they are based on well researched constructs and offer meaningful information. Tools that help the busy everyday generalist, particularly the primary care physician, and which do not have significant time and resource implications, can provide firstline management for many IBS sufferers. Equally, studies that are not hamstrung by unnecessarily reductionist approaches, rendering them remotely applicable to the clinical settings, should be welcomed.

Gut 2006;**55**:603–604.
doi: 10.1136/gut.2005.075606

Conflict of interest declared (the declaration can be viewed on the *Gut* website at http://www.gutjnl.com/supplement).

Correspondence to: Professor A P S Hungin, Centre for Integrated Health Care Research, Wolfson Research Institute, University of Durham, Queen's Campus, Stockton on Tees TS17 6BH, UK; A.P.S.Hungin@durham.ac.uk

REFERENCES

1 **Robinson A**, Lee V, Kennedy A, *et al.* A randomised controlled trial of self-help interventions in patients with a primary care diagnosis of irritable bowel syndrome. *Gut* 2006;**55**:643–8.
2 **Kennedy AP**, Robinson A, Rogers A. Incorporating patients' views and experiences of life with IBS in the development of an evidence based self-help guidebook. *Patient Educ Couns* 2003;**50**:303–10.
3 **Miller SM**, Brody DS, Summerton J. Styles of coping with threat: implications for health. *J Pers Soc Psychol* 1988;**54**:142–8.
4 **Ingham JG**, Miller P. Self referral to primary care: symptoms and social factors. *J Psychosom Res* 1986;**30**:49–56.
5 **Talley NJ**, Owen BK, Boyce P, *et al.* Psychological treatments for irritable bowel syndrome: a critique of controlled treatment trials. *Am J Gastroenterol* 1996;**91**:277–83.
6 **Svedlund J**. Functional gastrointestinal diseases. Psychotherapy is an efficient component to drug therapy. *Lakartidningen* 2002;**17**:172–4.
7 **Kennedy T**, Jones R, Darnley S, *et al.* Cognitive behaviour therapy in addition to antispasmodic treatment for irritable bowel syndrome: randomised controlled trial. *BMJ* 2005;**331**:435.
8 **Patel SM**, Statson WB, Legedza A, *et al.* The placebo effect in irritable bowel syndrome trials: a meta-analysis. *Neurogastroenterol Motil* 2005;**17**:332–40.
9 **Enck P**, Klosterhalfen S. The placebo response in functional bowel disorders: perspectives and putative mechanisms. *Neurogastroenterol Motil* 2005;**17**:325–31.
10 **Hungin APS**, Whorwell PJ, Tack J, *et al.* The prevalence, patterns and impact of IBS: an international survey of 40,000 subjects. *Aliment Pharmacol Ther* 2003;**17**:643–50.
11 **Kennedy P**, Nelson E, Reves D, *et al.* A randomised controlled trial to assess the effectiveness and cost of a patient orientated self management approach to chronic inflammatory bowel disease. *Gut* 2004;**53**:1639–45.

Colitis

T regulatory cell suppression of colitis: the role of TGF-β

R Duchmann, M Zeitz

Transforming growth factor β (TGF-β) and interleukin 2 may be involved in IBD peripheral regulatory T cell pathophysiology, raising the possibility of therapeutic application of TGF-β induced regulatory T cells in IBD patients

CD4+CD25+ regulatory T cells (T_{reg}) expressing the lineage marker Foxp3 control immune responses to self- and foreign antigens and are an intensely studied member of the heterogenous group of regulatory T cells. Although initially described as a population of suppressor T cells required to avoid organ specific autoimmunity, it has subsequently become clear that T_{reg} control immune responses in a much broader sense, including transplantation tolerance and immune responses to pathogens and tumours.[1–3] Relevant to inflammatory bowel diseases (IBD), T_{reg} prevent[4] and treat established[5] colitis in animal models of IBD and are numerically deficient in patients with active IBD.[6] This underlines the fact that the immune dys-equilibrium characteristic of chronic inflammatory diseases involves concomitant disturbances in inflammatory and suppressive immune mechanisms and opens up novel approaches for IBD therapy by strengthening T_{reg} mediated suppression.

Currently, efforts in laboratories worldwide are addressing the central questions of T_{reg} immunology, resolution of which will help us see more clearly the role of T_{reg} in disease pathogenesis and therapy. Some of these central questions are: where do T_{reg} come from; how are they generated, expanded, and maintained; how and where do they function; why do they fail to control immune responses in disease; and how can their therapeutic potential be used most efficiently and safely. As current data indicate that T_{reg} from IBD patients are functionally normal[6–8] but numerically deficient during active disease,[6] it will be interesting to understand the underlying mechanisms and, based on that knowledge, devise the most promising strategy to enlarge their numbers to therapeutic levels.

