

- testing correlates with symptom improvement in irritable bowel syndrome: a double-blind, randomized, placebo-controlled study. *Am J Gastroenterol* 2003;98:412–419.
20. Monnikes H, Tebbe JJ, Hildebrandt M, Arck P, Osmanoglou E, Rose M, Klapp B, Wiedenmann B, Heymann-Monnikes I. Role of stress in functional gastrointestinal disorders. Evidence for stress-induced alterations in gastrointestinal motility and sensitivity. *Dig Dis* 2001;19:201–211.
  21. Khastgir G, Studd JW, Catalan J. The psychological outcome of hysterectomy. *Gynecol Endocrinol* 2000;14:132–141.
  22. Hearing SD, Thomas LA, Heaton KW, Hunt L. Effect of cholecystectomy on bowel function: a prospective, controlled study. *Gut* 1999;45:889–894.
  23. Creed F. Life events and appendectomy. *Lancet* 1981;1:1381–1385.
  24. Rutgeerts P, D'Haens G, Hiele M, Geboes K, Vantrappen G. Appendectomy protects against ulcerative colitis. *Gastroenterology* 1994;106:1251–1253.
  25. Russel MG, Dorant E, Brummer RJ, van de Kruijs MA, Muris JW, Bergers JM, Goedhard J, Stockbrugger RW. Appendectomy and the risk of developing ulcerative colitis or Crohn's disease: results of a large case-control study. South Limburg Inflammatory Bowel Disease Study Group. *Gastroenterology* 1997;113:377–382.
  26. Andersson RE, Olaison G, Tysk C, Ekblom A. Appendectomy and protection against ulcerative colitis. *N Engl J Med* 2001;344:808–814.
  27. Gwee KA, Collins SM, Read NW, Rajnakova A, Deng Y, Graham JC, McKendrick MW, Moochhalal SM. Increased rectal mucosal expression of interleukin 1beta in recently acquired post-infectious irritable bowel syndrome. *Gut* 2003;52:523–526.
  28. Talley NJ, Spiller R. Irritable bowel syndrome: a little understood organic bowel disease? *Lancet* 2002;360:555–564.
  29. Heaton KW. Review article: epidemiology of gall-bladder disease—role of intestinal transit. *Aliment Pharmacol Ther* 2000;14(Suppl. 2):9–13.
  30. Veysey MJ, Thomas LA, Mallet AI, Jenkins PJ, Besser GM, Wass JA, Murphy GM, Dowling RH. Prolonged large bowel transit increases serum deoxycholic acid: a risk factor for octreotide induced gallstones. *Gut* 1999;44:675–681.
  31. Veysey MJ, Thomas LA, Mallet AI, Jenkins PJ, Besser GM, Murphy GM, Dowling RH. Colonic transit influences deoxycholic acid kinetics. *Gastroenterology* 2001;121:812–822.
  32. Azzaroli F, Mazzella G, Mazzeo C, Simoni P, Festi D, Colecchia A, Montagnani M, Martino C, Villanova N, Roda A, Roda E. Sluggish small bowel motility is involved in determining increased biliary deoxycholic acid in cholesterol gallstone patients. *Am J Gastroenterol* 1999;94:2453–2459.
  33. Kamath PS, Gaisano HY, Phillips SF, Miller LJ, Charboneau JW, Brown ML, Zinsmeister AR. Abnormal gallbladder motility in irritable bowel syndrome: evidence for target-organ defect. *Am J Physiol* 1991;260:G815–819.
  34. Misra SP, Dwivedi M, Mital M, Misra V. Gallbladder dynamics in patients with irritable bowel syndrome and essential dyspepsia. *J Clin Gastroenterol* 1991;13:65–68.
  35. Owens DM, Nelson DK, Talley NJ. The irritable bowel syndrome: long-term prognosis and the physician-patient interaction. *Ann Intern Med* 1995;122:107–112.
  36. MacFadyen BV Jr, Vecchio R, Ricardo AE, Mathis CR. Bile duct injury after laparoscopic cholecystectomy. The United States experience. *Surg Endosc* 1998;12:315–321.
  37. Glasgow RE, Mulvihill SJ. Surgical management of gallstone disease and postoperative complications. In: Feldman M, Friedman LS, Sleisenger MH, eds. *Sleisenger and Fordtran's gastrointestinal and liver disease*. 7th ed. Philadelphia: Saunders, 2002: 1091–1105.
  38. Talley NJ. *Helicobacter pylori* management: how to improve the therapeutic confusion in practice. *Can J Gastroenterol* 2003;17(Suppl. B):21B–24B.
  39. Huth EJ, Murray TJ, eds. *Medicine in quotations. Views of Health and Disease through the ages*. Philadelphia, PA: American College of Physicians, 2000.

