OPINION

IDO and regulatory T cells: a role for reverse signalling and non-canonical NF-κB activation

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Abstract | The immunoregulatory enzyme indoleamine 2,3-dioxygenase (IDO) suppresses T-cell responses and promotes immune tolerance in mammalian pregnancy, tumour resistance, chronic infection, autoimmunity and allergic inflammation. 'Reverse signalling' and 'non-canonical activation' of the transcription factor nuclear factor-κB (NF-κB) characterize the peculiar events that occur in dendritic cells when T-cell-engaged ligands work as signalling receptors and culminate in the induction of IDO expression by dendritic cells in an inhibitor of NF-κB (IκB) kinase-α (IKKα)-dependent manner. In this Opinion article, we propose that IDO acts as a bridge between dendritic cells and $CD4^+$ regulatory T cells, and that regulatory T cells use reverse signalling and non-canonical NF-κB activation for effector function and self-propagation. This mechanism may also underlie the protective function of glucocorticoids in pathological conditions.

A new paradigm emerging from studies of lymphocyte regulation proposes that cells of the immune system use a form of bidirectional communication, commonly referred to as reverse signalling, that allows pairs of 'co-receptors' on adjacent cells to engage in a crosstalk by reciprocally acting as ligands and receptors. (The term reverse signalling is used here conventionally to indicate a two-way communication between cells or cell types, whereby information is actually flowing in both directions, but one direction ('forward') is of greater or longer-standing importance.) Reverse signalling, thanks to primary ligands having evolved into ancillary receptors, enables an immediate feedback to the ligand-bearing cell in response to the forward signal, and it typically involves tumour-necrosis factor (TNF) family members[1].

Reverse signalling also applies to the triad of co-receptors consisting of cytotoxic T-lymphocyte antigen 4 (CTLA4), CD28 and B7 molecules (CD80 and CD86), and this enables bidirectional and univocal conditioning of T cells and dendritic cells (DCs)[2–4]. As a result, mouse and human DCs[5] respond to CTLA4 engagement of their surface B7 molecules with activation of the immunoregulatory pathway of tryptophan catabolism[6]. This pathway, initiated by the enzyme indoleamine 2,3-dioxygenase (IDO; encoded by *INDO*), is controlled by interferons (IFNs)[7], through the involvement of poorly characterized transcription factors of the nuclear factor-κB (NF-κB) family[2,8], and it is often associated with the expression of the anti-inflammatory cytokine interleukin-10 (IL-10)[9,10].

Renewed interest in regulatory T cells has focused on the $CD4^+CD25^+$ regulatory T (T_{Reg})-cell population. It has become increasingly clear that these cells not only exist as natural and adaptive subsets that contribute to the maintenance of self tolerance, but that they also have a potential for treating allergic and chronic inflammatory diseases. However, the origin, recognition properties and molecular basis for the suppressive activity of human and mouse T_{Reg} cells are controversial, as is their relationship to other populations of regulatory cells. Whereas some of the inhibitory effects appear to be mediated by the production of immunosuppressive cytokines — including IL-10 — other mechanisms, which mostly operate in the control of autoimmune and allergic conditions, involve direct interactions of regulatory T cells with responding T cells or antigen-presenting cells[11–14].

Reverse signalling in DCs leading to the activation of IDO expression following T-cell contact features as one of the contact-dependent effector mechanisms of natural regulatory T cells that express surface CTLA4 (REFS 15–17), thereby reconciling a long-established role of IDO in mammalian pregnancy[18] with the more recent role of IDO in T_{Reg}-cell function[19]. There is, however, increasing recognition of a broader and truly immunoregulatory role for IDO in physiopathology, far beyond its function in pregnancy or as a grossly immunosuppressive mechanism[20]. The role of IDO has shifted in importance from that of a metabolic regulator of tryptophan availability in local tissue microenvironments, to one that is central to immune homeostasis and the plasticity of the immune system (FIG. 1), with implications for many aspects of immunopathogenesis[21–25]. This Opinion article focuses on the nature and mechanisms of the mutual relationship between IDO and T_{Reg} cells.

Non-canonical NF-κB signalling

The NF-κB family comprises seven structurally related transcription factors that have a central role in the cellular stress response and in inflammation by controlling a network of gene expression[26]. Although the NF-κB subunits are ubiquitously expressed, their actions are regulated in a cell-type- and stimulus-specific manner, allowing for a diverse range of effects. Recent molecular dissection of NF-κB activation has shown that NF-κB can be induced by the so-called 'canonical' (classical) and 'non-canonical' (alternative) signalling pathways, leading to distinct patterns in the individual NF-κB subunits that are activated and the downstream genetic responses that are induced.

