Clinical and Experimental Immunology REVIEW ARTICLE doi:10.1111/j.1365-2249.2007.03356.x

The role of T helper 17 (Th17) and regulatory T cells (Treg) in human organ transplantation and autoimmune disease

B. Afzali, G. Lombardi, R. I. Lechler and G. M. Lord
Department of Nephrology and Transplantation, King's College London, Guy's and St Thomas' Hospital, UK

Summary

Uncommitted (naive) murine CD4$^+$ T helper cells (Thp) can be induced to differentiate towards T helper 1 (Th1), Th2, Th17 and regulatory (T_{reg}) phenotypes according to the local cytokine milieu. This can be demonstrated most readily both *in vitro* and *in vivo* in murine CD4$^+$ T cells. The presence of interleukin (IL)-12 [signalling through signal transduction and activator of transcription (STAT)-4] skews towards Th1, IL-4 (signalling through STAT-6) towards Th2, transforming growth factor (TGF)-β towards T_{reg} and IL-6 and TGF-β towards Th17. The committed cells are characterized by expression of specific transcription factors, *T-bet* for Th1, *GATA-3* for Th2, forkhead box P3 (FoxP3) for T_{regs} and *RORγt* for Th17 cells. Recently, it has been demonstrated that the skewing of murine Thp towards Th17 and T_{reg} is mutually exclusive. Although human Thp can also be skewed towards Th1 and Th2 phenotypes there is as yet no direct evidence for the existence of discrete Th17 cells in humans nor of mutually antagonistic development of Th17 cells and T_{regs}. There is considerable evidence, however, both in humans and in mice for the importance of interferon (IFN)-γ and IL-17 in the development and progression of inflammatory and autoimmune diseases (AD). Unexpectedly, some models of autoimmunity thought traditionally to be solely Th1-dependent have been demonstrated subsequently to have a non-redundant requirement for Th17 cells, notably experimental allergic encephalomyelitis and collagen-induced arthritis. In contrast, T_{regs} have anti-inflammatory properties and can cause quiescence of autoimmune diseases and prolongation of transplant function. As a result, it can be proposed that skewing of responses towards Th17 or Th1 and away from T_{reg} may be responsible for the development and/or progression of AD or acute transplant rejection in humans. Blocking critical cytokines *in vivo*, notably IL-6, may result in a shift from a Th17 towards a regulatory phenotype and induce quiescence of AD or prevent transplant rejection. In this paper we review Th17/IL-17 and T_{reg} biology and expand on this hypothesis.

Keywords: autoimmune disease, human, interleukin-17, lineage commitment, regulatory T cells, Th17, transcription factors

Accepted for publication 25 January 2007
Correspondence: Graham M. Lord, Department of Nephrology and Transplantation, King's College London, Guy's and St Thomas' Hospital, UK.
E-mail: graham.lord@kcl.ac.uk

Introduction

Despite repertoire restriction in the thymus ('central tolerance') [1], the generation of a diverse T cell repertoire inevitably results in the production of T cell receptors with specificity for self-antigens [2,3]. Naive thymic emigrants therefore have the potential not only to respond to foreign antigens but also to components of self. Maturation of naive T cells depends critically on their interaction with the physicochemical environment and results in the development of cells with an effector (and memory) or regulatory function and the tolerization of autoreactive cells. It is critically important for the prevention of autoimmune diseases, therefore, that self-reactive naive T cells are not induced to mature into effector cells.

Murine experiments have demonstrated that naive CD4$^+$ helper T cells (Thp) can develop into at least four types of committed helper T cells, namely T helper 1 (Th1), Th2,

Th17 and regulatory T cells (T_{regs}) (see below). In humans, there is evidence for the existence of all but discrete Th17 cells, although helper T cells secreting interleukin (IL)-17 have clearly been described [4]. IL-17 has a proinflammatory role and has been implicated in many inflammatory conditions in humans and mice, while T_{regs} have an anti-inflammatory role and maintain tolerance to self-components (see below).

Naive T cells can be induced to commit to particular lineages based on mode of stimulation, antigen concentration, costimulation and cytokine milieu [5]. The pathways of differentiation towards Th1 and Th2 cells have been elucidated previously with IL-4 signalling through signal transduction and activator of transcription-6 (STAT-6) possibly the most important cytokine in inducing Th2 cell differentiation [6] and IL-12 signalling through STAT-4, the central cytokine for commitment towards a Th1 lineage [7]. Once differentiated, each lineage is characterized by its own cytokine profile (with interferon (IFN)-γ being the signature cytokine of Th1 cells and IL-4 the archetypal cytokine of Th2 cells) and transcription factors (*T-bet* for Th1 [8,9], *GATA-3* for Th2 [10], forkhead box P3 (FoxP3) for T_{regs} [11,12] and *RORγt* for Th17 cells [13]). Although Th1 responses have been implicated in the development of autoimmune diseases (AD) [14], reduction in IFN-γ signalling in mice (using IFN-γ knock-out strains or blocking IFN-γ) paradoxically worsens susceptibility to AD, most notably experimental allergic encephalomyelitis (EAE) and collagen-induced arthritis (CIA) [15,16] in the absence of exaggerated Th2 responses, implying the involvement of other effector populations. Recent evidence suggests that naive T cells (in mice) can also be induced to differentiate along a pathway favouring development of Th17 or T_{reg} cells in a mutually exclusive manner [17–19]. Indeed, the Th17 population is important in mediating autoimmune diseases in animals [20,21].

As a result, a novel hypothesis has been proposed [22] with regards to inflammatory and autoimmune diseases, namely that skewing of responses towards Th17 or Th1 and away from T_{reg} (and Th2) may be responsible for the development and progression of AD or transplant rejection in humans and that blockade of critical cytokines may result in a shift in this polarization from Th17/Th1 phenotypes towards T_{reg} and Th2 (i.e. that 'regulation and 'dysregulation' are inducible and remediable). Our own observations suggest that human effector T cells can be identified that produce mutually exclusive IFN-γ or IL-17 profiles. Additionally, the hypothesis predicts that blockade of critical cytokines for generation of Th17 (namely IL-6) can result in remission of AD. The purpose of this review is to discuss the relevance of Th17 and T_{reg} in human disease pathogenesis and progression.

IL-17

First cloned in 1993 from a murine cDNA library and known originally as CTLA-8 [23,24], IL-17A is a member of a family

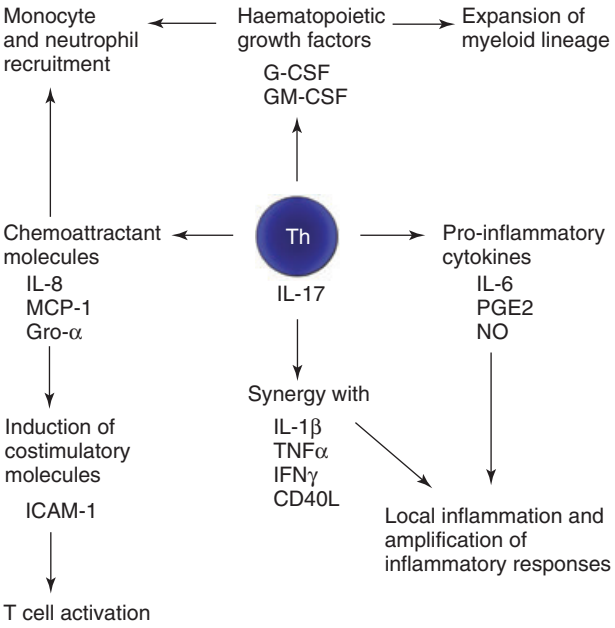

Fig. 1. Proinflammatory effects of interleukin-17.

of IL-17 cytokines (IL-17A–F [25–29]) which are structurally homologous to each other and to a gene in the herpesvirus saimiri [23,30]. It was described initially as a product of activated and memory $CD4^+$ T cells [23,30,30,31] but it is now known that the production of IL-17A is more ubiquitous and has been demonstrated in γδ T cells [32], $CD8^+$ memory T cells [31,33], eosinophils [34], neutrophils [31] and monocytes [35]. Nevertheless, the predominant source of IL-17A (henceforth referred to as IL-17) remains the $CD4^+$ memory T cell population [4,33].

The broad cell and tissue distribution of receptors for IL-17 (of which five have been described, namely IL-17R (the dominant receptor for IL-17A), IL-17RB, IL-17RC, IL-17RD and IL-17RE) in both humans and mice [27,29,30,36–38] and the diversity of expression through alternate splicing (reviewed in Moseley *et al.* 2003 [39]) argues for a pleiotropic spectrum of biological activity that may extend beyond the purely immunological, with the potential to act on many different cell types. Indeed, experiments in animals suggest that, unlike other cytokines, very little redundancy exists in the IL-17 network as IL-17R-deficient mice are very susceptible to lethal bacterial infections [40] and have inhibited T cell responses [41].

Nevertheless, the predominant function of IL-17 is thought to be as a proinflammatory mediator through a variety of mechanisms as summarized in Fig. 1. Locally, IL-17 stimulates production of IL-6, nitric oxide and prostaglandin E_2 (PGE_2) [4,30,42], while synergy with other inflammatory cytokines such as IL-1β, tumour necrosis factor (TNF)-α, IFN-γ [43–45] and CD40 ligand (by increasing surface levels of CD40) [46] leads to up-regulation of gene expression and progression and amplification of local inflammation. IL-17

mediates chemotaxis of neutrophils and monocytes to sites of inflammation through the chemoattractant mediators IL-8, monocyte chemoattractant protein (MCP)-1 and growth-related protein (Gro)-α [45,47–49] while enhancing production of haematopoietic growth factors, such as granulocyte-colony stimulating factor (G-CSF) and granulocyte-macrophage (GM)–CSF [26,50], which promote the growth and maturation of the recruited myeloid cells. Furthermore, IL-17 acts as a bridge between the innate and adaptive immune response by augmenting the induction of co-stimulatory molecules such as ICAM-1 by other cytokines [45,51], thereby supporting T cell activation.

Although much is now known regarding the biology of IL-17 in murine systems and there is compelling evidence for an important role of IL-17 in inflammatory/autoimmune conditions [52,53], attempts to overexpress IL-17A ubiquitously in mice have failed due to generalized overproduction being lethal to developing embryos [54], while overexpression of IL-17E leads to a generalized inflammatory syndrome [55]. In humans, there is also a considerable body of evidence suggesting an important role for IL-17 in the aetiopathogenesis of inflammatory and autoimmune diseases, as discussed below.

Rheumatoid arthritis (RA)

Many lines of evidence support the role of IL-17 in the pathogenesis of human RA [56]. Levels of IL-17 are elevated in the synovium of patients with RA [57,58] and synovial cultures from patients with RA spontaneously secrete IL-17 [59]; the source of this IL-17 is local production by T cells [59] and juxta-articular bone lymphocytes [60]. Pathologically, this cytokine can activate and enhance all mechanisms of tissue injury that have been described previously in rheumatoid arthritis. In particular, IL-17 can up-regulate and/or synergize with local inflammatory mediators such as IL-6 [61,62], IL-1β and TNF-α [61–63], pro-oxidants such as nitric oxide [64], as well as promoting extracellular matrix injury through stimulation of production of matrix metalloproteinases (MMP) [65,66] and inhibition of matrix repair components such as proteoglycans and collagens [67,68]. Furthermore, bone injury is enhanced [69] through promotion of osteoclastogenesis via osteoclast activating factor [57]. The combination of these factors has pathological effects on bone (resorption), extracellular matrix (degeneration), synovium (proliferation and inflammation), blood vessels (angiogenesis) and immune cells (recruitment and activation of monocytes and lymphocytes) in the rheumatoid joint. Not surprisingly, perhaps, intra-articular injection of IL-17 in normal mouse joints induces similar changes to RA [64] and excess local IL-17 (via adenovirus-mediated gene expression vectors) exacerbates significantly CIA [20].