---

Address correspondence to: Nicholas J. Talley, M.D., Ph.D., Charlton 8-127, Mayo Clinic, 200 First Street, Rochester, Minnesota 55905. e-mail: talley.nicholas@mayo.edu; fax: (507) 538-5820.

© 2004 by the American Gastroenterological Association  
0016-5085/04/\$30.00

doi:10.1053/j.gastro.2004.04.029

## The Other Way Round: Colitis Regulates Regulatory T Cells

See article on page 1759.

The gastrointestinal tract, with its resident flora, is the site of the most abundant encounters with antigens by the lymphoid system in a vertebrate. Regulation of the immune response in the gut is a balance between the need to mount protective immunity toward pathogens while not activating damaging inflammatory responses to the vast numbers of harmless antigens, including those derived from commensal bacteria and food contents. Indeed, the breakdown of this delicate balance is dramatically shown in inflammatory bowel disease (IBD).<sup>1</sup>

Animal models of IBD have provided much insight into the mechanisms governing this delicate balance

between immune activation and suppression.<sup>2</sup> One of the key observations derived from such studies was that a subpopulation of T lymphocytes serves to suppress (lately termed regulate) pathogenic T-cell activity that would otherwise lead to inflammation and a morphologic and pathophysiologic picture resembling human IBD. One of the key initial observations on regulatory T-cell activity in colitis came from transfer experiments: transfer of CD4<sup>+</sup>CD45RB<sup>high</sup> (naïve) T cells into immunodeficient *scid* mice led to progressive wasting disease characterized by colonic inflammation, which could be prevented by coadministration of CD4<sup>+</sup>CD45RB<sup>low</sup> (memory) T cells into such mice.<sup>3</sup> More recently, the regulatory activity of T cells could be identified to reside within the CD4<sup>+</sup>CD25<sup>+</sup> T regulatory (T<sub>reg</sub>) cell population in humans and mice (the CD4<sup>+</sup>CD45RB<sup>low</sup> population is

heterogeneous, containing memory/effector T cells and  $T_{reg}$  cells).<sup>4,5</sup> Further support for the notion that the failure of regulatory T-cell development (or activity) can cause the development of IBD came from data that showed that administration of  $T_{reg}$  cells to mice with established colitis can even cure disease via pathways involving interleukin (IL)-10 and transforming growth factor  $\beta$ .<sup>6,7</sup> Thus, IBD can be considered to be caused by either excess T-cell effector function or inadequate  $T_{reg}$  cell function.<sup>2</sup> Additionally,  $T_{reg}$  cells play important roles well beyond colitis in anti-infectious immunity, autoimmune diseases, transplantation tolerance, and antitumor immunity, to cite a few.<sup>4,8</sup>

In a normal healthy individual, these  $T_{reg}$  cells make up approximately 5%–10% of T cells in peripheral lymphoid organs. The transcriptional repressor Foxp3 has been identified as the master-control gene for the development of  $CD4^+CD25^+$   $T_{regs}$ , defining  $T_{regs}$  as a distinct lineage.<sup>9–11</sup> Indeed, a mutation in the gene encoding *foxp3* was identified as the genetic defect underlying autoimmune and inflammatory disease in scurfy mice and in humans with immune dysregulation, polyendocrinopathy, enteropathy X-linked syndrome, and X-linked autoimmunity allergic dysregulation syndrome, emphasizing the importance of  $T_{reg}$  cells in the maintenance of normal immune homeostasis.