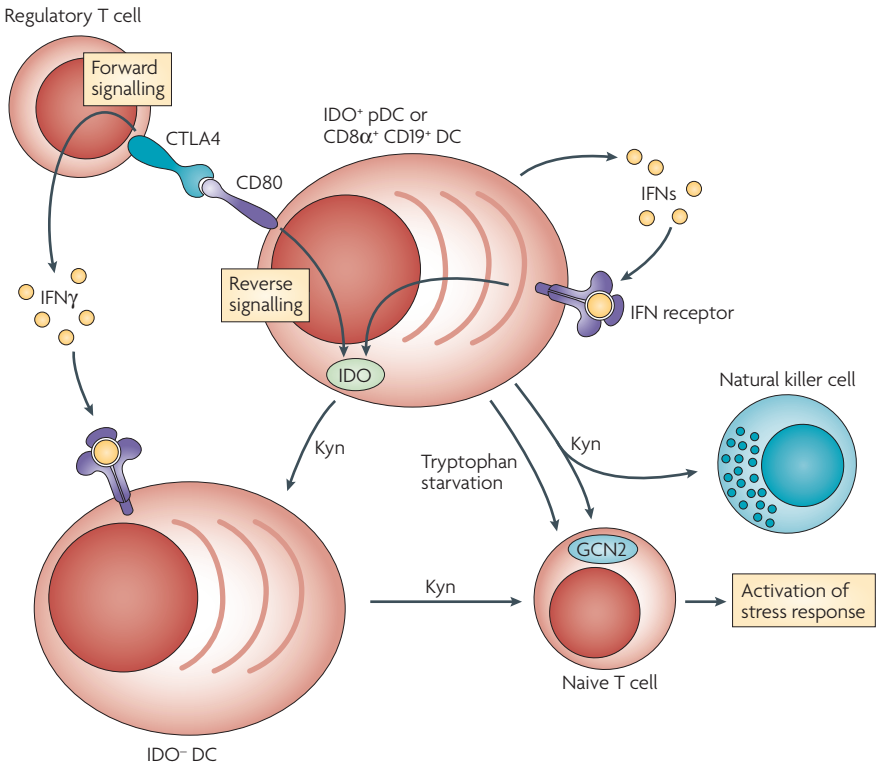

Figure 1 | **A model of crosstalk between dendritic cells and T cells via reverse signalling.** In local tissue microenvironments, the activity of indoleamine 2,3-dioxygenase (IDO)-expressing plasmacytoid dendritic cells (pDCs)[3,40] and/or CD8α⁺CD19⁺ DCs[43,87] driven by type I or type II interferons (IFNs) will result in the sustained IDO enzymatic production of tryptophan catabolites, collectively known as kynurenines (Kyn). In turn, Kyn could recruit other cell types to the regulatory response, including pDCs in which the function of IDO, but not of the Kyn pathway enzymes downstream of IDO, is inhibited post-translationally[64]. (Under conditions of post-translational blockade of IDO, the IFNγ-inducible enzymes of the Kyn pathway can be recruited to a tolerogenic response if cells take up and further metabolize external Kyn that are downstream of the initial, IDO-dependent degradation product of tryptophan.) The combined effects of tryptophan starvation, caused by IDO⁺ pDCs, and the high Kyn production, resulting from the actions of IDO⁺ and IDO⁻ pDCs, is expected to have various effects on target T cells[38,88] and other cell types[89,90], in part involving the stress-response kinase GCN2 (general control non-derepressible 2), which is a sensor of amino-acid deficiency[38,42]. Regulatory T cells could have a crucial role in establishing an IFNγ-rich environment that activates IDO⁻ and IDO⁺ pDCs, either by reverse signalling to pDCs or by direct production of the cytokine[91].

The canonical pathway involves activation of the inhibitor of NF-κB (IκB) kinase-β (IKKβ), which leads to phosphorylation-induced proteolysis of the inhibitor IκBα and consequent nuclear translocation of the REL-A (also known as p65; a subunit of NF-κB) transcriptional activator in the form of p50 (also known as NF-κB1)–REL-A dimers. In the non-canonical pathway, activation of IKKα by NF-κB-inducing kinase (NIK) results in the processing of p100 to p52 and consequent formation of p52 (also known as NF-κB2)–REL-B dimers, which translocate into the nucleus and activate gene transcription[27].

Although much attention has been focused on the pro-inflammatory signalling of NF-κB, recent data indicate that IKKα and IKKβ could have opposing roles. Whereas IKKβ mediates NF-κB activation in response to pro-inflammatory stimuli, IKKα accelerates both the turnover of REL-A and its removal from pro-inflammatory gene promoters[28]. As a result, IKKβ is indispensable in the canonical pathway, whereas IKKα is pivotal in the non-canonical activation that leads to resolution of the early inflammatory process and to the onset of tolerance to self[29] or adaptive immunity to foreign antigens[27,28]. Thus, the cross-regulation between canonical and non-canonical signalling pathways is crucial in promoting an optimally protective response that is balanced between inflammation and tolerance[28].