Many of these effects of IL-17 on synovium and bone can be antagonized *in vitro* by treatment with IL-4, IL-13 or anti-IL-17 blocking antibody. These interventions can reduce IL-17-driven production of inflammatory factors such as leukaemia inhibitory factor [61], CCL20/MIP3α [70] as well as decreasing matrix metalloproteinase (MMP) production and increasing tissue inhibitors of MMPs [67]. Some of the effects of IL-17 on articular cartilage can be attenuated by this approach *in vivo* [64], while blockade of IL-17 abrogates completely the spontaneous development of inflammatory arthritis in IL-1R antagonist-deficient mice [71], and mice lacking IL-17 are highly resistant to CIA [72].

Respiratory diseases

The importance of IL-17 to airways immunity is highlighted by the susceptibility of IL-17R knock-out mice to fatal pulmonary infections [40], which correlates with impaired neutrophil mobilization and bacterial clearance [48,73]. Similarly, the pulmonary response to local introduction of *Escherichia coli* endotoxin requires the presence of IL-17 for neutrophil accumulation in the bronchoalveolar space [47,73]. In mice and rats, there is evidence that activated lymphocytes from lung tissues can produce substantial amounts of IL-17 [47,74] and that the effect of IL-17 on the respiratory epithelium is to produce chemokines that favour a neutophilic infiltrate [47,75] and that increase neutrophil activity *in vivo* (as measured by myeloperoxidase and elastase release) [54,73].

Given these findings, it is perhaps not surprising that exaggerated IL-17 responses are implicated in the pathogenesis of inflammatory airways diseases. Human respiratory epithelial cells (and even some nonepithelial cells [34,75]), in a similar manner to murine ones, are responsive to IL-17 and can be stimulated to produce the same chemoattractant molecules [49,50,75–77], although there is now evidence supporting the notion that, under normal physiological conditions, the human bronchoalveolar space releases only very low amounts of IL-17 [78]. Severe respiratory inflammation precipitated by exposure to organic dust in humans is characterized by a marked increase in IL-17 levels in the broncheoalveolar lavage and a 50-fold increase in neutrophil recruitment to the lung [78]. Similarly, there are suggestions that in pulmonary asthma there is not only an increase in the number of IL-17-producing cells (T lymphocytes and eosinophils in broncheoalveolar lavage) in comparison to healthy controls both locally and within the circulation [34], but also higher levels of intracellular IL-17 in IL-17-producing cells than their healthy counterparts [34], although not all studies agree on this [79].

Pathologically, IL-17 is likely to exert its effects through exaggerated physiological mechanisms (highlighted above), namely synergy with other cytokines and the recruitment of neutrophils to lung tissues. Indeed, studies in allergen-sensitized mice suggest that neutrophil accumulation in the lungs following encounter with allergen is orchestrated by IL-17 transcription [80]. This latter mechanism may be critically important as neutrophilic infiltrates and neutrophil

enzymatic activity correlate with the degree of bronchial hyperreactivity in patients with asthma [81]. IL-17-induced IL-6 release may have dual pathological significance, as IL-6 promotes neutrophil elastase release [82] (elastase activity is thought to be a key mediator in the pathogenesis of chronic airway diseases [83,84] and reciprocally controls activity of neutrophil IL-6 [85]) and is one of the mechanisms by which IL-17 stimulates release of mucin by respiratory epithelial cells [86]. Furthermore, IL-6 is important in the generation of IL-17-producing cells (mouse data, see below). Also, many of the effects of IL-17 on lung tissues can be antagonized by glucorticoids [34,75], which form the mainstay of treatment for inflammatory and allergic pulmonary diseases in humans.

Despite the evidence in favour of a significant role for IL-17 in inflammatory respiratory diseases in humans, most of the evidence is circumstantial and there is no direct evidence that this cytokine is the causative mediator or the central participant (i.e. that production of IL-17 is the trigger that precipitates these pulmonary diseases) and it may be that an elevated IL-17 response is simply part of the generalized inflammatory milieu. Certainly more evidence regarding the role of the cytokine in lung pathology is required.

Allograft rejection

Rejection of transplanted tissues involves interplay between mechanisms that maintain tolerance to the graft and factors that promote rejection. While immunological factors are important for both, the process of rejection is very much an inflammatory one and, as a consequence, the production of many proinflammatory cytokines, such as IFN-γ, IL-2, IL-6 and IL-15, locally from infiltrating lymphocytes and resident cells [e.g. proximal renal tubular epithelium (PTEC)], is increased during acute renal graft rejection [87–89]. Studies in acute rat rejection models have also identified an elevation in IL-17 mRNA (in the renal allograft) and IL-17 protein (in infiltrating mononuclear cells) as early as day 2 post-transplant [90]. Similarly, IL-17 protein is elevated in human renal allografts during borderline (subclinical) rejection together with detectable IL-17 mRNA in the urinary MNC sediment of these patients; in control (non-rejecting) patients, IL-17 is not detectable in either the biopsy sample nor the urinary sediment [90]. These findings have also been described previously [91].

IL-17 induces IL-6, IL-8, MCP-1 and complement component C3 but not regulated upon activated normal T cell exposed expressed and secreted (RANTES) nor other complement components [91] by PTECs [90–92] via the src/mitogen-activated protein kinase (MAPK) pathways [92]. An additional mechanism, through synergy with CD40-ligand and proceeding via nuclear factor (NF)-κB activation, has also been described [46].

In human lung organ transplantation, IL-17 has also been reported as being elevated during acute rejection [93], while rat models have demonstrated that collagen type V-specific lymphocytes can mediate lung allograft rejection and express IL-17 locally at the site of rejection [94]. In cardiac allograft models, antagonism of the IL-17 network (via expression of an IL-17R-immunoglobin fusion protein) can reduce intragraft production of inflammatory cytokines (namely IFN-γ) and prolong graft survival [95]. This approach, however, is more successful at preventing acute, rather than chronic, vascular rejection [96] and may indicate a more important role for IL-17 in mediating early rather than late cardiac rejection. There may, in addition, be a role for IL-17 in inducing the maturation of alloreactive dendritic cells [97].

Systemic lupus erythematosis (SLE) and other conditions

IL-17 has been implicated in a variety of other chronic human diseases, largely through demonstrations that the cytokine is overexpressed in these conditions. Examples include SLE [98], psoriasis [99,100], multiple sclerosis [101], systemic sclerosis [102] and chronic inflammatory bowel disease [103]. As this evidence is largely circumstantial, the exact role of IL-17 in these conditions is unclear.

With regard to infectious agents, it is possible that IL-17 has a role to play as a virulence factor, particularly given its homology to the gene in herpesvirus saimiri [23,30], a virus that is tropic for T cells [104]. While expression of murine IL-17 gene in a vaccinia virus does increase significantly its virulence [105], IL-17 knock-out strains of herpesvirus saimiri have unaltered pathogenicity [106], therefore the role of IL-17 in viral infections is unclear. Nevertheless, certain bacterial infections, notably *Helicobacter pylori* [107], *Bacteroides fragilis* [108] and periodontitis [109] are associated with particularly high levels of IL-17.

Similarly, there are suggestions that IL-17 may be involved in tumorigenesis, as IL-17 can both promote (e.g. human cervical cancer cell lines transplanted into nude mice) [110,111] and inhibit growth (e.g. haematopoietic tumours in immunocompetent mice) [112,113] of tumours in experimental animals. Although the role of IL-17 in tumour biology is unclear and evidence remains conflicting, IL-17 can be detected in some (such as ovarian, skin and prostatic cancers) [114–116] but not all (e.g. acute myeloid leukaemia) [117] human tumours and one emerging theme is that, when present, it promotes angiogenesis [111,114], for example through the up-regulation of angiogenic factors such as CXCL1, CXCL5, CXCL6 and CXCL8 [118], thereby facilitating tumour growth and invasion. The alternative explanation is the possibility that vascular tumours may be better able to recruit activated/memory T cells, some of which will be IL-17 producers and, by virtue of being highly vascular, may portend a poorer prognosis.

In either event, there is now considerable evidence that IL-17 is involved in the aetiopathogenesis of many human

disorders. Whether IL-17 is a key causative factor or is involved in the amplification of inflammatory responses has yet to be elucidated.

Regulatory T cells

A number of cell types with immunoregulatory capacity have been described in the literature. These include IL-10-secreting Tr1 cells [119], transforming growth factor (TGF)-β-secreting Th3 cells [120], Qa-1 restricted $CD8^+$ cells [121], $CD8^+$ $CD28^-$ T cells [122], $CD8^+$ $CD122^+$ T cells [123], γδ T cell receptor (TCR) T cells [124], natural killer (NK) cells [125,126], dendritic cells [127], apoptotic neutrophils [128], $CD8^+$ $CD28^-$ cells [129–132], $CD3^+$ $CD4^-$ $CD8^-$ cells [133,134] and naturally occurring $CD4^+$ $CD25^+$ T cells [135]. Given that both human and murine knock-outs for $CD4^+$ $CD25^+$ cells develop severe autoimmune diseases [135–138], the focus of attention in the literature has been mainly on these regulatory T cells (referred to as T_{regs} in this paper).

In vitro, T_{regs} have the ability to inhibit proliferation and production of cytokines by responder ($CD4^+$ $CD25^-$ and $CD8^+$) T cells [139–141] to polyclonal stimuli, as well as to down-modulate the responses of $CD8^+$ T cells, NK cells and $CD4^+$ cells to specific antigens [139,142]. These predicates translate *in vivo* to a greater number of functions other than the maintenance of tolerance to self-components (i.e. prevention of autoimmune disease) [143] and include control of allergic diseases [144] and regulation of responses to microbial pathogens [145,146], as well as the ability to prevent transplant rejection [147] and to maintain gastrointestinal tolerance [148] and maternal tolerance to semi-allogeneic fetal antigens [149]. Indeed, donor-specific T_{regs} can prevent allograft rejection in some models of murine transplant tolerance [150–152] through a predominant effect on the indirect alloresponse [153].

Although mutations in *Foxp3*, a forkhead-winged-helix transcription factor, are responsible for the loss of T_{reg} function in both mice [137] and humans [154] and overexpression of *Foxp3* in mouse cells leads to development of a T_{reg} phenotype [154–156] and can act as a phenotypic marker [12,157], FoxP3 expression may not be an ideal marker for T_{regs} in humans [158] as FoxP3 is induced during TCR stimulation [159] (in much the same manner as CD25), and there is some debate as to whether the induced $CD4^+CD25^+FoxP3^+$ population is suppressive or anergic [159,160]. Recent evidence has also implicated the IL-7 receptor (CD127) as a possible biomarker of T_{regs} in humans as the combination of CD4 and CD25 together with low expression of CD127 identifies a group of peripheral blood T cells, which are highly suppressive in functional assays and the highest expressors of FoxP3 [161].