T_{REG} ORIGINATING IN THE THYMUS

It was first described that during ontogeny, CD4+CD25+ T_{reg} originate in the thymus, become detectable in the periphery of normal mice from around day 4 after birth, and increase to adult numbers by week 3.[9] Production of Foxp3 expressing T_{reg} by the thymus is considerably delayed relative to non-regulatory thymocytes,[10] and neonatally thymectomised mice show an early and substantial reduction in peripheral CD4+CD25+ T_{reg}, associated with the development of autoimmune diseases. It was demonstrated in transforming growth factor β1 (TGFβ1)[11] and interleukin 2 (IL-2)[12] deficient mice, that both cytokines, in contrast with their important function on mature peripheral T_{reg}, are dispensable for intrathymic T_{reg} development. Efficient generation of CD4+CD25+ thymocytes results from relatively high affinity interaction of the T cell receptor (TCR) with agonist ligands expressed in thymic epithelial cells.[13 14] This process requires CD28 dependent costimulation of developing thymocytes[15] and thymic stromal lymphopoietin expressed by human Hassall's corpuscles to activate thymic CD11c+ dendritic cells to express high levels of CD80 and CD86.[16] Whether a thymic defect contributes to T_{reg} pathophysiology in IBD is not known. Interestingly, however, thymic T_{reg} are functionally impaired in patients with autoimmune myasthenia gravis,[17] and it was shown in animal models that colitis induces aberrant thymic development with impaired T_{reg} cell production.[18]

T_{REG} ORIGINATING IN THE PERIPHERY

An important next step in our understanding of T_{reg} biology was when it was shown that Foxp3+ T cells with regulatory function in vivo do not only

originate intrathymically but can also be generated from peripheral CD4+CD25− cells. Apostolou and von Boehmer showed that prolonged subcutaneous infusion of low doses of peptide by means of osmotic pumps transforms mature T cells into CD4+25+ suppressor cells that can persist for long periods of time in the absence of antigen and confer specific immunological tolerance after challenge with antigen.[19] Conversion of peripheral CD25− T cells into T_{reg} in mice requires the presence of TGF-β and downregulation of the TGF-β signalling inhibitor Smad7[20] in addition to TCR and IL-2R mediated signals.[21 22] In contrast, studies by Walker and colleagues[23] suggest that human FOXP3+ T cells with regulatory function in vitro can be generated from peripheral CD4+CD25− T cells via CD3/CD28 signalling in the absence of TGF-β.

In the present issue of *Gut*, Fantini and colleagues[24] report that T_{reg} generated in vitro from CD25− T cells under the influence of TGF-β are functionally active and suppress Th1 mediated colitis induced in SCID mice after transfer of CD4+CD62L+ T cells ***(see page 671)***. Furthermore, the authors provide evidence that such TGF-β induced regulatory T (Ti-T_{reg}) cells require exogenous IL-2 for their in vivo expansion and long term maintenance. Ultimately, these data raise the question of the potential involvement of TGF-β and IL-2 in IBD peripheral T_{reg} pathophysiology and the potential therapeutic application of Ti-T_{reg} in IBD patients.

The picture that emerges from the present studies is that TGF-β and IL-2 have complementary but partly opposing functions in the biology of peripheral T_{reg}. Interestingly, in a system in which conversion of naive CD4+CD25− T cells into T_{reg} was achieved by minute antigen doses with suboptimal dendritic cell activation, both addition of TGF-β and absence of IL-2 production enhanced conversion but reduced proliferation.[25] A negative effect of IL-2 mediated expansion on the conversion rate was also supported by the observation that reduced proliferation in the absence of autocrine IL-2 production was associated with an increased conversion rate of the least divided cells.[25] On a similar line, elegant studies using mice containing the Foxp3GFP knock-in allele that were genetically deficient in either IL-2 (IL-2−/−) or CD25 (Ilra−/−), demonstrated that IL-2 signalling seems to be critically required for promoting the survival and population expansion of T_{reg} in vivo but is dispensable for induction of Foxp3 expression in developing thymocytes and for peripheral T_{reg} suppressive function.[26] The latter finding notwithstanding, it was shown with T_{reg} derived from wild-type mice that IL-2 uptake by T_{reg} is required for their suppressive activity in vitro.[27 28] In the face of increased IL-2 levels in active IBD lesions and no known defects in IL-2 signalling in IBD, it seems unlikely that the numerical T_{reg} deficit in IBD is due to a lack of IL-2 mediated T_{reg} expansion. Furthermore, the findings of Fantini and colleagues[24] that Ti-T_{reg} were maintained under the influence of IL-2 when transferred into colitic mice support the fact that natural T_{reg} expanded for therapeutic purposes and Ti-T_{reg} should be maintained after transfer in IBD patients. The reason for the discrepant behaviour of natural T_{reg}, which expand in lymphopenic hosts, and Ti-T_{reg}, which are not maintained when transferred into SCID mice under both syngenic and MHC mismatched conditions, remains to be clarified. It is similarly unknown whether the numerical deficit observed in IBD is a consequence of insufficient conversion of CD4+CD25− T cells into CD4−CD25+ T_{reg} or whether this is caused by insufficient TGF-β levels or signalling. The former does not seem very likely as colitis is associated with high TGF-β levels, whereas the latter might be possible as Smad 7, an inhibitor of TGF-β signalling, is expressed at high levels in IBD T cells.[29]

