Published in final edited form as:

Curr Opin Pharmacol. 2009 August ; 9(4): 447–453. doi:10.1016/j.coph.2009.04.008.

Anti- and Pro-inflammatory Roles of TGF- β , IL-10, and IL-22 In Immunity and Autoimmunity

Shomyseh Sanjabi¹, Lauren A. Zenewicz¹, Masahito Kamanaka¹, and Richard A. Flavell^{1,2,*}

¹Yale University School of Medicine, New Haven, CT 06520 (U.S.A.)

²Howard Hughes Medical Institute

Abstract

Cytokines play a major role in maintaining lymphocyte homeostasis under both steady-state and inflammatory conditions. Unregulated lymphocytes in steady-state conditions can lead to autoimmunity, whereas during inflammation they can cause excessive tissue damage. Regulatory cytokines function in combination with other environmental signals to properly modulate the function and the extent of lymphocyte activation. Many recent studies have highlighted the importance of regulatory cytokines in controlling the differentiation and function of lymphocytes under steady-state and inflammatory conditions, as well as minimizing tissue damage.

A healthy immune system will maintain tolerance against autoreactive T cells and commensal bacteria under steady-state conditions, mounts an effective immune response against foreign antigens to clear the infection, assures control of activated lymphocytes to minimize damage to the host, and once the infection is cleared it returns the immune cells and the tissues back to their homeostatic state. Here we will briefly describe the actions of three regulatory cytokines, TGF- β , IL-10, and IL-22, which all participate to orchestrate this complicated sequence of events under steady-state and infectious conditions.

TGF- β

Transforming growth factor β (TGF- β) is a pleiotropic cytokine with potent regulatory and inflammatory activity [1,2]. The multi-faceted effects of TGF- β on numerous immune functions are cellular and environmental context dependent [3]. TGF- β binds to TGF- β receptor II (TGF- β RII) triggering the kinase activity of the cytoplasmic domain that in turn activates TGF- β RI. The activated receptor complex leads to nuclear translocation of Smad molecules, and transcription of target genes [3]. The role of TGF- β as an immune modulator of T cell activity is best exemplified by the similarities between TGF- β 1 knockout and T cell specific TGF- β receptor II knockout mice [4–6]. The animals in both of these models develop severe multi-organ autoimmunity and succumb to death within a few weeks after birth [4–6]. In addition, in mice where TGF- β signaling is blocked specifically in T cells, the development of natural killer T (NKT) cells, natural regulatory T (nTreg) cells, and CD8⁺ T cells was shown to be dependent on TGF- β signaling in the thymus [4,5].

© 2009 Elsevier Ltd. All rights reserved.

*Correspondence should be addressed to: Richard A. Flavell, Ph.D., FRS, Department of Immunobiology, Yale School of Medicine, 300 Cedar Street, TAC S-569, PO Box 208011, New Haven, CT 06520, richard.flavell@yale.edu, Phone: 203-737-2216, Fax: 203-737-2958.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

TGF- β is best known for its regulatory activity and induction of peripheral tolerance. Some of the self-reactive T cells in the periphery form as a result of incomplete presentation of self-antigens in the thymus and are thus not eliminated through negative selection. Regulatory mechanisms in the periphery assure that these self-reactive T cells do not cause autoimmunity [7]. However, positively selected thymocytes also retain some self-reactivity, which is essential for survival of naïve T cells that requires TCR signaling and cytokines such as IL-7 [8]. Due to the intact peripheral tolerance mechanisms, this self-reactivity is generally not sufficient to cause autoimmunity. Thus, in the periphery, TGF- β is necessary for the survival of naïve T cells and it also maintains peripheral tolerance by inhibiting the proliferation and differentiation of self-reactive CD4⁺ and CD8⁺ T cells [2,3]. Protection from self-reactive T cells by TGF- β is best demonstrated by the studies where TGF- β knockout mice maintained under germ-free conditions still develop an autoimmune phenotype [9]. One of the mechanisms by which TGF- β is able to maintain peripheral tolerance is to maintain the survival of naturally occurring Treg cells [4,5]. In addition, in combination with IL-2 and retinoic acid (RA), TGF- β promotes the differentiation of induced Treg cells (iTreg) [10–13]. Interestingly, it has recently been shown that this is due to an indirect effect of RA, which reduces the expression of IL-4, IL-21, and IFN- γ by the CD4⁺ CD44^{hi} cell population, thus releasing their inhibitory effect on TGF- β -driven Foxp3 expression [14].