Regulatory T cells function in an antigen-presenting cell (APC)-independent (at least *in vitro*) [162] and antigen-non-specific manner [141,163]. However, they do respond to their cognate antigen [164–166] and, while anergic *in vitro* [139], T_{regs} can proliferate extensively in response to antigen *in vivo* [167,168]. Although the exact mechanism by which T_{regs} exert their effect is unknown, it is believed that their suppressive function is contact-dependent on the basis of transwell experiments, where suppression could be abrogated via separation of T_{regs} and effector T cells by a semi-permeable membrane [141,163,169], and demonstrations that signalling through the T cell receptor is critical to their function [170,171]. These observations are divergent with *in vivo* data showing an important role for TGF-β and IL-10 production as mediators of T_{reg} activity [172,173], and do not exclude the possibility that T_{reg} function may involve soluble mediators acting at very short distances from the cell or bound to the cell surface [174,175]. Despite suggestions that T_{regs} influence cells by direct contact, these cell-to-cell interactions are poorly mapped. Indeed, there is evidence that some of the activity of T_{regs} progresses through intermediaries, such as NK T [176] and mast cells [177], and that their effect on target T effector cells includes an arrest in cell cycle progression caused by uncoupling of IL-2 signalling [178,179]. Recently, a role for IFN-γ in the regulatory function of T_{regs} has also been proposed based on the up-regulation of IFN-γ mRNA in alloantigen-reactive T_{regs} *in vivo* hours after encounter with antigen and failure of skin graft tolerance in the presence of IFN-γ neutralization [180].

T_{regs} express constitutively CTLA-4 (cytotoxic T lymphocyte antigen-4) and there are suggestions that signalling through this pathway may be important, for their function as antibodies (or Fab) to CTLA-4 can inhibit T_{reg}-mediated suppression [181–183]. However, CTLA-4 is also inducibly expressed on $CD4^+$ $CD25^-$ cells [184] and therefore these observations may be the result of CTLA-4 antibodies acting on effector rather than regulatory cells and could explain why the initial reports have been so difficult to reproduce in mice [141] and humans [140,140,185]. For a review on CTLA-4 in T_{reg} biology, please see Sansom *et al.* [186].

Development and persistence of T_{regs} and IL-17-producing cells

T_{regs}

It is now clear that $CD4^+$ $CD25^+$ T_{regs} can be derived from two sources, namely those developing within the thymus (whose contribution may therefore diminish with age) and those generated in the periphery. Thymically derived T_{regs} are thought to originate at the transition between the double-positive and single-positive stages following encounter between thymocytes that bear high affinity TCRs for self-peptide with their cognate antigens [165,187], but this assertion has been challenged recently by observations that thymic commitment to a T_{reg} phenotype may occur at an earlier developmental stage [188]. The autoreactive T_{reg} repertoire may be entrained by deletion following interaction

with endogenous superantigens and APCs of both thymic and bone marrow origin [164], but the peripheral T_{reg} repertoire retains a higher frequency of autospecific than alloreactive cells [164]. How T_{reg} precursors commit to a T_{reg} lineage in the thymus is unknown, but recent evidence points (in mice) to an interaction with a gene locus intimately linked with the MHC [189] (characterization of this locus and the genes involved is awaited) and may involve an important role for IL-2 signalling [190] and/or CD28 engagement [191].

Adults rendered temporarily lymphopenic have a greater propensity to develop autoimmune diseases [192,193]. Although this may reflect loss of a significant proportion of thymically derived T_{regs} (which are hard to regenerate given age-related thymic atrophy [194,195]) leading to loss of self-tolerance, one cannot ignore the fact that not everyone who is made lymphopenic develops autoimmune disease. One possibility is that the important determinant for maintenance of tolerance to self-components may be the relative frequency of effector cells to T_{regs}, as some chemotherapy agents have an equal effect on both [196]. However, depletion of $CD25^+$ T cells from mice [197] or the adoptive transfer of naive T cells into lymphopenic recipients [198] is not sufficient for the development of autoimmune phenomena. The second possibility is that T_{regs} are generated in the periphery, an attractive notion that is supported by data showing reconstitution of the $CD4^+$ $CD25^+$ T_{reg} population through conversion of $CD4^+$ $CD25^-$ T cells [199,200]. Although this is not a robust phenomenon [12,157], there are suggestions that discrepancies may be the result of competition between $CD4^+$ $CD25^-$ and $CD4^+$ $CD25^+$ T cells (i.e. that the rate of conversion is related to the relative frequency of the two cell types) [201] and that the number of T_{regs} may be linked to the availability of IL-2 (and, by inference, the number of IL-2-producing effector cells) [202]. Studies of human T_{reg} populations have shown these populations to be highly proliferative and senescent *in vivo* with very short telomeres [203], which is consistent with their memory/$CD45RO^+$ phenotype [140]. Their susceptibility to apoptosis and short telomeres (with low telomerase activity) means it is unlikely that they are capable of self-renewal; the more likely explanation is that T_{regs} are generated in the periphery.

Other studies have corroborated the importance of IL-2 for the development of T_{regs} [204] and are summarized in the review by Malek *et al.* [205]. Indeed, in an animal model of graft-*versus*-host disease (GvHD) where autoreactive T cells from donors deficient in T_{regs} (DO11·10 $Rag^{-/-}$ animals) are infused into athymic antigen-expressing lymphopenic recipients (sOVA transgenic thymectomized, lethally irradiated mice reconstituted with $Rag^{-/-}$ bone marrow), development and recovery from disease is reliant upon generation of antigen-specific effector cells followed by *de novo* generation of peripheral T_{regs} in a concomitant, IL-2-dependent manner [206]. The added implication of these findings is that both functional effector cells and T_{regs} can develop in parallel from the same population of T cells in response to a single antigen in the periphery.

The mechanism(s) by which T_{regs} are generated in the periphery are unknown. However, there are indications that, in the same manner as with Th1/Th2 cell polarization, the antigenic stimulus (linked to the amount of antigen present as well as the strength of interaction) may determine the commitment to a T_{reg} phenotype (low doses/weaker stimulus result in more T_{reg} generation) [207,208]. The mode of antigen encounter, namely through (immature or sub-optimally activated) dendritic cell presentation, also seems important for the conversion of naive T cells to a T_{reg} phenotype [208,209], as may interactions with anti-inflammatory molecules such as thrombospondin-1 [210]. The demonstration that TGF-β-deficient mice have reduced numbers of peripheral [211,212] but not thymic T_{regs} [213] and that TGF-β assists the conversion of naive $CD4^+$ $CD25^-$ T cells into T_{regs} both *in vivo* [209] and *in vitro* [214,215] argue in favour of the importance of this cytokine in the generation and maintenance of the peripheral T_{reg} pool. The potential importance of these observations to human disorders will be discussed in the context of Th17 development below.

IL-17-producing cells

In mice, a discrete population of $CD4^+$ helper T cells has been described as the predominant source of IL-17. These cells have been named Th17 cells. The initial basis of this nomenclature is the dichotomous effects of IL-12 and IL-23 (both members of the same family of IL-12 cytokines, sharing a common IL-12p40 subunit but differing second subunits, IL-12p35 and IL-12p19, respectively [216]) on the cytokine profile of CD4 cells. While IL-12 (signalling through signal transducer and activator of transcription, STAT-4) had been known to allow lineage commitment towards a Th1 phenotype producing IFN-γ as its signature cytokine (via the transcription factor *T-bet* [8,9]) [5,7,217] and inflammatory diseases had been viewed along the lines of a Th1/Th2 paradigm (e.g. IL-12p40 neutralization in mice ameliorates inflammatory diseases [218,219]), mice deficient in IFN-γ or IFN-γ signalling remained, paradoxically, susceptible to development of EAE and CIA [15,16]. Furthermore, IL-12p40 (lacking both IL-12 and IL-23) and IL-12p19 (lacking IL-23)-deficient mice were protected against EAE and CIA whereas IL-12p35 (IL-12)-deficient strains remained susceptible [220,221] suggesting that IL-23 rather than IL-12 was important in mediating the pathogenesis of these conditions. Shortly after these observations, it was reported that IL-23 stimulates production of IL-17 from a population of memory (but not naive) $CD4^+$ T cells in a manner that does not exhibit elevation of IFN-γ [21,222] and that IL-17 is linked to the inflammation seen in CIA and EAE [20,21,72]. Further evidence that IL-17 was derived in mice from a discrete population of Th cells that were distinct

from the Th1 lineage, termed Th17 cells, were provided by publications showing resistance of Th1 and Th2 cells *in vitro* to proliferation or production of IL-17 following stimulation with IL-23 and that development of IL-17-producing cells was inhibited by the presence of IFN-γ and/or IL-4 in the culture supernatant [223,224]. It has been proposed that production of IL-12 has greater importance for systemic responses and immunity to intracellular pathogens [225] while IL-23, produced from activated human macrophages and dendritic cells (DC) [226], has a more important role for mediating mucosal immune pathology through the promotion of Th1 and Th17 cytokine profiles, respectively [227].

It is now known that IL-6 [17–19] (see below), and not IL-23 [17,18], is critical for the induction of Th17 lineage commitment (which is supported by the fact that the IL-23 receptor is expressed exclusively on activated and memory T cells [222,228]), while IL-23 seems to be important for the selective expansion of these cells and production of IL-17 [229]. Indeed, other cytokines including IL-2, IL-15, IL-18 and IL-21 can also stimulate IL-17 production from (activated) human T cells and peripheral blood mononuclear cells (PBMC) [226,229], while IL-12 potently inhibits it [229]. Development of Th17 cells is dependent upon correct co-stimulation (ICOS and CD28 [224]) and the absence of IFN-γ and IL-4, both of which are inhibitory [223]. Furthermore, Th17 lineage differentiation can be inhibited by the Th1-specific transcription factor *T-bet* in the context of IL-4 blockade [230] and is characterized by the expression of the orphan nuclear receptor *RORγT* [13]. Because IL-12 specifies lineage commitment to Th1 and has a stimulatory effect on IFN-γ secretion by Th1 cells [229], IL-12 may play a critically important role as a regulator of the balance between Th1 and Th17 responses. This assertion is supported by *in vivo* mouse data in which IL-23 and IL-12 had divergent pro- and anti-inflammatory roles in a model of collagen-induced arthritis [221]. It is important to state that, despite these observations, the description of discrete Th17 cells is mouse-specific and to date no committed Th17 cells have been demonstrated in humans.

Figure 2 shows the cytokine network that is thought to be important in the development and expansion of Th17 cells and the dichotomous Th1/Th17 cytokine profile engendered by these cytokines. Three recent papers have shed some light on the mechanisms by which naive precursor T cells commit to a Th17 phenotype in mice [17–19]. The first, a publication by Veldhoen *et al.*, showed that naive $CD4^+$ T cells could be skewed towards a Th17 phenotype in the presence of dendritic cells and T_{regs} in an inflammatory milieu (lipopolysaccharide stimulation) [18]. Absence of T_{regs} leads to Th1 differentiation, presumably through interaction of naive T cells with DCs producing IL-12. In the presence of T_{regs} and DC, the important drivers of Th17 differentiation were T_{reg}-derived TGF-β and DC-derived IL-6, although both TNF-α and IL-1β (both DC-derived) also augmented the commitment to Th17. In this series of experiments, the IL-17-