T_{REG} FUNCTION

Once activated, peripheral Foxp3+ T_{reg} are robust suppressors and mediate inhibition of CD4+CD25− responder T cells by an as yet undefined mechanism. Current studies strongly indicate a critical role for TGF-β in this process. However, conflicting results in the literature make the exact nature of the involvement and cellular source of TGF-β a matter of current debate.[30–34] The important role of TGF-β in T_{reg} suppression was again evident from recent studies which demonstrated that T cells with a dominant negative TGF-β receptor which cannot respond to TGF-β escape control by T_{reg} in vivo.[33 35 36] Thus high levels of Smad7, an inhibitor of TGF-β signalling, in intestinal IBD T cells[29] may not only decrease conversion of CD25− T cells into CD25+ T_{reg}, as discussed above, but could also be responsible for the fact that CD25− T cells escape TGF-β mediated control. This however does not seem to be critical as peripheral T_{reg} from IBD patients[6 8] and intestinal T_{reg} from active IBD patients were functionally normal in their inhibition of CD25− T cells.[7]

T_{REG} THERAPY

Thymic generation of natural T_{reg} seems constitutive and relatively stable, with low levels of approximately 1–2% of human T_{reg} in peripheral blood lymphocytes and even lower levels in the intestine.[6] The potential for their therapeutic manipulation greatly increased when it was shown that human CD4+CD25+ T_{reg} can be expanded in vitro in the presence of high dose IL-2 and allogeneic feeder cells without loss of function.[37 38] Additional studies showed that their large scale in vitro expansion for clinical purposes can be achieved via repeated stimulation with anti-CD3, anti-CD28, and high dose IL-2.[39] Importantly, these expanded T_{reg} retain their phenotypic characteristics, maintain expression of lymph node homing receptors, and show an even increased suppressive activity compared with freshly isolated cells. Although this indicates that expansion of CD4+CD25+ T_{reg} has definite potential application in designing future clinical therapy for autoimmune diseases, inflammation, and transplantation, their physiological scarcity and numerical deficiency in IBD and/or their functional deficit in other human diseases support alternative approaches to convert normal naive CD4+CD25− T cells into CD4+CD25+ FOXP3 regulatory T cells. Here, several alternative approaches may be an option and are likely to compete depending on further information and specifics of T_{reg} pathophysiology in different diseases. These approaches include for example protocols which use TGF-β together with IL-2 and TCR mediated signals or nuclear factor κB blocked immature APC[40] for the ex vivo generation of T_{reg}, monoclonal antibodies,[41] or copolymer I,[42] or infusion of low dose antigen[19] for their in vivo generation and further strategies to strengthen T_{reg} mediated suppression locally.[43]

In summary, it is quite well established that TGF-β effectively promotes the generation of Ti- T_{reg} and has an essential role in T_{reg} function. Current data indicate that a colitis induced defect in the generation of thymic T_{reg} or a defect in TGF-β signalling involving high expression of Smad7 may play a role in IBD, but further studies are required to determine the molecular mechanisms of T_{reg} pathophysiology in IBD, which so far has been characterised as a numerical deficit in active disease. Treatment of IBD by strengthening T_{reg} mediated suppression has become a feasible option. Further studies will help to choose the best and least cumbersome approach towards achieving T_{reg} levels that are therapeutic and readjust over time.

ACKNOWLEDGEMENTS

The authors wish to thank the Deutsche Forschungsgemeinschaft and the Broad Medical Research Program of the Eli and

Edythe L Broad Foundation for their continued support of our research.