TGF- β also plays a major role under inflammatory conditions. TGF- β in the presence of IL-6 drives the differentiation of T helper 17 (Th17) cells, which can promote further inflammation and augment autoimmune conditions [15]. TGF- β orchestrates the differentiation of both Treg and Th17 cells in a concentration-dependent manner [16]. In addition, TGF- β in combination with IL-4, promotes the differentiation of IL-9- and IL-10-producing T cells, which lack suppressive function and also promote tissue inflammation [17,18]. The biological effects of TGF- β under inflammatory conditions on effector and memory CD8⁺ T cells are much less understood. In a recent study, it was shown that TGF- β has a drastically opposing role on naïve compared to antigen-experienced/memory CD8⁺ T cells [19]. When cultured *in vitro*, TGF- β suppressed naïve CD8⁺ T cell activation and IFN- γ production, whereas TGF- β enhanced survival of memory CD8⁺ T cells and increased the production of IL-17 and IFN- γ [19].

TGF- β also plays an important role in suppressing the cells of the innate immune system. Unlike when TGF- β signaling is blocked in T cells, blockade of TGF- β signaling in natural killer (NK) and dendritic cells (DCs) does not cause spontaneous disease. However, in response to *Leishmania* infection, blockade of TGF- β signaling in NK cells caused the accumulation of a large number of NK cells capable of secreting large amounts of IFN- γ , which resulted in enhanced skewing of CD4⁺ cells into a Th1 phenotype [20]. Inactivation of TGF- β signaling in DCs in combination with MOG TCR transgenic T cells caused an spontaneous EAE-like disease [21]. In addition, blocking TGF- β -Smad2/3 innate immune signaling in a Tg2576 Alzheimer's disease mouse model completely mitigates Tg2576-associated hyperactivity and partially mitigates defective spatial working memory [22]. Collectively, these studies suggest that TGF- β control of innate immune cells can have severe pathological consequences.

There is considerable interest in TGF- β as a therapeutic target, especially as a treatment for cancer [23]. Unlike most other cytokines, TGF- β is produced by many immune and non-immune cells and virtually all cell types are responsive to this pleiotropic cytokine [3]. Many anti-TGF- β compounds have been developed and their efficacy has been tested in numerous animal models [24]. However, a better basic understanding of the exact effects of TGF- β in combination with other immunomodulatory molecules, as well as the differentiation state of the cells being targeted, will greatly aid the development of new immunotherapies to combat a broad range of inflammatory, autoimmune, cancer, and even Alzheimer's disease.

IL-10

IL-10 is a key regulator of the immune system by limiting the inflammatory response which could otherwise cause tissue damage. In addition, IL-10 is essential for homeostasis of the immune system; especially in the gastrointestinal tract. Mice deficient in IL-10 signaling are highly susceptible to colitis due to aberrant immune responses to commensal bacteria; this colitis is more severe when combined with deficiency in TGF- β signaling [25,26]. During acute infections, blocking IL-10 signaling can result in more severe pathology or even fatality of the host [27]. However, high production of IL-10 is associated with sustained chronic infections and its blockade promotes pathogen clearance [27].

The broad expression of IL-10 and its receptor by the cells of the immune system has complicated the detailed understanding of the exact role of IL-10 *in vivo*. IL-10 is expressed by many types of immune cells including Th2 cells, Treg cells, Tr1 cells (IL-10 producing Foxp3⁺ CD4⁺ T cells), Th3 cells (TGF- β and IL-10 producing CD4⁺ T cells induced in oral tolerance), as well as NK T cells, B cells, macrophages, and DCs [28]. IL-10 binds to its heterodimeric receptor, composed of the IL-10R α , which is unique to IL-10 signaling and is expressed on hematopoietic cells, and IL-10R β that is also shared by IL-22 and is ubiquitously expressed [28]. Ligation of IL-10 activates Jak1 and Tyk2 followed by Stat3 phosphorylation [29]. Although not completely sufficient, Stat3 is required for the inhibitory functions of IL-10 [30]. Importantly, the IL-10 promoter itself has a Stat3 binding site, suggesting a positive feed-back loop for IL-10-Stat3 signaling [30]. Stat3 induces the expression of Socs3 that regulates various cytokine signaling pathways including IL-6 [29]. Interestingly, Stat3 has a bi-modal function; it is anti-inflammatory for IL-10 while pro-inflammatory for IL-6 signaling [29]. In addition to modulating Stat3, other signaling pathways such as inhibition of NF- κ B play a role in the anti-inflammatory function of IL-10 [30]. Similar to TGF- β , responses to IL-10 vary depending on the target cell. Greatest focus has been on the effects of IL-10 on antigen-presenting cells, in particular macrophages. IL-10 downregulates IL-12 production and expression of co-stimulatory molecules in macrophages, thereby reducing the generation of a Th1 response [28]. A more detailed and complete understanding of IL-10 signaling on different subsets of immune cells requires further investigation.

