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1. Scott RD. The Direct Medical Costs of Healthcare-Associated Infections in U.S. Hospitals and the Benefits of Prevention, 2009. Division of Healthcare Quality Promotion, National Center for Preparedness, Detection, and Control of Infectious Diseases, Coordinating Center for Infectious Diseases, Centers for Disease Control and Prevention, February 2009.
2. Magauran CE, Salgado CD. Challenges and advances in infection control of hematopoietic stem cell transplant recipients [published online ahead of print August 10, 2010]. *Infect Disord Drug Targets*. doi:BSP/ID DT/E-Pub/-0039-10-5 [pii].
3. Salgado CD, Ison MG. Should clinicians worry about vancomycin-resistant *Enterococcus* bloodstream infections? *Bone Marrow Transplant*. 2006; 38(12):771–774.
4. Ubeda C, et al. Vancomycin-resistant *Enterococcus* domination of intestinal microbiota is enabled by antibiotic treatment in mice and precedes bloodstream invasion in humans. *J Clin Invest*. 2010; 120(12):4332–4341.
5. Artis D. Epithelial-cell recognition of commensal bacteria and maintenance of immune homeostasis in the gut. *Nat Rev Immunol*. 2008;8(6):411–420.
6. Zaph C, et al. Epithelial-cell-intrinsic IKK- β expression regulates intestinal immune homeostasis. *Nature*. 2007;446(7135):552–556.
7. Ivanov II, et al. Induction of intestinal Th17 cells by segmented filamentous bacteria. *Cell*. 2009; 139(3):485–498.
8. Lee YK, Menezes JS, Umesaki Y, Mazmanian SK. Microbes and Health Sackler Colloquium: Proinflammatory T-cell responses to gut microbiota promote experimental autoimmune encephalomyelitis [published online ahead of print July 28, 2010]. *Proc Natl Acad Sci U S A*. doi:10.1073/pnas.1000082107.
9. Stepankova R, et al. Segmented filamentous bacteria in a defined bacterial cocktail induce intestinal inflammation in SCID mice reconstituted with CD45RBhigh CD4⁺ T cells. *Inflamm Bowel Dis*. 2007; 13(10):1202–1211.
10. Dethlefsen L, Huse S, Sogin ML, Relman DA. The pervasive effects of an antibiotic on the human gut microbiota, as revealed by deep 16S rRNA sequencing. *PLoS Biol*. 2008;6(11):e280.
11. Brandl K, et al. Vancomycin-resistant enterococci exploit antibiotic-induced innate immune deficits. *Nature*. 2008;455(7214):804–807.
12. Rakoff-Nahoum S, Paglino J, Eslami-Varzaneh F, Edberg S, Medzhitov R. Recognition of commensal microflora by toll-like receptors is required for intestinal homeostasis. *Cell*. 2004;118(2):229–241.
13. Rakoff-Nahoum S, Hao L, Medzhitov R. Role of toll-like receptors in spontaneous commensal-dependent colitis. *Immunity*. 2006;25(2):319–329.
14. Nenci A, et al. Epithelial NEMO links innate immunity to chronic intestinal inflammation. *Nature*. 2007;446(7135):557–561.
15. Keilbaugh SA, et al. Activation of RegIII β /gamma and interferon gamma expression in the intestinal tract of SCID mice: an innate response to bacterial colonisation of the gut. *Gut*. 2005;54(5):623–629.
16. Cash HL, Whitham CV, Behrendt CL, Hooper LV. Symbiotic bacteria direct expression of an intestinal bactericidal lectin. *Science*. 2006; 313(5790):1126–1130.
17. Wang ML, et al. Regulation of RELM/FIZZ isoform expression by Cdx2 in response to innate and adaptive immune stimulation in the intestine. *Am J Physiol Gastrointest Liver Physiol*. 2005;288(5):G1074–G1083.
18. Hill DA, et al. Metagenomic analyses reveal antibiotic-induced temporal and spatial changes in intestinal microbiota with associated alterations in immune cell homeostasis. *Mucosal Immunol*. 2010;3(2):148–158.
19. Sekirov I, et al. Antibiotic-induced perturbations of the intestinal microbiota alter host susceptibility to enteric infection. *Infect Immun*. 2008; 76(10):4726–4736.
20. Crosswell A, Amir E, Teggatz P, Barman M, Salzman NH. Prolonged impact of antibiotics on intestinal microbial ecology and susceptibility to enteric *Salmonella* infection. *Infect Immun*. 2009;77(7):2741–2753.
21. Garner CD, et al. Perturbation of the small intestine microbial ecology by streptomycin alters pathology in a *Salmonella* enterica serovar typhimurium murine model of infection. *Infect Immun*. 2009; 77(7):2691–2702.
22. Cerutti A, Rescigno M. The biology of intestinal immunoglobulin A responses. *Immunity*. 2008; 28(6):740–750.
23. Strober W. The multifaceted influence of the mucosal microflora on mucosal dendritic cell responses. *Immunity*. 2009;31(3):377–388.
24. Abt MC, Artis D. The intestinal microbiota in health and disease: the influence of microbial products on immune cell homeostasis. *Curr Opin Gastroenterol*. 2009;25(6):496–502.
25. Mucida D, Park Y, Cheroutre H. From the diet to the nucleus: vitamin A and TGF- β join efforts at the mucosal interface of the intestine. *Semin Immunol*. 2009;21(1):14–21.