There is accumulating evidence that  $T_{reg}$  cells capable of modulating the function of conventional T cells are primarily derived from the thymus.<sup>4,12</sup> Coexpression of a specific MHC class II-restricted T-cell receptor (TCR), together with an agonist ligand within the same host, has been shown to result in the generation of  $CD4^+CD25^+$  regulatory T cells,<sup>13</sup> suggesting that  $T_{reg}$  cells are induced by self-antigen. Further experiments have shown that the thymic epithelium is the antigen presenting cell for  $T_{reg}$  selection because expression of agonist ligands by this cell type serve as potent, but not exclusive, inducers of  $T_{regs}$ .<sup>14,15</sup> Selection of  $CD4^+CD25^+$  thymocytes appears to require a TCR with intermediate affinity for a self-peptide because thymocytes that bear TCRs with low affinity do not undergo selection into this pathway.<sup>14</sup> With regard to  $T_{reg}$  cells induced by thymic epithelium, there is a need to identify agonistic TCR ligands on thymic epithelium that induce  $T_{reg}$  development under physiologic conditions. Besides these data supporting the thymus as a primary site of  $T_{reg}$  development, von Boehmer<sup>4</sup> recently presented evidence that  $T_{regs}$  can be induced in the periphery when peptides are continuously delivered to adult thymectomized TCR-transgenic mice on a recombination activating gene<sup>-/-</sup> background. This is in line with a recent report on the in vitro generation of Foxp3<sup>+</sup>,  $CD4^+CD25^+$  T cells from

$CD4^+CD25^-$  human peripheral lymphocytes after anti-CD3/CD28 activation.<sup>16</sup> These stimulation-induced  $CD4^+CD25^+$  T cells were indistinguishable from freshly isolated  $CD4^+CD25^+$   $T_{reg}$  cells that were likely derived from the thymus with regard to their suppressor activity, suggesting that peripheral pathways may exist that drive the development of  $T_{regs}$ .<sup>16,17</sup> This might be particularly relevant to the intestine, which must differentiate, as noted, between numerous nonpathogenic and potentially pathogenic foreign antigens.

In this issue of GASTROENTEROLOGY, Faubion et al.<sup>18</sup> provide intriguing evidence that development of colitis per se might impair production of regulatory T cells and that treatment of colitis by several means might be associated with restoration of thymic function and  $T_{reg}$  production. To conclude this, they used the tg $\epsilon$ 26 mouse model of colitis<sup>19</sup> to show the effect of colitis on  $T_{reg}$  development. Adult tg $\epsilon$ 26 mice develop colitis on reconstitution with wild-type bone marrow.<sup>19</sup> Tg $\epsilon$ 26 mice bear a transgenic human CD3- $\epsilon$  chain in which overexpression results in intrathymic T-cell and natural killer (NK)-cell death presumably because of excessive signal transduction during thymic development.<sup>2,19,20</sup> They also manifest a secondary defect in thymic stromal architecture because the development of the latter depends on the presence of normal thymocytes.<sup>21</sup> T-cell development in fetal mice bearing the human transgene, and neonatal mice as shown in the current report, can be rescued by transplantation of T cell-depleted normal bone marrow because such transplantation preserves stromal architecture; this is in contrast to adult mice which cannot be rescued because, by this time, the alteration in architecture cannot be reversed.<sup>2,21</sup> Thus, whereas bone marrow reconstitution of adult mice leads to the reversal of lymphoid depletion, the reconstituted mice contain a cell population that has developed in a defective thymic microenvironment and, hence, is capable of driving and/or inadequately regulating the development of colitis.

Faubion et al.<sup>18</sup> now report that a temporal window of a few days exists when newborn tg $\epsilon$ 26 mice can develop a normal thymus on bone-marrow transplantation and support the development of  $CD4^+CD25^+$   $T_{reg}$  cells in contrast to adult mice which do not. Although adult transplanted tg $\epsilon$ 26 mice show involution of their transiently developing thymus concomitantly with the onset of colitis, treatment with anti-tumor necrosis factor  $\alpha$  monoclonal antibody or lymphotoxin  $\beta$  receptor fusion protein prevented involution of the thymus, as did transplantation of STAT4<sup>-/-</sup> (introducing IL-12 nonresponsiveness of T cells) bone marrow or coadministration of bona fide  $CD4^+CD25^+$   $T_{reg}$  cells. More importantly,