We have recently found that non-canonical NF-κB activation is necessary for the induction of IDO expression in response to reverse signalling, a requirement that might be in part due to the presence of a putative binding site for p52–REL-B dimers in the *INDO* promoter[30]. The mouse *Indo* promoter contains a putative partial binding site (GGGAGA) at position –3,566 that is recognized by the non-canonical NF-κB dimer, p52–REL-B[31], and this site is conserved in the human gene (position –2,100). Recently, another enzyme with IDO-like activity, IDO-like protein 1 (INDOL1), has been described in both mice and humans. The mouse and human genes encoding INDOL1 and IDO have a similar genomic structure and are situated adjacent to each other on chromosome 8 of mice and humans[32]. Although IDO and INDOL1 have similar enzymatic activities, their expression patterns in tissues are different, yet both mouse *Indol1* (positions –3,180; –2,640; –2,024) and human *INDOL1* (positions –3,357; –993) have multiple GGGAGA sequences and therefore may also be regulated by non-canonical NF-κB.

Overall, these unanticipated findings could have significant implications in immune regulation, including a potential role for non-canonical NF-κB signalling in the development of T_{Reg}-cell responses.

The bipolar nature of DCs and IFNs

Immune receptors on plasmacytoid DCs (pDCs) are potent activators of innate immunity. Through their ability to produce type I IFNs, pDCs drive protective antiviral inflammation and promote the function of bystander myeloid DCs, B cells, T cells and natural killer cells[33]. These responses, however, have also been implicated in the induction and exacerbation of the inflammatory process associated with autoimmunity and allergy[34,35]. Nevertheless, a protective function of type I or type II IFNs has also been demonstrated in several experimental models of autoimmunity and allergy[6,36]. The protective functions of pDCs, and of the associated type I IFN response, appear to be an intrinsic ability of the immune system to co-activate cytostatic mechanisms, induce the death of pathogenic T cells and polarize T cells towards a T_{Reg}-cell phenotype[11,12]. Tryptophan catabolism may, in principle, fulfil all the requirements to mediate these functions, including an arrest in T-cell proliferation[20], induction of T helper 2 (T_H2)-cell apoptosis[37], reversible impairment of T-cell activity through downregulation of expression of the T-cell receptor ζ-chain[38] and the generation of IL-10-producing regulatory T cells[38].

Further regarding the relationship between type I IFNs and non-canonical NF-κB signalling in pDCs, it is interesting to note that although it is possible that type I IFNs contribute to non-canonical NF-κB activation by an inhibitory ligand[39], non-canonical NF-κB activation is also observed in pDCs from mice lacking type I IFN receptors (in other words, induction of the non-canonical pathway by the inhibitory ligand is not entirely dependent on IFNs)[30]. Reciprocally, transcriptional regulation of type I IFN genes in pDCs is controlled by IFN-regulatory factor 3 (IRF3) and/or IRF7 (REF. 34), and the *Irf3* promoter contains a non-canonical NF-κB binding site recognized by p52–REL-B dimers[31]. So it is possible that non-canonical NF-κB contributes to type I IFN production in response to an inhibitory ligand. Finally, owing perhaps to a required function of STAT1 (signal transducer and activator of transcription 1), concomitant IFN (type I or type II) and NF-κB signalling may be necessary for an inhibitory ligand to condition pDCs to express high levels of functional IDO[30,40].

In conclusion, true to their dual nature, increasing evidence links type I IFNs with resistance to specific forms of immunopathogenesis, including those associated with infection[10], by way of a potential bridge between pDCs and T_{Reg} cells[41]. Here, we raise the possibility that IDO represents the functional bridge between pDCs and T_{Reg} cells[38,42] and is, at the same time, a main participant in maintaining the tenuous balance between those opposing actions of IFNs in immune protection and pathology[43,44].

TLRs, NF-κB and IDO

Toll-like receptors (TLRs) trigger the induction of type I IFN production, thereby providing a crucial mechanism of antiviral defence. TLRs are evolutionarily conserved receptors that recognize similarly conserved pathogen-associated molecular patterns (PAMPs) present on various microorganisms. The role of TLRs as arbitrators of the discrimination between self and non-self implies that they have a central role in innate immunity, as well as in the initiation of adaptive immunity[35,45,46]. So far, 13 mouse TLRs have been described and most, if not all, of these can trigger signals to activate NF-κB[26]. TLRs have varied tissue distribution and recognize many different PAMPs, including lipopolysaccharide (LPS), double-stranded RNA (dsRNA), non-methylated CpG-containing DNA and flagellin. The intracellular domain of TLRs has a high degree of homology with that of the IL-1 receptor, and this shared Toll/IL-1 receptor (TIR) domain mediates interactions with downstream signalling adaptors that lead to the activation of three key transcription factors — NF-κB, activator protein 1 (AP1) and IRF3.