To determine which cells express IL-10 during immune homeostasis, several groups have generated IL-10 reporter mice [31–33]. Using IL-10 reporter mice, it was shown that the ubiquitous expression of IL-10 is most frequently found in the intestinal tissues. Intraepithelial lymphocytes (IEL) from the small intestine and lamina propria lymphocytes (LPL) from the colon showed the highest frequency of IL-10 expressing cells. By crossing IL-10 reporter mice to Foxp3 reporter mice, the transcription factor that characterizes Treg cells, evaluation of IL-10 expression in Foxp3-positive and -negative populations became possible [32,33]. IL-10 producers in the IEL population were negative for Foxp3 while those in the colonic LPL population were mostly Foxp3 positive. Of note, tolerance-inducing procedures using repetitive TCR stimulation, using an anti-CD3 antibody, led to IL-10 expression most strongly in the small intestine [32]. These studies demonstrated that the systemic tolerance induction procedure induces IL-10-expressing intestinal T cells and that the intestine may be a major site in the induction of immunological tolerance. In addition, *Leishmania major* infection of the reporter mice revealed that IL-10 expressing cells are preferentially induced in the inflammatory tissue more than in the draining lymph nodes, demonstrating an inhibitory, rather than a tolerizing, role for IL-10 under infectious conditions [32]. Recent findings show that macrophages in the lamina propria preferentially induce IL-10 producing cells while DCs promote the generation of Th17 cells [34]. Thus, the environment of the intestine favors the generation of IL-10 producing T cells that play a role in mediating tolerance against commensal bacteria, whereas the expression of IL-10 in peripheral tissues under infectious conditions leads to down-modulation of the immune response.

Many *in vitro* culture conditions have been described that generate IL-10 expressing T cells. Recently, IL-27 was reported to induce IL-10 producing T cells, and addition of TGF- β further enhanced the recovery of IL-10 producing cells [35–37]. *In vivo*, Th1 cells are a source of IL-10 in certain types of infections [38,39], where IL-10 expression depends on IL-27 [37]. In addition, a combination of TGF- β and IL-6 induces Th17 cells *in vitro*, but also strongly directs T cells to produce IL-10 [37,40]. Either TGF- β or IL-6 alone is not enough to produce IL-10 expressing cells. Interestingly, both regimens (IL-27 or IL-6 and TGF- β) require Stat3 for developing IL-10 producing cells, but only the former also requires Stat1 [37]. The exact significance of these *in vitro* culture conditions has to be further evaluated in more physiological *in vivo* models.

Since its discovery, almost 20 years ago, IL-10 has been intensively studied. Because of its strong immunomodulatory function, IL-10 and its signaling components are good candidates for immunological intervention for disease control. Indeed, several clinical studies administering IL-10 have been performed for various chronic inflammatory diseases such as Crohn's disease, psoriasis, and rheumatoid arthritis, albeit with limited success [41]. Further studies in understanding the IL-10 signaling mechanism and the regulation of IL-10 expression in a context dependent manner, will aid in the development of effective treatments for many infectious and autoimmune diseases.