CD4<sup>+</sup>CD25<sup>+</sup> T cells derived from untreated BM $\Rightarrow$ tg $\epsilon$ 26<sub>adult</sub> mice were incapable of exerting regulatory function (besides being substantially diminished in numbers), whereas those derived from treated mice were fully functional. Corresponding data derived from an adapted CD45RB<sup>high</sup> transfer model of colitis led the authors to postulate a broader role of these effects of colitis on T<sub>reg</sub> cell development and function.<sup>18</sup>

These studies support the novel concept that the effector T cells, which drive the colitis and/or the colitic environment, might negatively regulate the development of T<sub>reg</sub> cells and the thymus in general. This in turn would predict that the colitis phenotype might be self-perpetuating. This also raises the interesting possibility that an initial insult on the colon (such as an intestinal infection) in a genetically susceptible host destined to develop IBD could initiate a process that starts with colitis and is followed by alterations in thymic function and thymic T<sub>reg</sub> generation with the latter leading to perpetuation of colitis and, over time, to the development of IBD.

There are several different hypothetical models to explain how colitis might regulate thymic function, particularly T<sub>reg</sub> generation. One attractive possibility is that the effector T cells that drive colitis can come back to the thymus and directly attack the thymic epithelium that normally selects T<sub>regs</sub> and/or the thymocytes from which T<sub>regs</sub> develop. Another possibility is that the inflamed colonic mucosa might release cytokines that impede thymic T<sub>reg</sub> development. In this case, T cells or other inflammatory cells in the colon might remotely regulate thymic function. A nice example of this kind of regulation in another organ system is receptor activator of NF- $\kappa$ B ligand (RANKL) derived from activated T cells that remotely regulates bone turnover.<sup>22</sup> In this context, the question arises whether the type of thymic deterioration reported by Faubion et al.<sup>18</sup> is specific for colitis or a broader phenomenon also occurring with chronic inflammatory diseases other than colitis. Additionally, inflammatory mediators might directly inhibit both the regulatory activity and/or development of T<sub>regs</sub> by counteracting transforming growth factor  $\beta$  or IL-10 and their signalling pathways, hence providing a nonpermissive environment for T<sub>reg</sub> cell development and function.<sup>2</sup>

Although colitis apparently causes a deterioration in thymic function and T<sub>reg</sub> cell generation, it might concomitantly regulate other regulatory cell types. Mizoguchi et al.<sup>23</sup> recently showed that chronic intestinal inflammatory condition in TCR $\alpha$ -deficient mice leads to the generation of IL-10-producing regulatory B cells that are induced in gut-associated lymphoid tissue and characterized by CD1d expression. These cells act via

inhibition of IL-1 pathways and signal transducer and activator of transcription (STAT)3 activation rather than regulating secretion of proinflammatory cytokines by T helper cells.<sup>23</sup> The results by Faubion et al.<sup>18</sup> causes one to wonder whether other environments, such as the bone marrow, which are involved in the development of regulatory cells, might also be adversely affected by chronic intestinal inflammation.

The current report might also shed new light onto the observation that patients with Crohn's disease who have undergone allogeneic bone-marrow transplantation seem to be largely protected from further flares of IBD.<sup>24</sup> Might this be caused by reversing otherwise abnormal thymic function?

Oxazolone colitis, a murine model of ulcerative colitis, is regulated by TCR $\alpha$ -invariant NK T (iNKT) cells, which are positively selected by CD1d-expressing CD4<sup>+</sup>CD8<sup>+</sup> immature cortical thymocytes and not by thymic epithelial cells.<sup>25</sup> TCR $\alpha$ -invariant NK T cells exert several regulatory functions and may be considered to be an early link between innate and adaptive immunity.<sup>26</sup> NK T cells have been shown to both promote and inhibit colitis.<sup>27,28</sup> Given the profound effects of colitis on thymic function and cellularity reported by Faubion et al., one might predict that colitis might also affect iNKT T-cell numbers and function through an impact on the thymus with concomitant effects on disease phenotype.