Integral role of integrins in Th17 development

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A lineage of CD4⁺ T cells known as Th17 cells, which are derived by exposure of naive CD4⁺ T cells to IL-6 and TGF- β , have been implicated in several autoimmune diseases. In this issue of the *JCI*, studies by Acharya et al. and Melton et al. show that TGF- β is activated at the DC/CD4⁺ T cell synapse by α v integrins and that this activation is required for Th17 differentiation and autoimmunity in the central nervous system. Thus, these studies offer a potential therapeutic target in fighting autoimmune diseases.

Th17 cells are a recently identified and critical component of the adaptive immune system (1–3). They are characterized by the production of IL-17A and IL-17F as well as other cytokines such as IL-22. These effector cytokines have been shown to be critical for clearance of certain bacteria and fungal pathogens (4). In addition, vaccine-induced Th17 cells have been shown to have broad protective roles against extracellular patho-

gens such as *Streptococcus pneumoniae* and to control Th1 cell migration in the context of vaccination against the intracellular pathogen *Mycobacterium tuberculosis* (4). However, this protective aspect of the Th17 lineage comes at a cost, as these cells have been implicated in autoimmune diseases such as multiple sclerosis, psoriasis, and rheumatoid arthritis (1–3).

Several groups have shown that naive CD4⁺ T cells differentiate into Tregs in the presence of TGF- β (5, 6). However, in the presence of TGF- β and IL-6, naive CD4⁺ T cells differentiate into Th17 cells (6–8). Early work by Li et al. (9) showed that the

source of TGF- β in this context was the CD4⁺ T cell. However, TGF- β is secreted from cells in an inactive form, in which bioactive TGF- β is in a complex with its latency-associated peptide (LAP) through noncovalent bonds. Two studies in this issue of the *JCI* demonstrate that DCs activate TGF- β in an integrin-dependent fashion (10, 11), suggesting that the activation of TGF- β occurs at the DC/T cell synapse (Figure 1) and that this activation is required to drive the differentiation of Th17 T cells.

TGF- β and integrins

TGF- β is a multifunctional cytokine involved in many aspects of immunology, angiogenesis, and epithelial growth as well as in pathogenic states such as fibrosis (12). Activation of TGF- β has been an area of intense study. Mechanisms identified as leading to the disruption of the noncovalent interaction between LAP and bioactive TGF- β and thus activation of TGF- β

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Figure 1

Schematic representation of Th17 differentiation. Two studies in this issue of the *JCI* (10, 11) demonstrate that TGF- β is activated at the DC/CD4⁺ T cell synapse by α v integrins and that this activation is required for Th17 differentiation in vitro. Moreover, mice lacking α v integrins on DCs fail to develop EAE, a disease mediated by Th17 cells.

include low pH, heat, reactive oxygen species produced as a result of environmental exposures, and LAP cleavage by proteases such as thrombin, elastase, MMP-2, and MMP-9 (13). Because of the ubiquitous expression of TGF- β by many cell types, indiscriminate activation of TGF- β is not advantageous. A more spatially regulated activation occurs through latent TGF- β binding to integrins at the cell surface, which allows activation of TGF- β in a more regulated and localized manner (13).

Integrins are a family of heterodimeric cell surface receptors consisting of an α and a β subunit. There are 24 total integrin subunits (18 α and 6 β). Among the integrins, five share the α v subunit (α v β 1, α v β 3, α v β 5, α v β 6, and α v β 8) and are capable of binding the RGD tripeptide sequence on the LAP of TGF- β (1). Studies in mice that have a mutation converting the RGD sequence of LAP to RGE demonstrate the same embryonic lethality and inflammatory phenotype as mice lacking TGF- β (14), suggesting integ-

rin-mediated activation of TGF- β is critical in development. There are two proposed mechanisms of integrin-dependent activation of TGF- β . In the case of integrins that are bound to the cytoskeleton, such as integrin α v β 6, binding of TGF- β induces a conformational change upon the latent complex of TGF- β , allowing the active portion of TGF- β to be exposed to its receptor, without breaking the LAP/TGF- β bonds (15). Integrin α v β 8 lacks this cytoskeletal connection. In its case, the integrin acts as an anchor for TGF- β , allowing proteolysis by membrane-bound MMP-14 (also known as mt1-MMP) (16). Both of these integrin-related mechanisms allow TGF- β to be activated in a very focal manner, which may be important in the context of Th17 differentiation.

