliminary experiments, the absence of Notch-1 has no effect on the polyclonal activation of peripheral T-cells, their ability to generate IL-2 upon activation, nor their ability to function as cytotoxic T-cells. However, T-cell differentiation studies have not yet been reported, and, as the authors acknowledge, the absence of an effect on activation may be due to a redundancy of Notch receptor function, whereby alternative Notch receptors can mediate these functions in the absence of Notch-1. In the case of peripheral T-cell responses, answering the question “which Notch is which?” will most likely require additional knock-out mouse lines.

**5.1. A model for Notch signalling in peripheral T-cell differentiation**

The existing data from both in vivo transplantation models and in vitro experiments strongly suggest a central role for Notch signalling in mediating the fate choices made by CD4\(^+\) T-cells in response to a combination of antigen and environmental signals. Based on this data, we propose the model detailed in Fig. 1. As discussed above, Th1 and Th2 effector cells are generated by well-characterised differentiation pathways and mediators, whereas the signals responsible for T\(\text{reg}\) generation have remained elusive: in our model, Notch is one of these molecules. By simultaneously promoting IL-10 secretion and inhibiting effector cytokine production, Notch favours T\(\text{reg}\) formation.

At which stage within T-cell differentiation could Notch exert this influence and promote T\(\text{reg}\) differentiation? Results from several studies have suggested that T\(\text{reg}\) are more closely-related to Th2 than to Th1 cells. For example, the inducible co-stimulatory molecule ICOS is important for in vivo Th2 responses [40], and yet is also critical in the development of regulatory cells capable of inhibiting Th2-dependent airway hyper-reactivity [41]. Studies comparing transcript profiles from human Th2 and T\(\text{reg}\) clones will be necessary to address these questions.

![Fig. 1. A model for Notch regulation of peripheral T-cell differentiation. Classically, activation of naïve T-cells generates either Th1 or Th2 effector phenotypes which produce characteristic cytokine profiles. As discussed in the text, we propose that Notch can intervene to alter these differentiative programs. In this model, Notch signalling promotes differentiation to a regulatory phenotype whilst blocking effector differentiation. In addition, Notch may promote cell survival by inhibiting activation-induced apoptosis. This enhanced cell survival, together with blocked effector differentiation, might then permit T-cells to receive input from various environmental signals, including Notch ligands, that promote differentiation to T\(\text{reg}\) cells. In addition, the inhibition of effector cytokines themselves would also serve to block Th1 and Th2 generation, favouring T\(\text{reg}\) formation. IL-10 produced from T\(\text{reg}\) plays a central role in establishing and maintaining tolerance, and it is possible that contact-mediated suppression mediated by T\(\text{reg}\) might involve Notch ligands expressed on their surface.](Image)
with a regulatory murine CD4+CD25+ population have also suggested that Treg over-express a subset of transcripts seen in Th2 effector cells [42]. As shown in Fig. 1, we therefore suggest that Th2 and Treg populations might arise from a common arm of the T-cell differentiation pathway, with Notch perhaps functioning as an inhibitor of Th2 effector formation as well as exerting a positive influence upon Treg differentiation.

The concept under-pinning this model is an interaction between Notch and TCR signalling. These two signalling pathways have been shown to integrate with one another both during thymocyte development and also in a human T-cell line [43], and so it is not far-fetched to speculate that the same may be true in peripheral T-cells. Indeed, our studies with recombinant Delta-1 fusion proteins have shown that the TCR and Notch signals can be separated both temporally and spatially and yet still generate the high IL-10/low effector cytokine phenotype described earlier (EB, LY, manuscript in preparation). Thus, if a T-cell receives both Notch and TCR/TCR co-stimulation signals, it is likely that the outcome will be different from an isolated TCR signal. In our model, this combination of Notch and TCR signals pushes CD4+ T-cells away from an effector phenotype and toward a regulatory programme. Although Notch signals stimulate IL-10 and inhibit effector cytokines, it is unlikely that these changes in cytokine production alone are capable of driving Treg differentiation or function, so we must consider these processes in more detail.

Notch-1 Ic has been shown to inhibit TRC-induced cell death of T-cell lines by interacting with and inhibiting the orphan nuclear receptor Nur77 [44], a molecule known to mediate TCR-induced apoptosis. Notch signalling may therefore protect T-cells from activation-induced death, maintaining them in an undifferentiated state that remains receptive to further environmental signals. These additional signals may be capable of modifying their phenotype further. Candidates for this post-Notch signal influence might include CTLA-4 [22], TGF-β1 [35] and GITR [45,46], molecules which have been variously implicated in modulating endogenous Notch signalling in response to immune challenge could include downregulation of Notch receptors or ligands at the cell surface, or increases in molecules capable of regulating Notch signalling within the target cell, such as ubiquitin ligases [49]. In the differentiation of the neuroectoderm in Drosophila, for example, signalling between Notch and Delta appears to form a negative feedback loop, Notch signalling downregulating the pro-neural achaete-scute complex genes required for transcription of Delta [50]. It remains to be seen if similar regulatory mechanisms exist in peripheral immune cells.

6. Concluding remarks

It is possible to imagine the immune system as an "organism within an organism." During embryogenesis, cells of similar differentiation potency arrange themselves into defined structures by a combination of signal delivery and receipt. Notch plays a key role in these processes. Similarly, cells of the peripheral immune system must interact with one another and their environment in order to respond constantly to immunological challenges. The data reviewed here demonstrate that Notch might guide these events through a set of mechanisms analogous to those observed in embryogenesis, directing T-cells towards a regulatory phenotype by providing input into cell fate decisions. Access to the Notch signalling pathway may therefore make the generation or inhibition of regulatory functions within the immune system a therapeutic possibility.

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