In this regard, the current report may shed further light on the relapsing-remitting course of IBD. Interception of this potentially self-amplifying circuit of colitis and T<sub>reg</sub> depletion early in the onset of a flare might therefore be considered an effective approach to disease management. As the authors speculate, it will be particularly interesting to see whether the long-lasting remissions induced by anti-tumor necrosis factor  $\alpha$  agents such as infliximab or immunomodulators such as azathioprine/6-mercaptopurine<sup>29</sup> might be related to the restoration of thymic function by potently inhibiting colonic inflammation in Crohn's disease and/or restoring T<sub>reg</sub> development.<sup>30</sup>

In summary, the present article by Faubion et al.<sup>18</sup> could open a very novel way of viewing T<sub>reg</sub> cells in the course of colitis and, indeed, could direct the focus of therapeutic intervention toward a new target: the thymus. It will be extremely exciting to see how this new concept unfolds and perhaps translates into clinical medicine.

ARTHUR KASER  
RICHARD S. BLUMBERG  
*Gastroenterology Division  
Brigham and Women's Hospital  
Harvard Medical School  
Boston, Massachusetts*

## References

- Bouma G, Strober W. The immunological and genetic basis of inflammatory bowel disease. *Nat Rev Immunol* 2003;3:521–533.
- Strober W, Fuss IJ, Blumberg RS. The immunology of mucosal models of inflammation. *Annu Rev Immunol* 2002;20:495–549.
- Powrie F, Leach MW, Mauze S, Caddle LB, Coffman RL. Phenotypically distinct subsets of CD4+ T cells induce or protect from chronic intestinal inflammation in C. B-17 scid mice. *Int Immunol* 1993;5:1461–1471.
- Nagler-Anderson C, Bhan AK, Podolsky DK, Terhorst C. Control freaks: immune regulatory cells. *Nat Immunol* 2004;5:119–122.
- Maloy KJ, Powrie F. Regulatory T cells in the control of immune pathology. *Nat Immunol* 2001;2:816–822.
- Mottet C, Uhlig HH, Powrie F. Cutting edge: cure of colitis by CD4+CD25+ regulatory T cells. *J Immunol* 2003;170:3939–3943.
- Liu H, Hu B, Xu D, Liew FY. CD4+CD25+ regulatory T cells cure murine colitis: the role of IL-10, TGF-beta, and CTLA4. *J Immunol* 2003;171:5012–5017.
- Jonuleit H, Schmitt E. The regulatory T cell family: distinct subsets and their interrelations. *J Immunol* 2003;171:6323–6327.
- Hori S, Nomura T, Sakaguchi S. Control of regulatory T cell development by the transcription factor Foxp3. *Science* 2003;299:1057–1061.
- Fontenot JD, Gavin MA, Rudensky AY. Foxp3 programs the development and function of CD4+CD25+ regulatory T cells. *Nat Immunol* 2003;4:330–336.
- Khattri R, Cox T, Yasayko SA, Ramsdell F. An essential role for Scurfin in CD4+CD25+ T regulatory cells. *Nat Immunol* 2003;4:337–342.
- von Boehmer H, Aifantis I, Gounari F, Azogui O, Haughn L, Apostolou I, Jaeckel E, Grassi F, Klein L. Thymic selection revisited: how essential is it? *Immunol Rev* 2003;191:62–78.
- Jordan MS, Riley MP, von Boehmer H, Caton AJ. Anergy and suppression regulate CD4(+) T cell responses to a self peptide. *Eur J Immunol* 2000;30:136–144.
- Jordan MS, Boesteanu A, Reed AJ, Petrone AL, Hohenbeck AE, Lerman MA, Naji A, Caton AJ. Thymic selection of CD4+CD25+ regulatory T cells induced by an agonist self-peptide. *Nat Immunol* 2001;2:301–306.
- Apostolou I, Sarukhan A, Klein L, von Boehmer H. Origin of regulatory T cells with known specificity for antigen. *Nat Immunol* 2002;3:756–763.
- Walker MR, Kaspirowicz DJ, Gersuk VH, Benard A, Van Landeghen M, Buckner JH, Ziegler SF. Induction of FoxP3 and acquisition of T regulatory activity by stimulated human CD4+. *J Clin Invest* 2003;112:1437–1443.