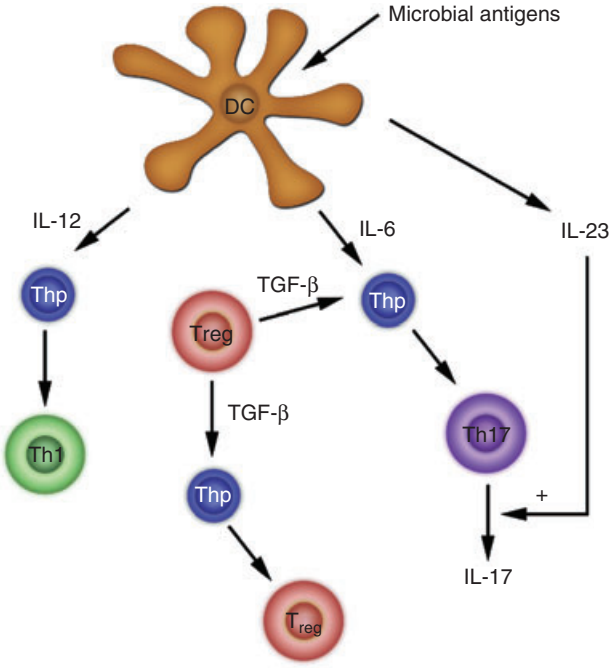

Fig. 2. Model of mouse helper T cell (Th) commitment to Th1, Th17 and T regulatory cell (T_{reg}) phenotypes following encounter with antigen. Production of transforming growth factor (TGF)-β by naturally occurring T_{regs} leads to lineage commitment of precursor helper T cells (Thp) towards T_{reg} phenotypes. Stimulation of dendritic cells (DC) by microbial antigens causes production of interleukin (IL)-6, IL-23 and/or IL-12. Predominant production of IL-12 promotes commitment of Thp to a Th1 phenotype while IL-6, in combination with T_{reg}-derived TGF-β promotes skewing of Thp towards a Th17 phenotype. IL-23 produced by DCs causes proliferation and cytokine production by Th17 cells.

producing cells did not express *T-bet* or *GATA-3* and addition of IL-12 and IL-4 or IL-18 inhibited Th17 development, but the most important determinant of commitment to a Th17 lineage was the presence of TGF-β, without requirement for cell-to-cell contact. These data were corroborated by Bettelli *et al.*, who demonstrated using cells from a *Foxp3*-GFP knock-in mouse strain that differentiation towards T_{reg} and Th17 phenotypes were mutually exclusive – activation of naive precursor cells using anti-CD3 in the presence of TGF-β lead to production of green fluorescent protein $(GFP)^+$ cells (i.e. T_{regs}) as per previous observations, but activation in the presence of IL-6 in addition to TGF-β completely abrogated this and led to development of Th17 cells (that were GFP^-). The differentiated cells were, respectively, functionally suppressive and inflammatory and development of the Th17 phenotype was independent of IL-23 [17]. The third paper, by Mangan *et al.* published simultaneously [19], showed that addition of TGF-β to naive $CD4^+$ T cells resulted in the development of Th17 cells, an effect which was augmented in the presence of neutralizing antibodies to Th1 and Th2 polarizing cytokines (IL-4 and IFN-γ) or the use of CD4 cells from IFN-γ

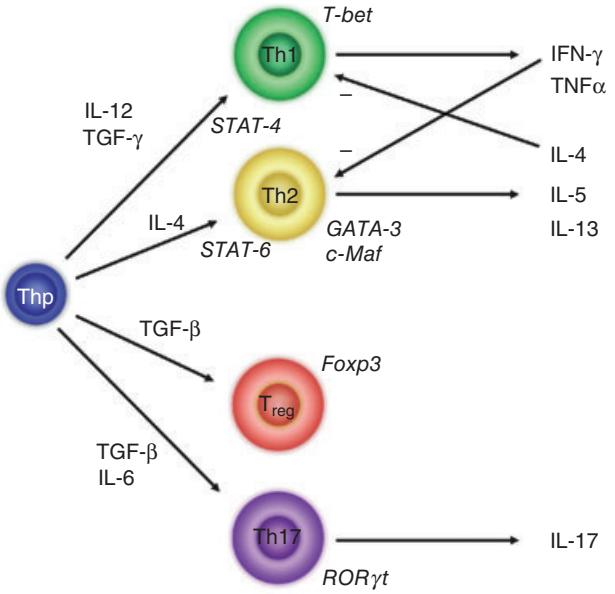

Fig. 3. T helper cell commitment towards specific lineages in mice. T helper cell precursors (Thp) can be skewed towards mutually exclusive Th1, Th2, Th17 and T regulatory cell (T_{reg}) phenotypes on the basis of the cytokine environment. Presence of interleukin (IL)-12 promotes skewing towards Th1 commitment by signalling through signal transduction and activator of transcription (STAT)-4. Th1 cells are characterized by expression of *T-bet* and produce interferon-γ and tumour necrosis factor-α. Th2 cell commitment is promoted by IL-4 via STAT-6 signalling. Th2 committed cells are characterized by expression of *GATA-3*. Development of T_{reg} and Th17 phenotypes both require the presence of transforming growth factor-β but the presence of IL-6 preferentially skews the response towards a Th17 phenotype. T_{regs} are characterized in mice by expression of *Foxp3*.

deficient animals [19]. Furthermore, they demonstrated that TGF-β up-regulated expression of the IL-23 receptor (which may explain the responsiveness of the Th17 population to IL-23). As before, supplementation of the culture conditions with exogenous IL-6 resulted in loss of all *Foxp3*$^+$ cells, while blockade of IL-6 enhanced T_{reg} development. Again, these findings point to mutually exclusive pathways for Th17 and T_{reg} development based on the availability of TGF-β and IL-6. A schematic for naive T cell commitment is represented in Fig. 3. It should be noted, once again, that these data are derived from mice and whether this pathway exists in humans has not been determined.

A model for the regulation of T cell polarity in humans

Although the majority of the data concerning commitment to T cell lineages has been derived from mice, there is a clear difference in lineage differentiation based on the mode of stimulation and the cytokine milieu. The presence of discrete IL-17-producing cells in humans has yet to be confirmed; however, IL-17 is likely to be an important cytokine in the mediation of many inflammatory diseases and allograft rejection in humans. As such, one can propose a hypothesis with regard to the pathogenesis of autoimmune/inflammatory diseases and allograft rejection in humans that is based on extrapolations of the mouse data on the assumption that human cells exhibit discrete IL-17-producing populations and can be skewed towards different lineages.

Specifically, the observation that many inflammatory or autoimmune diseases present clinically as episodes of inflammation (flares), with periods of quiescence in between these episodes, argues for the presence of intervening periods of 'equilibrium' where the immune system displays tolerance to self-components (i.e. that proinflammatory components are 'regulated'). During acute flares, a state of 'disequilibrium' ensues in which immune responses against self-components are dysregulated. The possibility arises that during these episodes Th cell phenotypes become skewed towards proinflamatory lineages (Th17 and Th1) (or that there is enhanced survival of these lineages) and away from anti-inflammatory phenotypes (T_{reg}) on the basis of the local cytokine environment and DC populations. The mechanism of such a change could either be loss of skewing towards T_{reg} phenotypes (with default towards Th1/Th17) or a primary shift towards the proinflammatory pathways. The central cytokine in this pathway, on the basis of the mouse data, may be IL-6, which is known to be elevated in most inflammatory conditions. Presumably, the balance is redressed during the recovery from flares and the equilibrium re-establishes itself. The part played by anti-inflammatory/immunosuppressive drugs in the resolution phase and the effects of these drugs on specific subsets of T cells is not known at present.

Conclusions

Interleukin 17 is a pleiotropic cytokine with multiple proinflammatory functions that is likely to be involved in either the causation or progression of inflammatory diseases and transplant rejection in humans. Regulatory T cells are an anti-inflammatory lineage of T cells that are derived naturally from the thymus and also generated in the periphery on the basis of the local environment. It is possible that acute flares of autoimmune diseases or acute episodes of transplant rejection may be explained by a change in the relative dominance of these pathways in the periphery, either through preferential differentiation towards proinflammatory lineages or enhanced survival of these phenotypes. A change in the local polarizing conditions may be important in the skewing of these responses.

References

1 Starr TK, Jameson SC, Hogquist KA. Positive and negative selection of T cells. Annu Rev Immunol 2003; **21**:139–76.
2 Zhang J, Markovic-Plese S, Lacet B, Raus J, Weiner HL, Hafler DA. Increased frequency of interleukin 2-responsive T cells specific for