Integrin-mediated Th17 development

In this issue of the *JCI*, two complementary papers demonstrate the requirement of integrin α v β 8 activation of TGF- β in the differentiation of Th17 cells (10, 11). Previ-

ous work using conditional knockout mice has shown that mice lacking either α v (17) or α v β 8 (18) in myeloid cells develop colitis and a spontaneous autoimmune disease, believed to be due to the inability of these mice to activate TGF- β and develop Tregs. Acharya et al. (10) have now considered the common requirement for TGF- β in the development of Tregs and Th17 cells and find that conditional knockout mice (which they generated using *tie2-cre* and termed α -*tie2* mice) that lack integrin α v on all hematopoietic cells have reduced proportions of Th17 cells in the lamina propria. However, CD4⁺ T cells from these mice were capable of differentiating into Th17 cells when supplied with exogenous TGF- β in vitro (10). By crossing mice with a floxed *Itgav* allele (i.e., the allele that encodes α v) to *LysM-cre* mice, which allowed expression of α v integrins on lymphoid cells but not on macrophages and DCs, the authors demonstrated that integrin α v expression on *LysM*-expressing cells was required for the TGF- β activation that is required for Th17 cell generation in α -*tie2* mice (10). While these data demonstrate the importance of α v, they do not completely identify which integrin is responsible, as mice lacking α v are incapable of making α v β 1, α v β 3, α v β 5, α v β 6, and α v β 8. In support of this work, Melton et al. (11) show a similar phenotype of markedly reduced numbers of Th17 cells in the lamina propria of mice lacking integrin β 8 expression on DCs (mice that they term β 8^{fl/fl} $\times$ CD11c-*cre* mice) (11). Using the experimental model of autoimmune encephalitis (EAE), which is Th17 dependant, neither the α -*tie2* mice (10) nor the β 8^{fl/fl} $\times$ CD11c-*cre* mice developed EAE (11). To understand what role integrin α v β 8 may have in Th17 development, both groups looked at cytokines involved in Th17 polarization. There were no differences in IL-6, IL-23, TGF- β (10, 11), or IL-1 β (10) expression after immunization in the EAE model. Further, IFN- γ , which inhibits Th17 development, was not increased in β 8^{fl/fl} $\times$ CD11c-*cre* mice, and, thus, the reduced Th17 polarization in vivo could not be explained by enhanced Th1 polarization (11). In vitro, both groups of investigators showed that DCs were required to activate TGF- β , as naive CD4⁺ T cells did not differentiate in the presence of latent TGF- β unless DCs were present (10, 11). Further, this activation did not occur in the presence of DCs from either α -*tie2* or β 8^{fl/fl} $\times$ CD11c-*cre* mice or in the presence of RGD mimetics (10) or TGF- β antibodies (11). Interestingly, this activation required cognate interaction

between the CD4⁺ T cells and DCs (Figure 1), as MHC class II-mismatched DCs, which are unable to present antigen to T cells, did not induce Th17 differentiation (10, 11).

Conclusions

Given the importance of IL-17 in autoimmune disease, the mechanisms of Th17 differentiation are under extensive study. The works presented by Acharya et al. (10) and Melton et al. (11) demonstrate a novel mechanism for the development of Th17 cells, in which naive CD4⁺ T cells recognize antigens presented by DCs in an MHC class II-dependent manner, while at the same time inducing the cell to differentiate to a Th17 cell through the activation of TGF- β by an integrin α v β 8-dependant mechanism (Figure 1). While these studies do not explain the production of IL-17 by other sources, such as γ δ T cells, they do offer insight into the development of an important cell lineage that is implicated in autoimmune states. They also suggest the use of RGD mimetics to block the activation of TGF- β could be a feasible therapy to reduce the severity of Th17-related diseases. Recently, however, work by Ghoreschi et al. demonstrates that Th17 cells can develop in the absence of TGF- β , and Th17 cells grown in these conditions show enhanced pathogenic potential after adoptive transfer (19). These data highlight the complexities of Th17 differentiation and suggest that it will be important to under-

stand the origins and phenotypes of Th17 cells (and their nuanced subsets) in order to develop therapeutic approaches.