Of interest, signalling through TLR4, the receptor for bacterial LPS, neither activates non-canonical NF-κB signalling *per se*[26] nor induces IDO expression[47,48]. By contrast, signalling through TLR9 activates IKKα (REF. 49) and induces IDO expression[37,50,51]. In a mouse model of systemic lupus erythematosus, the absence of TLR9 results in exacerbation of the autoimmune disease[52]. In addition, IKKα is required for the development of self tolerance[53], and NIK (an integral component of the non-canonical pathway) may be necessary for the generation of autoimmune-preventive T_{Reg} cells[54]. These data suggest that TLRs, NF-κB and IDO are intimately linked in the prevention of immunopathogenesis that involves T_{Reg}-cell function.

Reverse and non-canonical signalling

In T_{Reg}-cell generation. IDO has a role in the peripheral generation of regulatory T cells, under physiological[38] or pathological conditions[55]. Recently, a mechanism has been identified that intrinsically links maturing pDCs to the generation of IL-10-producing regulatory T cells, through TLR-dependent and TLR-independent pathways, and that may provide a means to prevent excessive inflammation during infection[14]. On the one hand, a potential role for TLRs, NF-κB and IDO in T_{Reg}-cell generation is consistent with the observations reported above. On the other hand, the TLR-independent mechanism appears to be mediated by CD40 signalling, and CD40 signalling activates NF-κB to induce IDO expression under environmental conditions that tip the balance in favour of the non-canonical pathway[56–59]. We have recently found that reverse signalling through glucocorticoid-inducible TNF receptor-related protein (GITR) ligand (GITRL) also activates non-canonical NF-κB signalling and IDO expression in pDCs[30].

Overall, these data indicate that different ligands acting on pDCs[60] — including TLR9 ligands[49,61,62], CTLA4 (REFS 2,3), CD200 (REF. 63), 4-1BB ligand[22], CD40 ligand[12,14,59] and GITR[30] — all contribute to IDO-mediated regulatory T-cell generation through pathways that converge on non-canonical NF-κB signalling. It has likewise been suggested that T_{Reg} cells use reverse signalling and consequent IDO production by pDCs to expand their own population in the periphery[30,38,64]. Accordingly, a model of T_{Reg}-cell generation by IDO^+ pDCs can be envisioned in which the combined actions of $CTLA4^+GITR^+$ T_{Reg} cells on the one hand, and NF-κB signalling in the pDCs on the other hand, are pivotal in maintaining a regulatory environment (FIG. 2).

In the gut and airways. Traditionally recognized for its role in infection[7], pregnancy[18,20], transplantation[2], autoimmunity[65] and neoplasia[21,66,67], the IDO mechanism has revealed an unexpected potential in the control of inflammation, allergy and allergic airway inflammation, which are all conditions in which pDCs could have a protective function. The first indication that IDO is expressed in the normal colon and is upregulated as a protective mechanism during inflammation came from an elegant study showing that inhibition of IDO activity during experimental colitis resulted in increased mortality and an augmentation of the normal inflammatory response[68]. Not surprisingly, therefore, the administration of soluble GITR is highly protective in this experimental setting[69], consistent with the ability of GITR to induce IDO expression in target cells[30]. As predicted by the 'hygiene hypothesis' — that is, a reduction in microbial burden at a young age may predispose individuals to allergy[70] and autoimmunity[71] — epidemiological and experimental data now suggest that certain microorganisms induce a state of protective tolerance[10]. Recognition of commensal bacteria by TLRs is crucial in maintaining intestinal homeostasis[72]. Moreover, the commensal flora could have anti-inflammatory effects through the inhibition of canonical NF-κB signalling[73,74]. A key role for DCs in probiotic functionality correlates with reduced colonic expression of pro-inflammatory genes and increased expression of IFNγ and IDO by DCs exposed to probiotic bacteria[75]. So TLR-mediated induction of IDO expression and inhibition of the canonical NF-κB signalling work together to maintain intestinal homeostasis in experimental settings.