myelin basic protein and proteolipid protein in peripheral blood and cerebrospinal fluid of patients with multiple sclerosis. J Exp Med 1994; **179**:973–84.
3 Markovic-Plese S, Fukaura H, Zhang J *et al.* T cell recognition of immunodominant and cryptic proteolipid protein epitopes in humans. J Immunol 1995; **155**:982–92.
4 Fossiez F, Djossou O, Chomarat P *et al.* T cell interleukin-17 induces stromal cells to produce proinflammatory and hematopoietic cytokines. J Exp Med 1996; **183**:2593–603.
5 Constant SL, Bottomly K. Induction of Th1 and Th2 CD4+ T cell responses: the alternative approaches. Annu Rev Immunol 1997; **15**:297–322.
6 Nelms K, Keegan AD, Zamorano J, Ryan JJ, Paul WE. The IL-4 receptor: signaling mechanisms and biologic functions. Annu Rev Immunol 1999; **17**:701–38.
7 Gately MK, Renzetti LM, Magram J *et al.* The interleukin-12/interleukin-12-receptor system: role in normal and pathologic immune responses. Annu Rev Immunol 1998; **16**:495–521.
8 Szabo SJ, Kim ST, Costa GL, Zhang X, Fathman CG, Glimcher LH. A novel transcription factor, T-bet, directs Th1 lineage commitment. Cell 2000; **100**:655–69.
9 Szabo SJ, Sullivan BM, Stemmann C, Satoskar AR, Sleckman BP, Glimcher LH. Distinct effects of T-bet in TH1 lineage commitment and IFN-gamma production in CD4 and CD8 T cells. Science 2002; **295**:338–42.
10 Zheng W, Flavell RA. The transcription factor GATA-3 is necessary and sufficient for Th2 cytokine gene expression in CD4 T cells. Cell 1997; **89**:587–96.
11 Fontenot JD, Gavin MA, Rudensky AY. Foxp3 programs the development and function of CD4+CD25+ regulatory T cells. Nature Immunol 2003; **4**:330–6.
12 Wan YY, Flavell RA. Identifying Foxp3-expressing suppressor T cells with a bicistronic reporter. Proc Natl Acad Sci USA 2005; **102**:5126–31.
13 Ivanov II, McKenzie BS, Zhou L *et al.* The orphan nuclear receptor RORgammat directs the differentiation program of proinflammatory IL-17+ T helper cells. Cell 2006; **126**:1121–33.
14 Liblau RS, Singer SM, McDevitt HO. Th1 and Th2 CD4+ T cells in the pathogenesis of organ-specific autoimmune diseases. Immunol Today 1995; **16**:34–8.
15 Matthys P, Vermeire K, Mitera T, Heremans H, Huang S, Billiau A. Anti-IL-12 antibody prevents the development and progression of collagen-induced arthritis in IFN-gamma receptor-deficient mice. Eur J Immunol 1998; **28**:2143–51.
16 Ferber IA, Brocke S, Taylor-Edwards C *et al.* Mice with a disrupted IFN-gamma gene are susceptible to the induction of experimental autoimmune encephalomyelitis (EAE). J Immunol 1996; **156**:5–7.
17 Bettelli E, Carrier Y, Gao W *et al.* Reciprocal developmental pathways for the generation of pathogenic effector TH17 and regulatory T cells. Nature 2006; **441**:235–8.
18 Veldhoen M, Hocking RJ, Atkins CJ, Locksley RM, Stockinger B. TGFbeta in the context of an inflammatory cytokine milieu supports de novo differentiation of IL-17-producing T cells. Immunity 2006; **24**:179–89.
19 Mangan PR, Harrington LE, O'Quinn DB *et al.* Transforming growth factor-beta induces development of the T (H) 17 lineage. Nature 2006; **441**:231–4.
20 Lubberts E, Joosten LA, van de Loo FA, Schwarzenberger P, Kolls J, van den Berg WB. Overexpression of IL-17 in the knee joint of collagen type II immunized mice promotes collagen arthritis and aggravates joint destruction. Inflamm Res 2002; **51**:102–4.
21 Langrish CL, Chen Y, Blumenschein WM *et al.* IL-23 drives a pathogenic T cell population that induces autoimmune inflammation. J Exp Med 2005; **201**:233–40.
22 Weaver CT, Harrington LE, Mangan PR, Gavrieli M, Murphy KM. Th17: an effector CD4 T cell lineage with regulatory T cell ties. Immunity 2006; **24**:677–88.
23 Rouvier E, Luciani MF, Mattei MG, Denizot F, Golstein P. CTLA-8, cloned from an activated T cell, bearing AU-rich messenger RNA instability sequences, and homologous to a herpesvirus saimiri gene. J Immunol 1993; **150**:5445–56.
24 Yao Z, Timour M, Painter S, Fanslow W, Spriggs M. Complete nucleotide sequence of the mouse CTLA8 gene. Gene 1996; **168**:223–5.
25 Li H, Chen J, Huang A *et al.* Cloning and characterization of IL-17B and IL-17C, two new members of the IL-17 cytokine family. Proc Natl Acad Sci USA 2000; **97**:773–8.
26 Starnes T, Broxmeyer HE, Robertson MJ, Hromas R. Cutting edge: IL-17D, a novel member of the IL-17 family, stimulates cytokine production and inhibits hemopoiesis. J Immunol 2002; **169**:642–6.
27 Lee J, Ho WH, Maruoka M *et al.* IL-17E, a novel proinflammatory ligand for the IL-17 receptor homolog IL-17Rh1. J Biol Chem 2001; **276**:1660–4.
28 Starnes T, Robertson MJ, Sledge G *et al.* Cutting edge: IL-17F, a novel cytokine selectively expressed in activated T cells and monocytes, regulates angiogenesis and endothelial cell cytokine production. J Immunol 2001; **167**:4137–40.
29 Shi Y, Ullrich SJ, Zhang J *et al.* A novel cytokine receptor-ligand pair. Identification, molecular characterization, and *in vivo* immunomodulatory activity. J Biol Chem 2000; **275**:19167–76.
30 Yao Z, Fanslow WC, Seldin MF *et al.* Herpesvirus saimiri encodes a new cytokine, IL-17, which binds to a novel cytokine receptor. Immunity 1995; **3**:811–21.
31 Ferretti S, Bonneau O, Dubois GR, Jones CE, Trifilieff A. IL-17, produced by lymphocytes and neutrophils, is necessary for lipopolysaccharide-induced airway neutrophilia: IL-15 as a possible trigger. J Immunol 2003; **170**:2106–12.
32 Lockhart E, Green AM, Flynn JL. IL-17 production is dominated by gammadelta T cells rather than CD4 T cells during *Mycobacterium tuberculosis* infection. J Immunol 2006; **177**:4662–9.
33 Shin HC, Benbernou N, Esnault S, Guenounou M. Expression of IL-17 in human memory CD45RO+ T lymphocytes and its regulation by protein kinase A pathway. Cytokine 1999; **11**:257–66.
34 Molet S, Hamid Q, Davoine F *et al.* IL-17 is increased in asthmatic airways and induces human bronchial fibroblasts to produce cytokines. J Allergy Clin Immunol 2001; **108**:430–8.
35 Zhou Q, Desta T, Fenton M, Graves DT, Amar S. Cytokine profiling of macrophages exposed to *Porphyromonas gingivalis*, its lipopolysaccharide, or its FimA protein. Infect Immun 2005; **73**:935–43.
36 Yao Z, Spriggs MK, Derry JM *et al.* Molecular characterization of the human interleukin (IL)-17 receptor. Cytokine 1997; **9**:794–800.
37 Silva WA Jr, Covas DT, Panepucci RA *et al.* The profile of gene expression of human marrow mesenchymal stem cells. Stem Cells 2003; **21**:661–9.
38 Haudenschild D, Moseley T, Rose L, Reddi AH. Soluble and transmembrane isoforms of novel interleukin-17 receptor-like protein by RNA splicing and expression in prostate cancer. J Biol Chem 2002; **277**:4309–16.

39 Moseley TA, Haudenschild DR, Rose L, Reddi AH. Interleukin-17 family and IL-17 receptors. Cytokine Growth Factor Rev 2003; **14**:155–74.

40 Ye P, Rodriguez FH, Kanaly S *et al.* Requirement of interleukin 17 receptor signaling for lung CXC chemokine and granulocyte colony-stimulating factor expression, neutrophil recruitment, and host defense. J Exp Med 2001; **194**:519–27.

41 Nakae S, Komiyama Y, Nambu A *et al.* Antigen-specific T cell sensitization is impaired in IL-17-deficient mice, causing suppression of allergic cellular and humoral responses. Immunity 2002; **17**:375–87.

42 Attur MG, Patel RN, Abramson SB, Amin AR. Interleukin-17 up-regulation of nitric oxide production in human osteoarthritis cartilage. Arthritis Rheum 1997; **40**:1050–3.

43 Ruddy MJ, Wong GC, Liu XK *et al.* Functional cooperation between interleukin-17 and tumor necrosis factor-alpha is mediated by CCAAT/enhancer-binding protein family members. J Biol Chem 2004; **279**:2559–67.

44 Albanesi C, Cavani A, Girolomoni G. IL-17 is produced by nickel-specific T lymphocytes and regulates ICAM-1 expression and chemokine production in human keratinocytes: synergistic or antagonist effects with IFN-gamma and TNF-alpha. J Immunol 1999; **162**:494–502.

45 Witowski J, Pawlaczyk K, Breborowicz A *et al.* IL-17 stimulates intraperitoneal neutrophil infiltration through the release of GRO alpha chemokine from mesothelial cells. J Immunol 2000; **165**:5814–21.

46 Woltman AMHS, Boonstra JG, Gobin SJ, van Daha MR, van Kooten C. Interleukin-17 and CD40-ligand synergistically enhance cytokine and chemokine production by renal epithelial cells. J Am Soc Nephrol 2000; **11**:2044–55.

47 Miyamoto M, Prause O, Sjostrand M, Laan M, Lotvall J, Linden A. Endogenous IL-17 as a mediator of neutrophil recruitment caused by endotoxin exposure in mouse airways. J Immunol 2003; **170**:4665–72.

48 Ye P, Garvey PB, Zhang P *et al.* Interleukin-17 and lung host defense against *Klebsiella pneumoniae* infection. Am J Respir Cell Mol Biol 2001; **25**:335–40.

49 Laan M, Lotvall J, Chung KF, Linden A. IL-17-induced cytokine release in human bronchial epithelial cells *in vitro*: role of mitogen-activated protein (MAP) kinases. Br J Pharmacol 2001; **133**:200–6.

50 Jones CE, Chan K. Interleukin-17 stimulates the expression of interleukin-8, growth-related oncogene-alpha, and granulocyte-colony-stimulating factor by human airway epithelial cells. Am J Respir Cell Mol Biol 2002; **26**:748–53.

51 Yao Z, Painter SL, Fanslow WC *et al.* Human IL-17: a novel cytokine derived from T cells. J Immunol 1995; **155**:5483–6.

52 McGeachy MJ, Anderton SM. Cytokines in the induction and resolution of experimental autoimmune encephalomyelitis. Cytokine 2005; **32**:81–4.

53 Lubberts E, Koenders MI, van den Berg WB. The role of T-cell interleukin-17 in conducting destructive arthritis: lessons from animal models. Arthritis Res Ther 2005; **7**:29–37.

54 Schwarzenberger P, La RV, Miller A *et al.* IL-17 stimulates granulopoiesis in mice: use of an alternate, novel gene therapy-derived method for *in vivo* evaluation of cytokines. J Immunol 1998; **161**:6383–9.

55 Pan G, French D, Mao W *et al.* Forced expression of murine IL-17E induces growth retardation, jaundice, a Th2-biased response, and multiorgan inflammation in mice. J Immunol 2001; **167**:6559–67.

56 Miossec P. Interleukin-17 in rheumatoid arthritis: if T cells were to contribute to inflammation and destruction through synergy. Arthritis Rheum 2003; **48**:594–601.

57 Kotake S, Udagawa N, Takahashi N *et al.* IL-17 in synovial fluids from patients with rheumatoid arthritis is a potent stimulator of osteoclastogenesis. J Clin Invest 1999; **103**:1345–52.

58 Hwang SY, Kim HY. Expression of IL-17 homologs and their receptors in the synovial cells of rheumatoid arthritis patients. Mol Cells 2005; **19**:180–4.

59 Chabaud M, Durand JM, Buchs N *et al.* Human interleukin-17: a T cell-derived proinflammatory cytokine produced by the rheumatoid synovium. Arthritis Rheum 1999; **42**:963–70.

60 Chabaud M, Lubberts E, Joosten L, van Den BW, Miossec P. IL-17 derived from juxta-articular bone and synovium contributes to joint degradation in rheumatoid arthritis. Arthritis Res 2001; **3**:168–77.

61 Chabaud M, Fossiez F, Taupin JL, Miossec P. Enhancing effect of IL-17 on IL-1-induced IL-6 and leukemia inhibitory factor production by rheumatoid arthritis synoviocytes and its regulation by Th2 cytokines. J Immunol 1998; **161**:409–14.

62 Katz Y, Nadiv O, Beer Y. Interleukin-17 enhances tumor necrosis factor alpha-induced synthesis of interleukins 1,6, and 8 in skin and synovial fibroblasts: a possible role as a 'fine–tuning cytokine' in inflammation processes. Arthritis Rheum 2001; **44**:2176–84.

63 LeGrand A, Fermor B, Fink C *et al.* Interleukin-1, tumor necrosis factor alpha, and interleukin-17 synergistically up-regulate nitric oxide and prostaglandin E2 production in explants of human osteoarthritic knee menisci. Arthritis Rheum 2001; **44**:2078–83.

64 Lubberts E, Joosten LA, van de Loo FA, van den Gersselaar LA, van den Berg WB. Reduction of interleukin-17-induced inhibition of chondrocyte proteoglycan synthesis in intact murine articular cartilage by interleukin-4. Arthritis Rheum 2000; **43**:1300–6.

65 Jovanovic DV, Di Battista JA, Martel-Pelletier J *et al.* Modulation of TIMP-1 synthesis by antiinflammatory cytokines and prostaglandin E2 in interleukin 17 stimulated human monocytes/macrophages. J Rheumatol 2001; **28**:712–8.

66 Jovanovic DV, Martel-Pelletier J, Di Battista JA *et al.* Stimulation of 92-kd gelatinase (matrix metalloproteinase 9) production by interleukin-17 in human monocyte/macrophages: a possible role in rheumatoid arthritis. Arthritis Rheum 2000; **43**:1134–44.

67 Chabaud M, Garnero P, Dayer JM, Guerne PA, Fossiez F, Miossec P. Contribution of interleukin 17 to synovium matrix destruction in rheumatoid arthritis. Cytokine 2000; **12**:1092–9.

68 Chevrel G, Garnero P, Miossec P. Addition of interleukin 1 (IL1) and IL17 soluble receptors to a tumour necrosis factor alpha soluble receptor more effectively reduces the production of IL6 and macrophage inhibitory protein-3alpha and increases that of collagen in an *in vitro* model of rheumatoid synoviocyte activation. Ann Rheum Dis 2002; **61**:730–3.

69 Van bezooijen RL, Farih-Sips HC, Papapoulos SE, Lowik CW. Interleukin-17: a new bone acting cytokine *in vitro*. J Bone Miner Res 1999; **14**:1513–21.