Gut 2006;**55**:604–606.
doi: 10.1136/gut.2005.083592

Authors' affiliations
R Duchmann, M Zeitz, Medizinische Klinik I, Campus Benjamin Franklin, Charité-Universitätsmedizin Berlin, Berlin, Germany

Correspondence to: Professor R Duchmann, Medizinische Klinik I, Campus Benjamin Franklin, Charité-Universitätsmedizin Berlin, Hindenburgdamm 30, D-12200 Berlin, Germany; rainer.duchmann@charite.de

Conflict of interest: None declared.

REFERENCES

1 **Taylor PA**, Friedman TM, Korngold R, *et al.* Tolerance induction of alloreactive T cells via ex vivo blockade of the CD40:CD40L costimulatory pathway results in the generation of a potent immune regulatory cell. *Blood* 2002;**99**:4601–9.
2 **Belkaid Y**, Piccirillo CA, Mendez S, *et al.* CD4+CD25+ regulatory T cells control Leishmania major persistence and immunity. *Nature* 2002;**420**:502–7.
3 **Sakaguchi S**, Sakaguchi N. Regulatory T cells in immunologic self-tolerance and autoimmune disease. *Int Rev Immunol* 2005;**24**:211–26.
4 **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.
5 **Mottet C**, Uhlig HH, Powrie F. Cutting edge: cure of colitis by CD4+CD25+ regulatory T cells. *J Immunol* 2003;**170**:3939–43.
6 **Maul J**, Loddenkemper C, Mundt P, *et al.* Peripheral and intestinal regulatory CD4+ CD25(high) T cells in inflammatory bowel disease. *Gastroenterology* 2005;**128**:1868–78.
7 **Makita S**, Kanai T, Oshima S, *et al.* CD4+CD25bright T cells in human intestinal lamina propria as regulatory cells. *J Immunol* 2004;**173**:3119–30.
8 **Viglietta V**, Baecher-Allan C, Weiner HL, *et al.* Loss of functional suppression by CD4+CD25+ regulatory T cells in patients with multiple sclerosis. *J Exp Med* 2004;**199**:971–9.
9 **Asano M**, Toda M, Sakaguchi N, *et al.* Autoimmune disease as a consequence of developmental abnormality of a T cell subpopulation. *J Exp Med* 1996;**184**:387–96.
10 **Fontenot JD**, Dooley JL, Farr AG, *et al.* Developmental regulation of Foxp3 expression during ontogeny. *J Exp Med* 2005;**202**:901–6.
11 **Marie JC**, Letterio JJ, Gavin M, *et al.* TGF-beta1 maintains suppressor function and Foxp3 expression in CD4+CD25+ regulatory T cells. *J Exp Med* 2005;**201**:1061–7.
12 **D'Cruz LM**, Klein L. Development and function of agonist-induced CD25+Foxp3+ regulatory T cells in the absence of interleukin 2 signaling. *Nat Immunol* 2005;**6**:1152–9.
13 **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.
14 **Bensinger SJ**, Bandeira A, Jordan MS, *et al.* Major histocompatibility complex class II-positive cortical epithelium mediates the selection of CD4(+)25(+) immunoregulatory T cells. *J Exp Med* 2001;**194**:427–38.
15 **Tai X**, Cowan M, Feigenbaum L, *et al.* CD28 costimulation of developing thymocytes induces Foxp3 expression and regulatory T cell differentiation independently of interleukin 2. *Nat Immunol* 2005;**6**:152–62.
16 **Watanabe N**, Wang YH, Lee HK, *et al.* Hassall's corpuscles instruct dendritic cells to induce CD4+CD25+ regulatory T cells in human thymus. *Nature* 2005;**436**:1181–5.
17 **Balandina A**, Lecart S, Dartevelle P, *et al.* Functional defect of regulatory CD4(+)CD25+ T cells in the thymus of patients with autoimmune myasthenia gravis. *Blood* 2005;**105**:735–41.
18 **Faubion WA**, De Jong YP, Molina AA, *et al.* Colitis is associated with thymic destruction attenuating CD4+25+ regulatory T cells in the periphery. *Gastroenterology* 2004;**126**:1759–70.
19 **Apostolou I**, von Boehmer H. In vivo instruction of suppressor commitment in naive T cells. *J Exp Med* 2004;**199**:1401–8.
20 **Fantini MC**, Becker C, Monteleone G, *et al.* Cutting edge: TGF-beta induces a regulatory phenotype in CD4+CD25− T cells through Foxp3 induction and down-regulation of Smad7. *J Immunol* 2004;**172**:5149–53.