However, the model that most clearly shows the protective effects of reverse signalling and IKKα-dependent induction of IDO expression in mucosal epithelia is that of experimental asthma. Allergic asthma is characterized by chronic inflammation associated with airway remodelling. This process results in subepithelial fibrosis, an increase in smooth muscle mass and an increase in the number of mucous glands. Chronically allergic mice develop sustained eosinophilic airway inflammation and airway

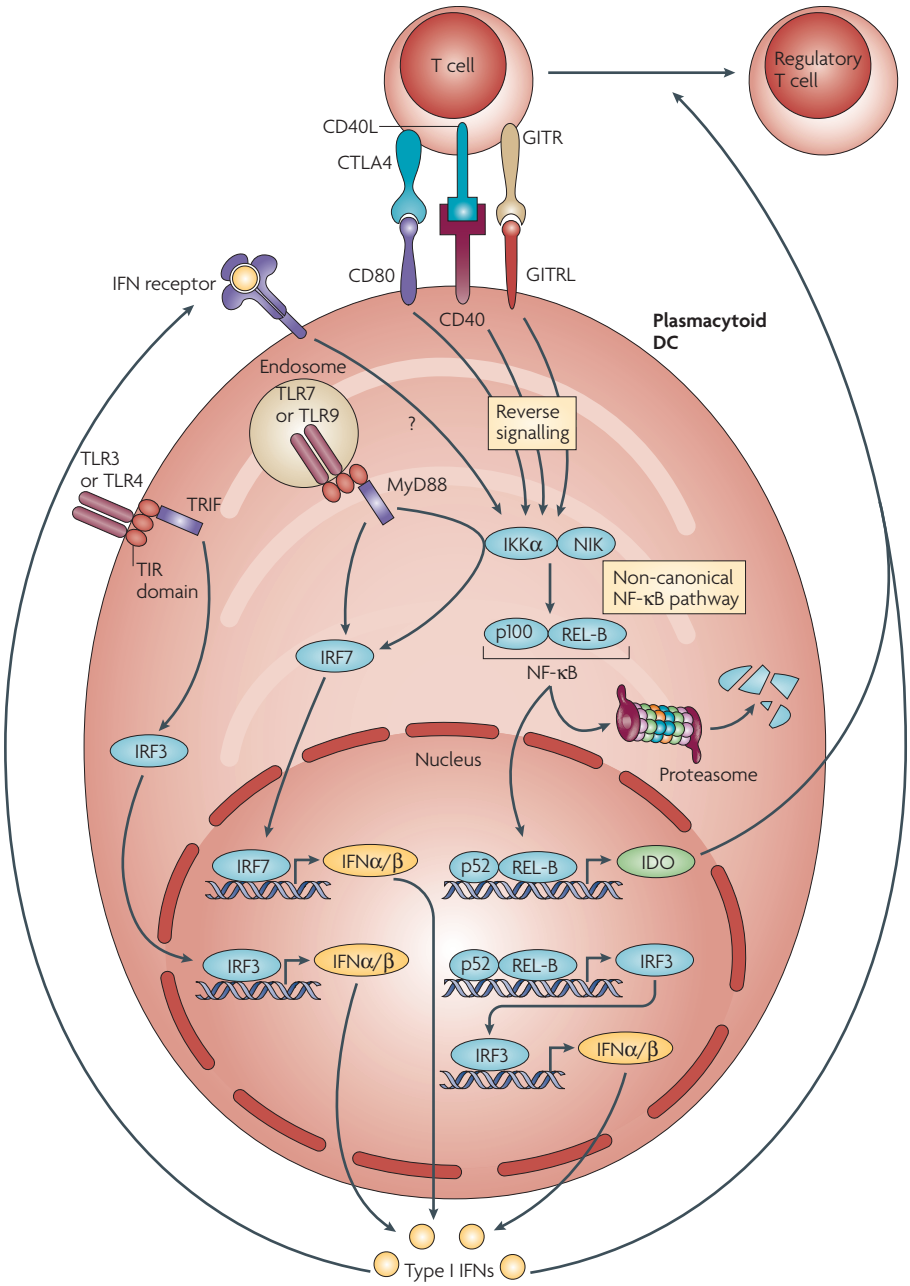

Figure 2 | **Regulatory T-cell generation via reverse and non-canonical signalling to pDCs.** In plasmacytoid dendritic cells (pDCs), several different signals emanating from different receptors may tip the balance of canonical and non-canonical pathways of nuclear factor-κB (NF-κB) activation in favour of inhibitor of NF-κB (IκB) kinase-α (IKKα)-dependent signalling, leading to p52–REL-B-driven transcription of *Indo* and *Irf3*, which encode IDO (indoleamine 2,3-dioxygenase) and IRF3 (interferon-regulatory factor 3), respectively. The resulting production of IDO, boosted by the autocrine type I interferons (that is, IFNα and IFNβ), generates a regulatory environment through the combined effects of the integrated stress response and immunoregulatory tryptophan catabolites. Reverse and CD40 signalling via co-receptor systems — that is, the pairs of cytotoxic T-lymphocyte antigen 4 (CTLA4) and CD80; glucocorticoid-induced tumour-necrosis factor receptor (GITR) and GITR ligand (GITRL); and CD40 ligand (CD40L) and CD40 — may sustain IKKα-dependent induction of IDO expression. Signalling through specific Toll-like receptors (TLRs) is expected to reinforce or mimic these events, either by directly affecting the balance of canonical and non-canonical pathways of NF-κB activation (as in the case of TLR7 and TLR9, which signal through the intracellular adaptor protein myeloid differentiation primary-response gene 88 (MyD88) in association with IRF7) or by activating IRF3 (as is the case for TLR3 and TLR4, which act through TRIF (Toll/interleukin-1 receptor (TIR)-domain-containing adaptor protein inducing IFNβ). Type I IFNs may, in turn, activate non-canonical NF-κB signalling.

hyperresponsiveness to inhaled antigen[76]. In two experimental models of allergic asthma, soluble CTLA4 (REF. 77) and a TLR9 ligand[37] can independently inhibit airway eosinophilia and hyperresponsiveness by regulating T_H-cell subsets. In a third model, T_{Reg} cells[78], pDCs[61] and IDO expression[10] all contribute to protection from allergic asthma. In this model, we found that effective anti-inflammatory treatment inhibited T_H2-cell responses and allergy, and induced the expression of forkhead box P3 (FOXP3; a T_{Reg}-lineage specification factor) in $CD4^+$ T cells through mechanisms dependent on IKKα-induced IDO[30]. These data suggest that modulation of IDO expression by components of non-canonical NF-κB signalling — in balance with canonical signalling counterparts — is essential for the maintenance of TLR-driven immune homeostasis in the airways (FIG. 3a,b). Clinical trials of TLR9-based immunotherapy are presently ongoing and suggest that TLR9 ligands may significantly improve the treatment of allergic diseases[79].