70 Chabaud M, Page G, Miossec P. Enhancing effect of IL-1, IL-17, and TNF-alpha on macrophage inflammatory protein-3alpha production in rheumatoid arthritis: regulation by soluble receptors and Th2 cytokines. J Immunol 2001; **167**:6015–20.

71 Nakae S, Saijo S, Horai R, Sudo K, Mori S, Iwakura Y. IL-17 production from activated T cells is required for the spontaneous development of destructive arthritis in mice deficient in IL-1 receptor antagonist. Proc Natl Acad Sci USA 2003; **100**:5986–90.

72 Nakae S, Nambu A, Sudo K, Iwakura Y. Suppression of immune induction of collagen-induced arthritis in IL-17-deficient mice. J Immunol 2003; **171**:6173–7.

73 Hoshino H, Laan M, Sjostrand M, Lotvall J, Skoogh BE, Linden A. Increased elastase and myeloperoxidase activity associated with neutrophil recruitment by IL-17 in airways *in vivo*. J Allergy Clin Immunol 2000; **105**:143–9.

74 Happel KI, Zheng M, Young E *et al.* Cutting edge: roles of Toll-like receptor 4 and IL-23 in IL-17 expression in response to *Klebsiella pneumoniae* infection. J Immunol 2003; **170**:4432–6.

75 Laan M, Cui ZH, Hoshino H *et al.* Neutrophil recruitment by human IL-17 via C-X-C chemokine release in the airways. J Immunol 1999; **162**:2347–52.

76 Kawaguchi M, Kokubu F, Kuga H *et al.* Modulation of bronchial epithelial cells by IL-17. J Allergy Clin Immunol 2001; **108**:804–9.

77 Rahman MS, Yamasaki A, Yang J, Shan L, Halayko AJ, Gounni AS. IL-17A induces eotaxin-1/CC chemokine ligand 11 expression in human airway smooth muscle cells: role of MAPK (Erk1/2, JNK, P38) pathways. J Immunol 2006; **177**:4064–71.

78 Laan M, Palmberg L, Larsson K, Linden A. Free, soluble interleukin-17 protein during severe inflammation in human airways. Eur Respir J 2002; **19**:534–7.

79 Barczyk A, Pierzchala W, Sozanska E. Interleukin-17 in sputum correlates with airway hyperresponsiveness to methacholine. Respir Med 2003; **97**:726–33.

80 Hellings PW, Kasran A, Liu Z *et al.* Interleukin-17 orchestrates the granulocyte influx into airways after allergen inhalation in a mouse model of allergic asthma. Am J Respir Cell Mol Biol 2003; **28**:42–50.

81 Jatakanon A, Uasuf C, Maziak W, Lim S, Chung KF, Barnes PJ. Neutrophilic inflammation in severe persistent asthma. Am J Respir Crit Care Med 1999; **160**:1532–9.

82 Bank U, Reinhold D, Kunz D *et al.* Effects of interleukin-6 (IL-6) and transforming growth factor-beta (TGF-beta) on neutrophil elastase release. Inflammation 1995; **19**:83–99.

83 Skold CM, Liu X, Umino T *et al.* Human neutrophil elastase augments fibroblast-mediated contraction of released collagen gels. Am J Respir Crit Care Med 1999; **159**:1138–46.

84 Suzuki T, Wang W, Lin JT, Shirato K, Mitsuhashi H, Inoue H. Aerosolized human neutrophil elastase induces airway constriction and hyperresponsiveness with protection by intravenous pretreatment with half-length secretory leukoprotease inhibitor. Am J Respir Crit Care Med 1996; **153**:1405–11.

85 Bank U, Kupper B, Reinhold D, Hoffmann T, Ansorge S. Evidence for a crucial role of neutrophil-derived serine proteases in the inactivation of interleukin-6 at sites of inflammation. FEBS Lett 1999; **461**:235–40.

86 Chen Y, Thai P, Zhao YH, Ho YS, DeSouza MM, Wu R. Stimulation of airway mucin gene expression by interleukin (IL)-17 through IL-6 paracrine/autocrine loop. J Biol Chem 2003; **278**:17036–43.

87 Merville P, Pouteil-Noble C, Wijdenes J, Potaux L, Touraine JL, Banchereau J. Detection of single cells secreting IFN-gamma, IL-6, and IL-10 in irreversibly rejected human kidney allografts, and their modulation by IL-2 and IL-4. Transplantation 1993; **55**:639–46.

88 Merville P, Pouteil-Noble C, Wijdenes J, Potaux L, Touraine JL, Banchereau J. Cells infiltrating rejected human kidney allografts secrete IFN-gamma, IL-6, and IL-10, and are modulated by IL-2 and IL-4. Transplant Proc 1993; **25**:111–13.

89 Pavlakis M, Strehlau J, Lipman M, Shapiro M, Maslinski W, Strom TB. Intragraft IL-15 transcripts are increased in human renal allograft rejection. Transplantation 1996; **62**:543–5.

90 Loong CC, Hsieh HG, Lui WY, Chen A, Lin CY. Evidence for the early involvement of interleukin 17 in human and experimental renal allograft rejection. J Pathol 2002; **197**:322–32.

91 van KC, Boonstra JG, Paape ME *et al.* Interleukin-17 activates human renal epithelial cells *in vitro* and is expressed during renal allograft rejection. J Am Soc Nephrol 1998; **9**:1526–34.

92 Hsieh HG, Loong CC, Lin CY. Interleukin-17 induces src/MAPK cascades activation in human renal epithelial cells. Cytokine 2002; **19**:159–74.

93 Vanaudenaerde BM, Dupont LJ, Wuyts WA *et al.* The role of interleukin-17 during acute rejection after lung transplantation. Eur Respir J 2006; **27**:779–87.

94 Yoshida S, Haque A, Mizobuchi T *et al.* Anti-type V collagen lymphocytes that express IL-17 and IL-23 induce rejection pathology in fresh and well-healed lung transplants. Am J Transplant 2006; **6**:724–35.

95 Li J, Simeoni E, Fleury S *et al.* Gene transfer of soluble interleukin-17 receptor prolongs cardiac allograft survival in a rat model. Eur J Cardiothorac Surg 2006; **29**:779–83.

96 Tang JL, Subbotin VM, Antonysamy MA, Troutt AB, Rao AS, Thomson AW. Interleukin-17 antagonism inhibits acute but not chronic vascular rejection. Transplantation 2001; **72**:348–50.

97 Antonysamy MA, Fanslow WC, Fu F *et al.* Evidence for a role of IL-17 in organ allograft rejection: IL-17 promotes the functional differentiation of dendritic cell progenitors. J Immunol 1999; **162**:577–84.

98 Wong CK, Ho CY, Li EK, Lam CW. Elevation of proinflammatory cytokine (IL-18, IL-17, IL-12) and Th2 cytokine (IL-4) concentrations in patients with systemic lupus erythematosus. Lupus 2000; **9**:589–93.

99 Arican O, Aral M, Sasmaz S, Ciragil P. Serum levels of TNF-alpha, IFN-gamma, IL-6, IL-8, IL-12, IL-17, and IL-18 in patients with active psoriasis and correlation with disease severity. Mediat Inflammation 2005; **2005**:273–9.

100 Homey B, eu-Nosjean MC, Wiesenborn A *et al.* Up-regulation of macrophage inflammatory protein-3 alpha/CCL20 and CC chemokine receptor 6 in psoriasis. J Immunol 2000; **164**:6621–32.

101 Matusevicius D, Kivisakk P, He B *et al.* Interleukin-17 mRNA expression in blood and CSF mononuclear cells is augmented in multiple sclerosis. Mult Scler 1999; **5**:101–4.

102 Kurasawa K, Hirose K, Sano H *et al.* Increased interleukin-17 production in patients with systemic sclerosis. Arthritis Rheum 2000; **43**:2455–63.

103 Fujino S, Andoh A, Bamba S *et al.* Increased expression of interleukin 17 in inflammatory bowel disease. Gut 2003; **52**:65–70.

104 Biesinger B, Muller-Fleckenstein I, Simmer B *et al.* Stable growth transformation of human T lymphocytes by herpesvirus saimiri. Proc Natl Acad Sci USA 1992; **89**:3116–9.

105 Patera AC, Pesnicak L, Bertin J, Cohen JI. Interleukin 17 modulates the immune response to vaccinia virus infection. Virology 2002; **299**:56–63.

106 Knappe A, Hiller C, Niphuis H *et al.* The interleukin-17 gene of herpesvirus saimiri. J Virol 1998; **72**:5797–801.

107 Luzza F, Parrello T, Monteleone G *et al.* Up-regulation of IL-17 is associated with bioactive IL-8 expression in *Helicobacter pylori*-infected human gastric mucosa. J Immunol 2000; **165**:5332–7.

108 Chung DR, Kasper DL, Panzo RJ *et al.* CD4+ T cells mediate abscess formation in intra-abdominal sepsis by an IL-17-dependent mechanism. J Immunol 2003; **170**:1958–63.

109 Johnson RB, Wood N, Serio FG. Interleukin-11 and IL-17 and the pathogenesis of periodontal disease. J Periodontol 2004; **75**:37–43.

110 Tartour E, Fossiez F, Joyeux I *et al.* Interleukin 17, a T-cell-derived cytokine, promotes tumorigenicity of human cervical tumors in nude mice. Cancer Res 1999; **59**:3698–704.

111 Numasaki M, Fukushi J, Ono M *et al.* Interleukin-17 promotes angiogenesis and tumor growth. Blood 2003; **101**:2620–7.

112 Benchetrit F, Ciree A, Vives V *et al.* Interleukin-17 inhibits tumor cell growth by means of a T-cell-dependent mechanism. Blood 2002; **99**:2114–21.

113 Hirahara N, Nio Y, Sasaki S *et al.* Reduced invasiveness and metastasis of Chinese hamster ovary cells transfected with human interleukin-17 gene. Anticancer Res 2000; **20**:3137–42.

114 Kato T, Furumoto H, Ogura T *et al.* Expression of IL-17 mRNA in ovarian cancer. Biochem Biophys Res Commun 2001; **282**:735–8.

115 Steiner GE, Newman ME, Paikl D *et al.* Expression and function of pro-inflammatory interleukin IL-17 and IL-17 receptor in normal, benign hyperplastic, and malignant prostate. Prostate 2003; **56**:171–82.

116 Ciree A, Michel L, Camilleri-Broet S *et al.* Expression and activity of IL-17 in cutaneous T-cell lymphomas (mycosis fungoides and Sezary syndrome). Int J Cancer 2004; **112**:113–20.

117 Wrobel T, Mazur G, Jazwiec B, Kuliczkowski K. Interleukin-17 in acute myeloid leukemia. J Cell Mol Med 2003; **7**:472–4.

118 Numasaki M, Watanabe M, Suzuki T *et al.* IL-17 enhances the net angiogenic activity and *in vivo* growth of human non-small cell lung cancer in SCID mice through promoting CXCR-2-dependent angiogenesis. J Immunol 2005; **175**:6177–89.

119 Grazia Roncarolo M, Gregori S, Battaglia M, Bacchetta R, Fleischhauer K, Levings MK. Interleukin-10-secreting type 1 regulatory T cells in rodents and humans. Immunol Rev 2006; **212**:28–50.

120 Weiner HL. Induction and mechanism of action of transforming growth factor-beta-secreting Th3 regulatory cells. Immunol Rev 2001; **182**:207–14.

121 Lu L, Werneck MBF, Cantor H. The immunoregulatory effects of Qa-1. Immunol Rev 2006; **212**:51–9.

122 Cortesini R, LeMaoult J, Ciubotariu R, Cortesini NSF. CD8+CD28- T suppressor cells and the induction of antigen-specific, antigen-presenting cell-mediated suppression of Th reactivity. Immunol Rev 2001; **182**:201–6.