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1. Weaver CT, Hatton RD, Mangan PR, Harrington LE. IL-17 family cytokines and the expanding diversity of effector T cell lineages. *Annu Rev Immunol.* 2007;25:821–852.
2. Korn T, Bettelli E, Oukka M, Kuchroo VK. IL-17 and Th17 cells. *Annu Rev Immunol.* 2009;27:485–517.
3. Dong C. TH17 cells in development: an updated view of their molecular identity and genetic programming. *Nat Rev Immunol.* 2008;8(5):337–348.
4. Khader SA, Gaffen SL, Kolls JK. Th17 cells at the crossroads of innate and adaptive immunity against infectious diseases at the mucosa. *Mucosal Immunol.* 2009;2(5):403–411.
5. Shevach EM. Mechanisms of foxp3⁺ T regulatory cell-mediated suppression. *Immunity.* 2009;30(5):636–645.
6. Bettelli E, et al. Reciprocal developmental pathways for the generation of pathogenic effector TH17 and regulatory T cells. *Nature.* 2006;441(7090):235–238.
7. Mangan PR, et al. Transforming growth factor- β induces development of the TH17 lineage. *Nature.* 2006;441(7090):231–234.
8. Veldhoen M, Hocking RJ, Atkins CJ, Locksley RM,

Stocker B. TGF β in the context of an inflammatory cytokine milieu supports de novo differentiation of IL-17-producing T cells. *Immunity.* 2006;24(2):179–189.

9. Li MO, Wan YY, Flavell RA. T cell-produced transforming growth factor- β 1 controls T cell tolerance and regulates Th1- and Th17-cell differentiation. *Immunity.* 2007;26(5):579–591.
10. Acharya M, et al. α v Integrin expression by DCs is required for Th17 cell differentiation and development of experimental autoimmune encephalomyelitis in mice. *J Clin Invest.* 2010;120(12):4445–4452.
11. Melton AC, Bailey-Bucktrout SL, Travis MA, Fife BT, Bluestone JA, Sheppard D. Expression of α v β 8 integrin on dendritic cells regulates Th17 cell development and experimental autoimmune encephalomyelitis in mice. *J Clin Invest.* 2010;120(12):4436–4444.
12. Mantel PY, Schmidt-Weber CB. Transforming growth factor- β : recent advances on its role in immune tolerance. *Methods Mol Biol.* 2011;677:303–338.
13. Annes JP, Munger JS, Rifkin DB. Making sense of latent TGF β activation. *J Cell Sci.* 2003;116(pt 2):217–224.
14. Yang Z, et al. Absence of integrin-mediated TGF- β 1 activation in vivo recapitulates the phenotype of TGF β 1-null mice. *J Cell Biol.* 2007;176(6):787–793.
15. Munger JS, et al. The integrin α v β 6 binds and activates latent TGF β 1: a mechanism for regulating pulmonary inflammation and fibrosis. *Cell.* 1999;96(3):319–328.
16. Mu D, et al. The integrin α (v) β 8 mediates epithelial homeostasis through MT1-MMP-dependent activation of TGF- β 1. *J Cell Biol.* 2002;157(3):493–507.
17. Lacy-Hulbert A, et al. Ulcerative colitis and autoimmunity induced by loss of myeloid α v integrins. *Proc Natl Acad Sci U S A.* 2007;104(40):15823–15828.
18. Travis MA, et al. Loss of integrin α (v) β 8 on dendritic cells causes autoimmunity and colitis in mice. *Nature.* 2007;449(7160):361–365.
19. Ghoreschi K, et al. Generation of pathogenic T(H)17 cells in the absence of TGF- β signalling. *Nature.* 2010;467(7318):967–971.

A tincture of hepcidin cures all: the potential for hepcidin therapeutics

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Iron overload as a result of blood transfusions and excessive intestinal iron absorption can be a complication of chronic anemias such as β -thalassemia. Inappropriately low levels of hepcidin, a negative regulator of iron absorption and recycling, underlie the pathophysiology of the intestinal hyperabsorption. In this issue of the *JCI*, Gardenghi et al. demonstrate that increasing hepcidin expression to induce iron deficiency in murine β -thalassemia not only mitigates the iron overload, but also the severity of the anemia. These data illustrate the therapeutic potential of modulating hepcidin expression in diseases associated with altered iron metabolism.

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Hepcidin, iron, and erythropoiesis

Erythropoiesis consumes the majority of the iron present in the human body (1). Most of this iron is obtained from the

recycling of effete red blood cells by macrophages found in the liver, spleen, and bone marrow. Interruption of iron export from macrophages leads to functional iron deficiency and iron-limited erythropoiesis. At equilibrium, only a small amount of iron is absorbed in the duodenum from the diet each day. Further, there is no physiologically regulated mechanism of eliminating excess iron from the body. Consequently, the proper regulation of dietary iron absorption as well as iron recycling is essential to maintaining iron homeostasis and to sustaining erythropoiesis.