In glucocorticoid function. Although glucocorticoids have been widely used since the late 1940s, the molecular mechanisms responsible for their anti-inflammatory activity are still under investigation. Since the discovery of NF-κB in 1986, and the cloning of the genes encoding the NF-κB components and IκB proteins, several molecular studies have demonstrated that these widely used drugs, known for their varied therapeutic activities, inhibit NF-κB activity, usually among other biological effects. Glucocorticoids act by binding to the glucocorticoid receptor that, upon activation, translocates to the nucleus and either stimulates or inhibits the expression of genes encoding anti-inflammatory proteins or pro-inflammatory transcription factors. It is widely believed that the anti-inflammatory properties of glucocorticoids, as well as non-steroidal anti-inflammatory drugs, are in part related to their inhibition of NF-κB[80]. A recent study in patients with asthma suggested that glucocorticoid treatment is not only immunosuppressive and anti-inflammatory, but that it also promotes or initiates the differentiation of naive T cells towards a T_{Reg}-cell phenotype in a FOXP3-dependent manner[81].

Using naive mice, we found that glucocorticoid treatment *in vivo* increased the amount of GITR expressed by $CD4^+$ T cells and of GITRL expressed by pDCs[30]. Glucocorticoid treatment also conferred immunoregulatory properties on pDCs that were dependent on GITR expression by the host and required functional IDO *in vivo*.

So it seems likely that through synergistic effects on T-cell expression of GITR and pDC expression of GITRL, glucocorticoid acts on the GITR–GITRL co-receptor system to induce IDO expression via non-canonical NF-κB signalling. In addition, in the model of allergic asthma, we found that glucocorticoids inhibited T_H2-cell responses and allergy, and induced FOXP3 expression in $CD4^+$ T cells by mechanisms dependent on tryptophan catabolism[30] (FIG. 3c). This supports the view that glucocorticoids, pDC expression of GITRL and T_{Reg}-cell activity are linked by a positive feedback loop, whereby $CTLA4^+GITR^+$ T_{Reg} cells would expand their own population by inducing the production of IDO by pDCs. More generally, these data suggest that glucocorticoids function by taking advantage of reverse signalling through GITRL to activate the non-canonical NF-κB pathway, oppose the canonical NF-κB pathway and induce IDO expression. This mechanism would explain the apparently paradoxical observation in humans of an enhanced IDO-dependent antimicrobial effect by glucocorticoids[82], which are otherwise immunosuppressive. Perhaps more importantly, these data might explain the unexpected finding of increased IL-10-dependent T_{Reg}-cell activity, induced by IDO, in asthmatic patients treated with glucocorticoids[81].