21 **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.
22 **Fu S**, Zhang N, Yopp AC, *et al.* TGF-beta induces Foxp3 + T-regulatory cells from CD4+ CD25− precursors. *Am J Transplant* 2004;**4**:1614–27.
23 **Walker MR**, Kasprowicz DJ, Gersuk VH, *et al.* Induction of FoxP3 and acquisition of T regulatory activity by stimulated human CD4+CD25− T cells. *J Clin Invest* 2003;**112**:1437–43.
24 **Fantini MC**, Becker C, Tubbe I, *et al.* Transforming growth factor β induced FoxP3+ regulatory T cells suppress Th1 mediated experimental colitis. *Gut* 2006;**55**:671–80.
25 **Kretschmer K**, Apostolou I, Hawiger D, *et al.* Inducing and expanding regulatory T cell populations by foreign antigen. *Nat Immunol* 2005;**6**:1219–27.
26 **Fontenot JD**, Rasmussen JP, Gavin MA, *et al.* A function for interleukin 2 in Foxp3-expressing regulatory T cells. *Nat Immunol* 2005;**6**:1142–51.
27 **Scheffold A**, Huhn J, Hofer T. Regulation of CD4+CD25+ regulatory T cell activity: it takes (IL-)two to tango. *Eur J Immunol* 2005;**35**:1336–41.
28 **Thornton AM**, Donovan EE, Piccirillo CA, *et al.* Cutting edge: IL-2 is critically required for the in vitro activation of CD4+CD25+ T cell suppressor function. *J Immunol* 2004;**172**:6519–23.
29 **Monteleone G**, Kumberova A, Croft NM, *et al.* Blocking Smad7 restores TGF-beta1 signaling in chronic inflammatory bowel disease. *J Clin Invest* 2001;**108**:601–9.
30 **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.
31 **Piccirillo CA**, Letterio JJ, Thornton AM, *et al.* CD4(+)CD25(+) regulatory T cells can mediate suppressor function in the absence of transforming growth factor beta1 production and responsiveness. *J Exp Med* 2002;**196**:237–46.
32 **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.
33 **Fahlen L**, Read S, Gorelik L, *et al.* T cells that cannot respond to TGF-beta escape control by CD4(+)CD25(+) regulatory T cells. *J Exp Med* 2005;**201**:737–46.
34 **Kullberg MC**, Hay V, Cheever AW, *et al.* TGF-beta1 production by CD4+ CD25+ regulatory T cells is not essential for suppression of intestinal inflammation. *Eur J Immunol* 2005;**35**:2886–95.
35 **Green EA**, Gorelik L, McGregor CM, *et al.* CD4+CD25+ T regulatory cells control anti-islet CD8+ T cells through TGF-beta-TGF-beta receptor interactions in type 1 diabetes. *Proc Natl Acad Sci U S A* 2003;**100**:10878–83.
36 **Chen ML**, Pittet MJ, Gorelik L, *et al.* Regulatory T cells suppress tumor-specific CD8 T cell cytotoxicity through TGF-beta signals in vivo. *Proc Natl Acad Sci U S A* 2005;**102**:419–24.
37 **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.
38 **Koenen HJ**, Fasse E, Joosten I. CD27/CFSE-based ex vivo selection of highly suppressive alloantigen-specific human regulatory T cells. *J Immunol* 2005;**174**:7573–83.
39 **Hoffmann P**, Eder R, Kunz-Schughart LA, *et al.* Large-scale in vitro expansion of polyclonal human CD4(+)CD25high regulatory T cells. *Blood* 2004;**104**:895–903.
40 **Cong Y**, Konrad A, Iqbal N, *et al.* Generation of antigen-specific, Foxp3-expressing CD4+ regulatory T cells by inhibition of APC proteosome function. *J Immunol* 2005;**174**:2787–95.
41 **Gorczynski RM**, Lee L, Boudakov I. Augmented induction of CD4+CD25+ Treg using monoclonal antibodies to CD200R. *Transplantation* 2005;**79**:1180–3.
42 **Hong J**, Li N, Zhang X, *et al.* Induction of CD4+CD25+ regulatory T cells by copolymer-I through activation of transcription factor Foxp3. *Proc Natl Acad Sci U S A* 2005;**102**:6449–54.
43 **Jaeckel E**, von Boehmer H, Manns M. Antigen-specific FoxP3-transduced T-cells can control established type 1 diabetes. *Diabetes* 2005;**54**:306–10.