- Sakaguchi S. The origin of FOXP3-expressing CD4+ regulatory T cells: thymus or periphery. *J Clin Invest* 2003;112:1310–1312.
- Faubion WA, de Jong YP, Molina AA, Ji H, Clarke K, Wang B, Mizoguchi E, Simpson SJ, Bhan AK, Terhorst C. Colitis is associated with thymic destruction attenuating CD4+25+ regulatory T cells in the periphery. *Gastroenterology* 2004;126:1759–1771.
- Hollander GA, Simpson SJ, Mizoguchi E, Nichogiannopoulou A, She J, Gutierrez-Ramos JC, Bhan AK, Burakoff SJ, Wang B, Terhorst C. Severe colitis in mice with aberrant thymic selection. *Immunity* 1995;3:27–38.
- Wang B, Levelt C, Salio M, Zheng D, Sancho J, Liu CP, She J, Huang M, Higgins K, Sunshine MJ. Over-expression of CD3 epsilon transgenes blocks T lymphocyte development. *Int Immunol* 1995;7:435–448.
- Wang B, Simpson SJ, Hollander GA, Terhorst C. Development and function of T lymphocytes and natural killer cells after bone marrow transplantation of severely immunodeficient mice. *Immunol Rev* 1997;157:53–60.
- Ashcroft AJ, Cruickshank SM, Croucher PI, Perry MJ, Rollinson S, Lippitt JM, Child JA, Dunstan C, Felsburg PJ, Morgan GJ, Carding SR. Colonic dendritic cells, intestinal inflammation, and T cell-mediated bone destruction are modulated by recombinant osteoprotegerin. *Immunity* 2003;19:849–861.
- Mizoguchi A, Mizoguchi E, Takedatsu H, Blumberg RS, Bhan AK. Chronic intestinal inflammatory condition generates IL-10-producing regulatory B cell subset characterized by CD1d upregulation. *Immunity* 2002;16:219–230.
- Lopez-Cubero SO, Sullivan KM, McDonald GB. Course of Crohn's disease after allogeneic marrow transplantation. *Gastroenterology* 1998;114:433–440.
- Bendelac A. Positive selection of mouse NK1+ T cells by CD1-expressing cortical thymocytes. *J Exp Med* 1995;182:2091–2096.
- Kronenberg M, Gapin L. The unconventional lifestyle of NKT cells. *Nat Rev Immunol* 2002;2:557–568.
- Heller F, Fuss IJ, Nieuwenhuis EE, Blumberg RS, Strober W. Oxazolone colitis, a Th2 colitis model resembling ulcerative colitis, is mediated by IL-13-producing NK-T cells. *Immunity* 2002;17:629–638.
- Sauberermann LJ, Beck P, de Jong YP, Pitman RS, Ryan MS, Kim HS, Exley M, Snapper S, Balk SP, Hagen SJ, Kanauchi O, Motoki K, Sakai T, Terhorst C, Koezuka Y, Podolsky DK, Blumberg RS. Activation of natural killer T cells by alpha-galactosylceramide in the presence of CD1d provides protection against colitis in mice. *Gastroenterology* 2000;119:119–128.
- Tiede I, Fritz G, Strand S, Poppe D, Dvorsky R, Strand D, Lehr HA, Wirtz S, Becker C, Atreya R, Mudter J, Hildner K, Bartsch B, Holtmann M, Blumberg R, Walczak H, Iven H, Galle PR, Ahmadian MR, Neurath MF. CD28-dependent Rac1 activation is the molecular target of azathioprine in primary human CD4+ T lymphocytes. *J Clin Invest* 2003;111:1133–1145.
- Targan SR, Hanauer SB, van Deventer SJ, Mayer L, Present DH, Braakman T, DeWoody KL, Schaible TF, Rutgeerts PJ. A short-term study of chimeric monoclonal antibody cA2 to tumor necrosis factor alpha for Crohn's disease. Crohn's Disease cA2 Study Group. *N Engl J Med* 1997;337:1029–1035.

---

Address correspondence to: Richard S. Blumberg, M.D., Gastroenterology Division, Brigham and Women's Hospital, Harvard Medical School, Thorn Research Building, Room 1419, 20 Shattuck St, Boston, Massachusetts 02115. e-mail: rblumberg@partners.org; fax: (617) 264-5185.

Supported by National Institutes of Health Grants DK44319, DK51362, and DK53056 (to R.S.B.).

A.K. was supported by the Max Kade foundation.

© 2004 by the American Gastroenterological Association

0016-5085/04/\$30.00

doi:10.1053/j.gastro.2004.04.027