123 Rifa'i M, Kawamoto Y, Nakashima I, Suzuki H. Essential roles of CD8+CD122+ regulatory T cells in the maintenance of T cell homeostasis. J Exp Med 2004; **200**:1123–34.

124 Hanninen A, Harrison LC. Gamma delta T cells as mediators of mucosal tolerance: the autoimmune diabetes model. Immunol Rev 2000; **173**:109–19.

125 Zhang C, Zhang J, Tian ZG. The regulatory effect of natural killer cells: do 'NK-reg cells' exist? Cell Mol Immunol 2006; **3**:241–54.

126 Yu G, Xu X, Vu MD, Kilpatrick ED, Li XC. NK cells promote transplant tolerance by killing donor antigen-presenting cells. J Exp Med 2006; **203**:1851–8.

127 Ochando JC, Homma C, Yang Y *et al.* Alloantigen-presenting plasmacytoid dendritic cells mediate tolerance to vascularized grafts. Nat Immunol 2006; **7**:652–62.

128 Ariel A, Fredman G, Sun YP *et al.* Apoptotic neutrophils and T cells sequester chemokines during immune response resolution through modulation of CCR5 expression. Nat Immunol 2006; **7**:1209–16.

129 Liu J, Liu Z, Witkowski P *et al.* Rat CD8+ FOXP3+ T suppressor cells mediate tolerance to allogeneic heart transplants, inducing PIR-B in APC and rendering the graft invulnerable to rejection. Transpl Immunol 2004; **13**:239–47.

130 Suciu-Foca N, Manavalan JS, Scotto L *et al.* Molecular characterization of allospecific T suppressor and tolerogenic dendritic cells: review. Int Immunopharmacol 2005; **5**:7–11.

131 Liu Z, Tugulea S, Cortesini R, Suciu-Foca N. Specific suppression of T helper alloreactivity by allo-MHC class I-restricted CD8+. Int Immunol 1998; **10**:775–83.

132 Kapp JA, Honjo K, Kapp LM, Xu XY, Cozier A, Bucy RP. TCR transgenic CD8+ T cells activated in the presence of TGF{beta} express FoxP3 and mediate linked suppression of primary immune responses and cardiac allograft rejection. Int Immunol 2006; **18**:1549–62.

133 Zhang ZX, Young K, Zhang L. CD3+CD4-CD8- alphabeta-TCR+ T cell as immune regulatory cell. J Mol Med 2001; **79**:419–27.

134 Zhang ZX, Yang L, Young KJ, DuTemple B, Zhang L. Identification of a previously unknown antigen-specific regulatory T cell and its mechanism of suppression. Nat Med 2000; **6**:782–9.

135 Sakaguchi S, Sakaguchi N, Asano M, Itoh M, Toda M. Immunologic self-tolerance maintained by activated T cells expressing IL-2 receptor alpha-chains (CD25). Breakdown of a single mechanism of self-tolerance causes various autoimmune diseases. J Immunol 1995; **155**:1151–64.

136 Sakaguchi S, Toda M, Asano M, Itoh M, Morse SS, Sakaguchi N. T cell-mediated maintenance of natural self-tolerance: its breakdown as a possible cause of various autoimmune diseases. J Autoimmun 1996; **9**:211–20.

137 Chatila TA, Blaeser F, Ho N *et al.* JM2, encoding a fork head-related protein, is mutated in X-linked autoimmunity-allergic disregulation syndrome. J Clin Invest 2000; **106**:R75–R81.

138 Wildin RS, Ramsdell F, Peake J *et al.* X-linked neonatal diabetes mellitus, enteropathy and endocrinopathy syndrome is the human equivalent of mouse scurfy. Nat Genet 2001; **27**:18–20.

139 Dieckmann D, Plottner H, Berchtold S, Berger T, Schuler G. *Ex vivo* isolation and characterization of CD4(+) CD25(+) T cells with regulatory properties from human blood. J Exp Med 2001; **193**:1303–10.

140 Jonuleit H, Schmitt E, Stassen M, Tuettenberg A, Knop J, Enk AH. Identification and functional characterization of human CD4(+) CD25(+) T cells with regulatory properties isolated from peripheral blood. J Exp Med 2001; **193**:1285–94.

141 Thornton AM, Shevach EM. CD4+CD25+ immunoregulatory T cells suppress polyclonal T cell activation *in vitro* by inhibiting interleukin 2 production. J Exp Med 1998; **188**:287–96.

142 Wing K, Lindgren S, Kollberg G *et al.* CD4 T cell activation by myelin oligodendrocyte glycoprotein is suppressed by adult but not cord blood CD25+ T cells. Eur J Immunol 2003; **33**:579–87.

143 Baecher-Allan C, Hafler DA. Human regulatory T cells and their role in autoimmune disease. Immunol Rev 2006; **212**:203–16.

144 Umetsu DT, DeKruyff RH. The regulation of allergy and asthma. Immunol Rev 2006; **212**:238–55.

145 Rouse BT, Sarangi PP, Suvas S. Regulatory T cells in virus infections. Immunol Rev 2006; **212**:272–86.

146 Belkaid Y, Blank RB, Suffia I. Natural regulatory T cells and parasites: a common quest for host homeostasis. Immunol Rev 2006; **212**:287–300.

147 Waldmann H, Adams E, Fairchild P, Cobbold S. Infectious tolerance and the long-term acceptance of transplanted tissue. Immunol Rev 2006; **212**:301–13.

148 Izcue A, Coombes JL, Powrie F. Regulatory T cells suppress systemic and mucosal immune activation to control intestinal inflammation. Immunol Rev 2006; **212**:256–71.

149 Aluvihare VR, Kallikourdis M, Betz AG. Regulatory T cells mediate maternal tolerance to the fetus. Nat Immunol 2004; **5**:266–71.

150 Edinger M, Hoffmann P, Ermann J *et al.* CD4+CD25+ regulatory T cells preserve graft-versus-tumor activity while inhibiting graft-versus-host disease after bone marrow transplantation. Nat Med 2003; **9**:1144–50.

151 Hoffmann P, Ermann J, Edinger M, Fathman CG, Strober S. Donor-type CD4(+) CD25(+) regulatory T cells suppress lethal acute graft-versus-host disease after allogeneic bone marrow transplantation. J Exp Med 2002; **196**:389–99.

152 Graca L, Thompson S, Lin CY, Adams E, Cobbold SP, Waldmann H. Both CD4(+) CD25(+) and CD4(+) CD25(-) regulatory cells mediate dominant transplantation tolerance. J Immunol 2002; **168**:5558–65.

153 Game DS, Hernandez-Fuentes MP, Chaudhry AN, Lechler RI. CD4+CD25+ regulatory T cells do not significantly contribute to direct pathway hyporesponsiveness in stable renal transplant patients. J Am Soc Nephrol 2003; **14**:1652–61.

154 Hori S, Nomura T, Sakaguchi S. Control of regulatory T cell development by the transcription factor Foxp3. Science 2003; **299**:1057–61.

155 Fontenot JD, Gavin MA, Rudensky AY. Foxp3 programs the development and function of CD4+CD25+ regulatory T cells. Nat Immunol 2003; **4**:330–6.

156 Khattri R, Cox T, Yasayko SA, Ramsdell F. An essential role for Scurfin in CD4+CD25+ T regulatory cells. Nat Immunol 2003; **4**:337–42.

157 Fontenot JD, Rasmussen JP, Williams LM, Dooley JL, Farr AG, Rudensky AY. Regulatory T cell lineage specification by the forkhead transcription factor foxp3. Immunity 2005; **22**:329–41.

158 Ziegler SF. FOXP3: of mice and men. Annu Rev Immunol 2006; **24**:209–26.

159 Walker MR, Kasprowicz DJ, Gersuk VH *et al.* Induction of FoxP3 and acquisition of T regulatory activity by stimulated human CD4+. J Clin Invest 2003; **112**:1437–43.

160 Gavin MA, Torgerson TR, Houston E *et al.* Single-cell analysis of normal and FOXP3-mutant human T cells: FOXP3 expression without regulatory T cell development. Proc Natl Acad Sci U S A 2006; **103**:6659–64.

161 Liu W, Putnam AL, Xu-Yu Z *et al.* CD127 expression inversely correlates with FoxP3 and suppressive function of human CD4+ T reg cells. J Exp Med 2006; **203**:1701–11.

162 Piccirillo CA, Shevach EM. Cutting edge. control of CD8+ T cell activation by CD4+CD25+ immunoregulatory cells. J Immunol 2001; **167**:1137–40.

163 Takahashi T, Kuniyasu Y, Toda M *et al.* Immunologic self-tolerance maintained by CD25+CD4+ naturally anergic and suppressive T cells: induction of autoimmune disease by breaking their anergic/suppressive state. Int Immunol 1998; **10**:1969–80.

164 Romagnoli P, Hudrisier D, van Meerwijk JP. Preferential recognition of self antigens despite normal thymic deletion of CD4(+) CD25(+) regulatory T cells. J Immunol 2002; **168**:1644–8.

165 Jordan MS, Boesteanu A, Reed AJ *et al.* Thymic selection of CD4+CD25+ regulatory T cells induced by an agonist self-peptide. Nat Immunol 2001; **2**:301–6.

166 Cozzo C, Larkin J III, Caton AJ. Cutting edge. self-peptides drive the peripheral expansion of CD4+CD25+ regulatory T cells. J Immunol 2003; **171**:5678–82.

167 Gavin MA, Clarke SR, Negrou E, Gallegos A, Rudensky A. Homeostasis and anergy of CD4(+) CD25(+) suppressor T cells *in vivo*. Nat Immunol 2002; **3**:33–41.

168 Fisson S, rrasse-Jeze G, Litvinova E *et al.* Continuous activation of autoreactive CD4+ CD25+ regulatory T cells in the steady state. J Exp Med 2003; **198**:737–46.

169 Ng WF, Duggan PJ, Ponchel F *et al.* Human CD4(+) CD25(+) cells: a naturally occurring population of regulatory T cells. Blood 2001; **98**:2736–44.

170 Thornton AM, Shevach EM. Suppressor effector function of CD4+CD25+ immunoregulatory T cells is antigen nonspecific. J Immunol 2000; **164**:183–90.

171 Thornton AM, Piccirillo CA, Shevach EM. Activation requirements for the induction of CD4+CD25+ T cell suppressor function. Eur J Immunol 2004; **34**:366–76.

172 Dieckmann D, Bruett CH, Ploettner H, Lutz MB, Schuler G. Human CD4(+) CD25(+) regulatory, contact-dependent T cells induce interleukin 10-producing, contact-independent type 1-like regulatory T cells [corrected]. J Exp Med 2002; **196**:247–53.

173 Longhi MS, Hussain MJ, Mitry RR *et al.* Functional study of CD4+CD25+ regulatory T cells in health and autoimmune hepatitis. J Immunol 2006; **176**:4484–91.

174 Nakamura K, Kitani A, Fuss I *et al.* TGF-beta 1 plays an important role in the mechanism of CD4+CD25+ regulatory T cell activity in both humans and mice. J Immunol 2004; **172**:834–42.

175 Nakamura K, Kitani A, Strober W. Cell contact-dependent immunosuppression by CD4(+) CD25(+) regulatory T cells is mediated by cell surface-bound transforming growth factor beta. J Exp Med 2001; **194**:629–44.

176 Ly D, Mi QS, Hussain S, Delovitch TL. Protection from type 1 diabetes by invariant NK T cells requires the activity of CD4+CD25+ regulatory T cells. J Immunol 2006; **177**:3695–704.