IL-22

Interleukin-22 (IL-22) is a member of the IL-10 related cytokine family, which also includes IL-19, IL-20, IL-24 and IL-26 [42–44]. The IL-22 receptor is highly expressed within tissues, such as epithelial cells of the gastrointestinal tract and skin [45,46]. IL-22 signals through a heterodimer comprised of IL-22R and IL-10R β [47]. Similar to IL-10, IL-22 activates Stat3 signaling pathways in target cells. IL-22 signaling leads to activation of proliferative and/or anti-apoptotic programs, allowing maintenance of epithelial barriers, such as those of the lungs or gastrointestinal tract during inflammation [48–50]. IL-22 also induces tissue expression of acute inflammatory proteins, mucins or antimicrobial peptides, which are important for tissues to maintain their integrity during inflammation, allowing for proper organ function and sequestration of potential pathogens [43,51–53].

IL-22 is highly expressed in the adaptive immune system by the recently identified Th17 cell subset [51]. These CD4⁺ T cells were first classified by their high expression of IL-17, hence their name, but have also been shown to selectively express IL-22 [54]. However, IL-22 appears to be differentially regulated from the other cytokines, which are highly dependent on the transcription factor retinoic acid orphan receptor γ t (ROR γ t) for their expression [54]. Instead, IL-22 expression is dependent on the transcription factor aryl hydrocarbon receptor (AHR) [55], best known for its role in dioxin toxicity. AHR ligands enhance Th17 cytokine production; interestingly however, the transcription factor AHR is essential for IL-22 expression, but not for expression of other Th17 cytokines [55].

IL-22 is also expressed by cells of the innate immune system. Recent studies have identified a subset of NK cells in the colon that express IL-22 [44,56–59]. Unlike traditionally described NK cells, these cells do not express high levels of IFN γ and are not highly cytotoxic [56]. IL-23, an important heterodimeric cytokine in Th17 biology that shares a subunit with IL-12, a traditional activator of NK cells, induces IL-22 expression in NK cells [44,56]. The identification of signaling pathways and transcription factors important for this expression is a matter of ongoing research.

IL-22 has a dual nature, protective versus inflammatory, in modulating the responses of the tissue during an immune response. In a mouse model of dermal inflammation in which IL-23 is directly applied to the ear, IL-22 was essential in mediating inflammation [60]. Stimulation

of keratinocytes with IL-22 leads to induction of the pro-inflammatory molecules such as S100A7 (also termed psoriasin), that promote psoriasis, as well as induction of antimicrobial peptides such as β -defensin [48,53]. The cytokine also induces keratinocyte migration, leading to the hyperplasia of keratinocyte layers, and results in a thickening of the epidermis, which are thought to modulate tissue responses and enhance the inflammation observed in psoriasis [48]. On the other hand, IL-22 can be protective in acute inflammatory models, such as hepatitis, as well as during chronic inflammation such as inflammatory bowel disease [44,52, 61]. IL-22 is also important for control of pathogenic bacteria that need to translocate through host epithelial barriers for their dissemination. For example, it has been shown that IL-22 is critical for control of pulmonary *Klebsiella pneumoniae* infection or infection of the gastrointestinal tract with *Citrobacter rodentium* [62,63]. Thus, the dual nature of IL-22 in modulating tissue immune responses, likely depends on the inflammatory context. This includes, but is not limited to, the duration and amount of IL-22 present, the overall cytokine milieu, and the tissues involved.

IL-22 may be a potential therapeutic for chronic inflammatory diseases due to its selective modulation on tissue responses. Treatment with recombinant cytokine or gene therapy delivery of IL-22 may alleviate tissue destruction during inflammatory responses [50,52,64]. Suppressing the immune system via anti-inflammatory treatments such as TNF α inhibitors can lead to unwanted dampening of the immune response, weakening its ability to respond to infections. In contrast, IL-22 is an ideal therapeutic candidate since it will specifically modulate tissue responses and not have direct effects on the immune response.

Concluding remarks

Under steady-state conditions, TGF- β signaling in lymphocytes assures that these cells do not become activated in response to self antigens (Figure 1A), while IL-10 producing T cells in the gut maintain tolerance against commensal bacteria and food antigens (Figure 1B). Under inflammatory conditions, activation of DCs by PAMPs leads to the production of IL-10 that dampens the immune response of lymphocytes to pathogenic infections, minimizing damage to the host. Meanwhile, in combination with other inflammatory cytokines, TGF- β promotes the differentiation of Th17 cells that in turn produce a unique set of cytokines including IL-22. In a context dependent matter, IL-22 can cause further inflammation, or contribute to healing of damaged tissue (Figure 2). Unlike TGF- β and IL-10 which directly modulate the immune response, IL-22 does not have direct effects on immune cells since these cells lack the expression for IL-22 receptor (IL-22R). Similarly, unlike TGF- β and IL-10 that are involved in maintaining immune homeostasis under steady-state conditions, IL-22 is instead highly expressed during chronic inflammatory diseases and modulates tissue responses to inflammation. All three of these cytokines provide unique and attractive angles for immune modulation and immunotherapy. However, the dual and multifaceted role of these regulatory cytokines under steady, inflammatory, and anti-inflammatory conditions warrants a better understanding of the function of these cytokines in combination with other immune modulatory factors under various conditions.