Future directions

Although our appreciation of both the complexity and potential for therapeutic intervention of the IDO mechanism has expanded enormously in recent years, key unanswered questions still remain. These relate to the nature of the adverse effect of soluble CTLA4 on T_{Reg}-cell survival after short-term administration of the recombinant protein[83]; the mechanisms through which DAP12-associated receptors[48,84] (such as specific isoforms of the CD200 receptor) and the combined effects of IL-6 and SOCS3 (suppressor of cytokine signalling 3)[4,85] restrain IDO activity; and how the integrated stress response[38,42,43] and various — natural or synthetic — tryptophan catabolites[24,64] contribute to IDO-dependent regulatory responses *in vivo*. Despite these limitations, it is possible to introduce a conceptually new model that effectively incorporates non-canonical NF-κB signalling in tolerance mechanisms for IDO and glucocorticoids in a T_{Reg}-cell-dominated scenario. The resulting

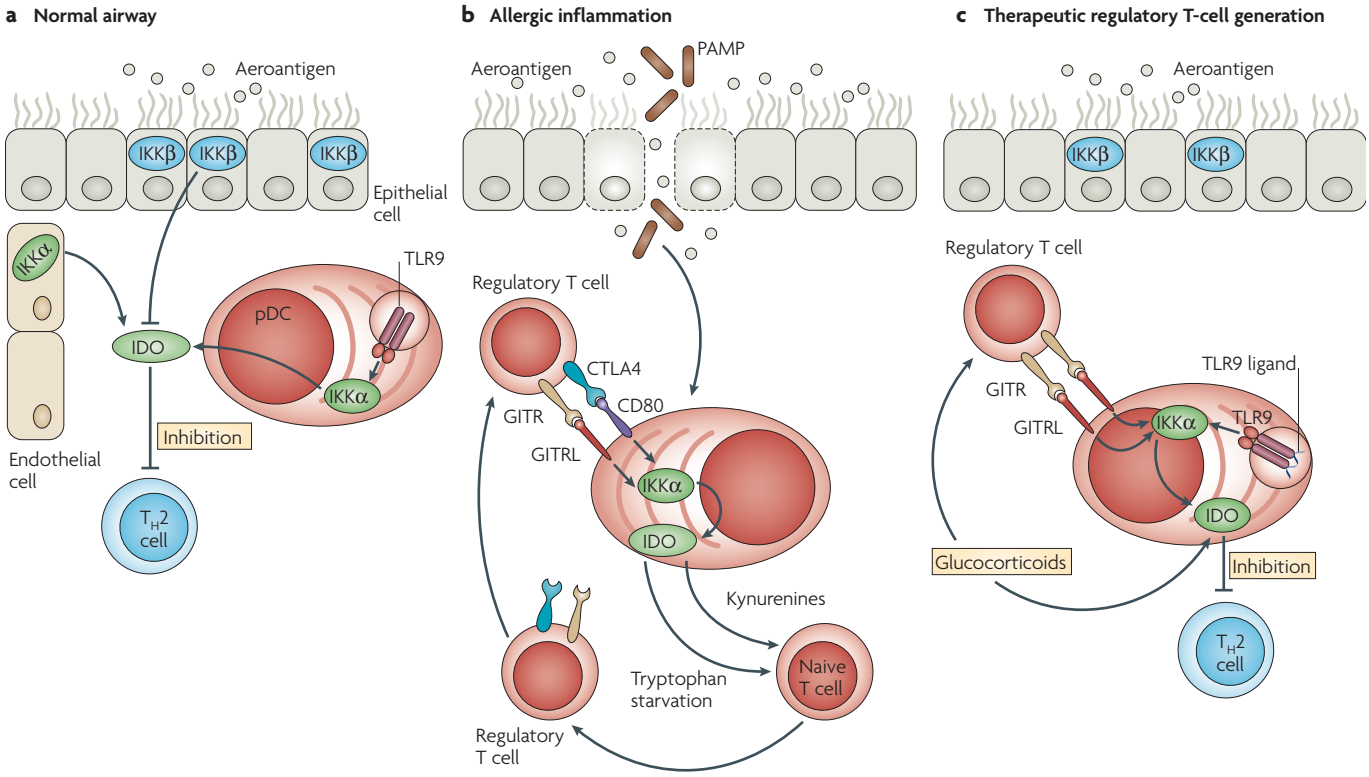

Figure 3 | **Non-canonical NF-κB-mediated induction of IDO expression is essential for the maintenance of immune homeostasis in the airways. a** | Harmless aeroantigens are prevented from initiating airway inflammation by the integrity and antimicrobial defence of the epithelium, in an environment in which Toll-like receptor 9 (TLR9)-driven expression of IDO by lung plasmacytoid dendritic cells (pDCs)[4,85] and other cell types[92,93] will inhibit the expansion and activation of T helper 2 (T_H2) cells[30]. Although some degree of activation of the canonical nuclear factor-κB (NF-κB) signalling pathway (epitomized here by inhibitor of NF-κB kinase-β (IKKβ)) probably contributes towards maintaining the integrity of the epithelial-cell barrier, non-canonical NF-κB signalling (that is, activated through IKKα) could contribute IDO-dependent regulatory effects to achieve an overall local immune homeostasis. **b** | Under pathological conditions, allergic inflammation may develop, resulting in a breach in the epithelial-cell barrier and the production of pro-inflammatory cytokines such as interleukin-6 (IL-6; not shown). Canonical NF-κB signalling favours IL-6-dependent suppression of IDO[94] and T_H2-cell expansion[95]. However, regulatory T (T_{Reg}) cells — expressing surface cytotoxic T-lymphocyte antigen 4 (CTLA4) and glucocorticoid-induced tumour-necrosis factor receptor (GITR) — could accumulate and further expand their own population through reverse signalling in pDCs, after engagement of CD80 and GITR ligand (GITRL), respectively. **c** | Used therapeutically, glucocorticoids and TLR9 ligands or modulators could greatly help to restore local homeostasis, by directly inhibiting the canonical NF-κB pathway (as is the case for glucocorticoids) or by promoting GITRL- and TLR9-dependent activation of the non-canonical NF-κB pathway and T_{Reg}-cell generation. PAMP, pathogen-associated molecular pattern.

paradigm is that reverse and non-canonical signalling is an essential component of T-cell regulatory function, whether in mucosal homeostasis of the gut and airways, or in the action of glucocorticoids as physiological mediators or therapeutic agents. Ligands[79] and modulators[61,84,86] of TLR9 signalling, soluble forms of co-stimulation antagonists[15,69], and inhibitors of canonical NF-κB signalling[80,81] may follow tolerogenic pathways that converge on T_{Reg}-cell generation. All of this could ultimately offer considerable promise in facilitating our understanding of the general mechanisms of tolerance.