177 Lu LF, Lind EF, Gondek DC *et al.* Mast cells are essential intermediaries in regulatory T-cell tolerance. Nature 2006; **442**:997–1002.

178 Duthoit CT, Mekala DJ, Alli RS, Geiger TL. Uncoupling of IL-2 signaling from cell cycle progression in naive CD4+ T cells by regulatory CD4+CD25+ T lymphocytes. J Immunol 2005; **174**:155–63.

179 Duthoit CT, Nguyen P, Geiger TL. Antigen nonspecific suppression of T cell responses by activated stimulation-refractory CD4+ T cells. J Immunol 2004; **172**:2238–46.

180 Sawitzki B, Kingsley CI, Oliveira V, Karim M, Herber M, Wood KJ. IFN-gamma production by alloantigen-reactive regulatory T cells is important for their regulatory function *in vivo*. J Exp Med 2005; **201**:1925–35.

181 Takahashi T, Tagami T, Yamazaki S *et al.* Immunologic self-tolerance maintained by CD25(+) CD4(+) regulatory T cells constitutively expressing cytotoxic T lymphocyte-associated antigen 4. J Exp Med 2000; **192**:303–10.

182 Read S, Greenwald R, Izcue A *et al.* Blockade of CTLA-4 on CD4+CD25+ regulatory T cells abrogates their function in vivo. J Immunol 2006; **177**:4376–83.

183 Read S, Malmstrom V, Powrie F. Cytotoxic T lymphocyte-associated antigen 4 plays an essential role in the function of CD25(+) CD4(+) regulatory cells that control intestinal inflammation. J Exp Med 2000; **192**:295–302.

184 Jago CB, Yates J, Camara NO, Lechler RI, Lombardi G. Differential expression of CTLA-4 among T cell subsets. Clin Exp Immunol 2004; **136**:463–71.

185 Levings MK, Sangregorio R, Roncarolo MG. Human CD25(+) CD4(+) T regulatory cells suppress naive and memory T cell proliferation and can be expanded *in vitro* without loss of function. J Exp Med 2001; **193**:1295–302.

186 Sansom DM, Walker LSK. The role of CD28 and cytotoxic T-lymphocyte antigen-4 (CTLA-4) in regulatory T-cell biology. Immunol Rev 2006; **212**:131–48.

187 Kawahata K, Misaki Y, Yamauchi M *et al.* Generation of CD4(+) CD25(+) regulatory T cells from autoreactive T cells simultaneously with their negative selection in the thymus and from nonautoreactive T cells by endogenous TCR expression. J Immunol 2002; **168**:4399–405.

188 Pennington DJ, Silva-Santos B, Silberzahn T *et al.* Early events in the thymus affect the balance of effector and regulatory T cells. Nature 2006; **444**:1073–7.

189 Tellier J, van Meerwijk JP, Romagnoli P. An MHC-linked locus modulates thymic differentiation of CD4+CD25+Foxp3+ regulatory T lymphocytes. Int Immunol 2006; **18**:1509–19.

190 Bayer AL, Yu A, Adeegbe D, Malek TR. Essential role for interleukin-2 for CD4(+) CD25(+) T regulatory cell development during the neonatal period. J Exp Med 2005; **201**:769–77.

191 Tang Q, Henriksen KJ, Boden EK *et al.* Cutting edge: CD28 controls peripheral homeostasis of CD4+CD25+ regulatory T cells. J Immunol 2003; **171**:3348–52.

192 Kirk AD, Hale DA, Swanson SJ, Mannon RB. Autoimmune thyroid disease after renal transplantation using depletional induction with alemtuzumab. Am J Transplant 2006; **6**:1084–5.

193 Coles AJ, Wing M, Smith S *et al.* Pulsed monoclonal antibody treatment and autoimmune thyroid disease in multiple sclerosis. Lancet 1999; **354**:1691–5.

194 Hakim FT, Memon SA, Cepeda R *et al.* Age-dependent incidence, time course, and consequences of thymic renewal in adults. J Clin Invest 2005; **115**:930–9.

195 Mackall CL, Gress RE. Thymic aging and T-cell regeneration. Immunol Rev 1997; **160**:91–102.

196 Zhang H, Chua KS, Guimond M *et al.* Lymphopenia and interleukin-2 therapy alter homeostasis of CD4+CD25+ regulatory T cells. Nat Med 2005; **11**:1238–43.

197 McHugh RS, Shevach EM. Cutting edge. depletion of CD4+CD25+ regulatory T cells is necessary, but not sufficient, for induction of organ-specific autoimmune disease. J Immunol 2002; **168**:5979–83.

198 Barthlott T, Kassiotis G, Stockinger B. T cell regulation as a side effect of homeostasis and competition. J Exp Med 2003; **197**:451–60.

199 Curotto de Lafaille MA, Lino AC, Kutchukhidze N, Lafaille JJ. CD25- T cells generate CD25+Foxp3+ regulatory T cells by peripheral expansion. J Immunol 2004; **173**:7259–68.

200 Liang S, Alard P, Zhao Y, Parnell S, Clark SL, Kosiewicz MM. Conversion of CD4+ CD25- cells into CD4+ CD25+ regulatory T cells *in vivo* requires B7 costimulation, but not the thymus. J Exp Med 2005; **201**:127–37.

201 Almeida AR, Zaragoza B, Freitas AA. Competition controls the rate of transition between the peripheral pools of CD4+. Int Immunol 2006; **18**:1607–13.

202 Almeida AR, Zaragoza B, Freitas AA. Indexation as a novel mechanism of lymphocyte homeostasis. the number of CD4+CD25+ regulatory T cells is indexed to the number of IL-2-producing cells. J Immunol 2006; **177**:192–200.

203 Vukmanovic-Stejic M, Zhang Y, Cook JE *et al.* Human CD4+ CD25hi Foxp3+ regulatory T cells are derived by rapid turnover of memory populations *in vivo*. J Clin Invest 2006; **116**:2423–33.

204 Yu A, Malek TR. Selective availability of IL-2 is a major determinant controlling the production of CD4+CD25+Foxp3+ T regulatory cells. J Immunol 2006; **177**:5115–21.

205 Malek TR, Bayer AL. Tolerance, not immunity, crucially depends on IL-2. Nat Rev Immunol 2004; **4**:665–74.

206 Knoechel B, Lohr J, Kahn E, Bluestone JA, Abbas AK. Sequential development of interleukin 2-dependent effector and regulatory T cells in response to endogenous systemic antigen. J Exp Med 2005; **202**:1375–86.

207 Thorstenson KM, Khoruts A. Generation of anergic and potentially immunoregulatory CD25+CD4 T cells *in vivo* after induction of peripheral tolerance with intravenous or oral antigen. J Immunol 2001; **167**:188–95.

208 Apostolou I, von Boehmer H. *In vivo* instruction of suppressor commitment in naive T cells. J Exp Med 2004; **199**:1401–8.

209 Kretschmer K, Apostolou I, Hawiger D, Khazaie K, von Nussenzweig MC, von Boehmer H. Inducing and expanding regulatory T cell populations by foreign antigen. Nat Immunol 2005; **6**:1219–27.

210 Grimbert P, Bouguermouh S, Baba N *et al.* Thrombospondin/CD47 interaction: a pathway to generate regulatory T cells from human CD4+. J Immunol 2006; **177**:3534–41.

211 Huber S, Schramm C, Lehr HA *et al.* Cutting edge: TGF-beta signaling is required for the in vivo expansion and immunosuppressive capacity of regulatory CD4+CD25+ T cells. J Immunol 2004; **173**:6526–31.

212 Schramm C, Huber S, Protschka M *et al.* TGFbeta regulates the CD4+CD25+ T-cell pool and the expression of Foxp3 *in vivo*. Int Immunol 2004; **16**:1241–9.

213 Marie JC, Letterio JJ, Gavin M, Rudensky AY. TGF-beta1 maintains suppressor function and Foxp3 expression in CD4+CD25+ regulatory T cells. J Exp Med 2005; **201**:1061–7.

214 Chen W, Jin W, Hardegen N *et al.* Conversion of peripheral CD4+CD25- naive T cells to CD4+CD25+ regulatory T cells by TGF-beta induction of transcription factor Foxp3. J Exp Med 2003; **198**:1875–86.

215 Fantini MC, Becker C, Monteleone G, Pallone F, Galle PR, Neurath MF. Cutting edge: TGF-beta induces a regulatory phenotype in CD4+. J Immunol 2004; **172**:5149–53.

216 Oppmann B, Lesley R, Blom B *et al.* Novel p19 protein engages IL-12p40 to form a cytokine, IL-23, with biological activities similar as well as distinct from IL-12. Immunity 2000; **13**:715–25.

217 Trinchieri G. Interleukin-12 and the regulation of innate resistance and adaptive immunity. Nat Rev Immunol 2003; **3**:133–46.

218 Leonard JP, Waldburger KE, Goldman SJ. Prevention of experimental autoimmune encephalomyelitis by antibodies against interleukin 12. J Exp Med 1995; **181**:381–6.

219 Constantinescu CS, Wysocka M, Hilliard B *et al.* Antibodies against IL-12 prevent superantigen-induced and spontaneous relapses of experimental autoimmune encephalomyelitis. J Immunol 1998; **161**:5097–104.

220 Cua DJ, Sherlock J, Chen Y *et al.* Interleukin-23 rather than interleukin-12 is the critical cytokine for autoimmune inflammation of the brain. Nature 2003; **421**:744–8.

221 Murphy CA, Langrish CL, Chen Y *et al.* Divergent pro- and anti-inflammatory roles for IL-23 and IL-12 in joint autoimmune inflammation. J Exp Med 2003; **198**:1951–7.

222 Aggarwal S, Ghilardi N, Xie MH, de Sauvage FJ, Gurney AL. Interleukin-23 promotes a distinct CD4 T cell activation state characterized by the production of interleukin-17. J Biol Chem 2003; **278**:1910–4.

223 Harrington LE, Hatton RD, Mangan PR *et al.* Interleukin 17-producing CD4+ effector T cells develop via a lineage distinct from the T helper type 1 and 2 lineages. Nat Immunol 2005; **6**:1123–32.

224 Park H, Li Z, Yang XO *et al.* A distinct lineage of CD4 T cells regulates tissue inflammation by producing interleukin 17. Nat Immunol 2005; **6**:1133–41.

225 Ottenhoff TH, Verreck FA, Lichtenauer-Kaligis EG, Hoeve MA, Sanal O, van Dissel JT. Genetics, cytokines and human infectious disease: lessons from weakly pathogenic mycobacteria and salmonellae. Nat Genet 2002; **32**:97–105.

226 Verreck FABT, Langenberg DM *et al.* Human IL-23-producing type 1 macrophages promote but IL-10-producing type 2 macrophages subvert immunity to (myco) bacteria. Proc Natl Acad Sci USA 2004; **101**:4560–5.

227 Uhlig HH, McKenzie BS, Hue S *et al.* Differential activity of IL-12 and IL-23 in mucosal and systemic innate immune pathology. Immunity 2006; **25**:309–18.

228 Parham C, Chirica M, Timans J *et al.* A receptor for the heterodimeric cytokine IL-23 is composed of IL-12Rbeta1 and a novel cytokine receptor subunit, IL-23R. J Immunol 2002; **168**:5699–708.

229 Hoeve MA, de Savage ND, BT et al. Divergent effects of IL-12 and IL-23 on the production of IL-17 by human T cells. Eur J Immunol 2006; **36**:661–70.

230 Rangachari M, Mauermann N, Marty RR *et al.* T-bet negatively regulates autoimmune myocarditis by suppressing local production of interleukin 17. J Exp Med 2006; **203**:2009–19.