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doi:10.1038/nri2163
Published online 3 September 2007

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Acknowledgements
We thank G. Andrielli for help with the original art work. Support for the work in our laboratory came in part from grants from the Juvenile Diabetes Research Foundation (U.G. and P.P.) and the Italian Association for Cancer Research (P.P.).

Competing interests statement
The authors declare no competing financial interests.

DATABASES
Entrez Gene:
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CD28 | CD80 | CD86 | CTLA4 | GITR | GITRL | IDO | INDOL1

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VIEWPOINT

Reflections on the clonal-selection theory

Melvin Cohn, N. Av Mitchison, William E. Paul, Arthur M. Silverstein, David W. Talmage and Martin Weigert

Abstract | How do we account for the immune system's ability to produce antibodies in response to new antigens? It has been 50 years since F. Macfarlane Burnet published his answer to this question: the clonal-selection theory of antibody diversity. The idea that specificity for diverse antigens exists before these antigens are encountered was a radical notion at the time, but one that became widely accepted. In this article, *Nature Reviews Immunology* asks six key scientists for their thoughts and opinions on the clonal-selection theory, from its first proposal to their views of it today.

What was revolutionary about the clonal-selection theory as a solution to the specificity-of-antibody problem?

Melvin Cohn. The clonal-selection theory (CST), as it was viewed by F Macfarlane Burnet[1], meant nothing more than cellular selection (not clonal selection) even as late as 1961 (REF. 2). In any case, it made no contribution to the debate of the instructionist theory versus the selectionist theory. In fact, it was the experiments of Luria and Delbrück[3], Newcombe[4] and Joshua and Esther Lederberg[5] that were the bases for disproving instructionism.

Burnet himself rejected Niels K. Jerne's selectionist theory[6] based on the implausibility of a self-replicating antibody molecule, and instead suggested that the B-cell receptor (BCR) was located on cells that replicate. I, myself, was unaware of Burnet's paper[1] until 1959 when it was referenced in Burnet's book[7]. I had independently concluded, as had David W. Talmage[8], that antibodies had to act as receptors on cells, and by 1959 my colleagues and I were in the middle of an experiment that was derived from the demise of instructionism — namely, to determine the number of antibodies that a single cell could make. The moment one considers that an antigen receptor is located on cells, its genetics and the pathway of its expression come into play. At one extreme, instructionism required that one cell produce all antibodies. At the other extreme, selectionism required that one cell produce one antibody. Today, I prefer to refer to selectionism as somatic evolution and cite Burnet's CST as but one of the initiating sparks.

N. Av Mitchison. Biology in the 1950s was coming to terms with 'DNA and all that'. We saw the problem of antibody synthesis in terms of read-out from constant DNA (that is, the germline theory, which states that the information that is required for the production of all necessary antibodies is provided by the genome), although Linus Pauling's