Acknowledgments

The authors would like to apologize for omitting the citation of many important and significant primary articles, as they were limited by number of citations. R.A.F is an investigator of the Howard Hughes Medical Institute. This work is supported by post-doctoral fellowship grants from the Cancer Research Institute (S.S.), the American Liver Foundation and the American Cancer Society (L.A.Z), with additional support from NIH grants CA121974, DK051665, and P01AI36529 (R.A.F.), JDRF grant 32-2008-352 (R.A.F.), and the American College of Rheumatology (R.A.F.). The authors declare that they have no competing financial interests.

References

1. Li MO, Flavell RA. Contextual regulation of inflammation: a duet by transforming growth factor-beta and interleukin-10. *Immunity* 2008;28:468–476. [PubMed: 18400189]
2. Li MO, Flavell RA. TGF-beta: a master of all T cell trades. *Cell* 2008;134:392–404. [PubMed: 18692464]
3. Li MO, Wan YY, Sanjabi S, Robertson AK, Flavell RA. Transforming growth factor-beta regulation of immune responses. *Annu Rev Immunol* 2006;24:99–146. [PubMed: 16551245]
4. Li MO, Sanjabi S, Flavell RA. Transforming growth factor-beta controls development, homeostasis, and tolerance of T cells by regulatory T cell-dependent and -independent mechanisms. *Immunity* 2006;25:455–471. [PubMed: 16973386]
5. Marie JC, Liggitt D, Rudensky AY. Cellular mechanisms of fatal early-onset autoimmunity in mice with the T cell-specific targeting of transforming growth factor-beta receptor. *Immunity* 2006;25:441–454. [PubMed: 16973387]
6. Shull MM, Ormsby I, Kier AB, Pawlowski S, Diebold RJ, Yin M, Allen R, Sidman C, Proetzel G, Calvin D, et al. Targeted disruption of the mouse transforming growth factor-beta 1 gene results in multifocal inflammatory disease. *Nature* 1992;359:693–699. [PubMed: 1436033]
7. Kronenberg M, Rudensky A. Regulation of immunity by self-reactive T cells. *Nature* 2005;435:598–604. [PubMed: 15931212]
8. Surh CD, Sprent J. Homeostasis of naive and memory T cells. *Immunity* 2008;29:848–862. [PubMed: 19100699]
9. Boivin GP, Ormsby I, Jones-Carson J, O'Toole BA, Doetschman T. Germ-free and barrier-raised TGF beta 1-deficient mice have similar inflammatory lesions. *Transgenic Res* 1997;6:197–202. [PubMed: 9167267]
10. Davidson TS, DiPaolo RJ, Andersson J, Shevach EM. Cutting Edge: IL-2 is essential for TGF-beta-mediated induction of Foxp3+ T regulatory cells. *J Immunol* 2007;178:4022–4026. [PubMed: 17371955]
11. Zheng SG, Wang J, Wang P, Gray JD, Horwitz DA. IL-2 is essential for TGF-beta to convert naive CD4+CD25- cells to CD25+Foxp3+ regulatory T cells and for expansion of these cells. *J Immunol* 2007;178:2018–2027. [PubMed: 17277105]
12. Sun CM, Hall JA, Blank RB, Bouladoux N, Oukka M, Mora JR, Belkaid Y. Small intestine lamina propria dendritic cells promote de novo generation of Foxp3 T reg cells via retinoic acid. *J Exp Med* 2007;204:1775–1785. [PubMed: 17620362]
13. Coombes JL, Siddiqui KR, Arancibia-Carcamo CV, Hall J, Sun CM, Belkaid Y, Powrie F. A functionally specialized population of mucosal CD103+ DCs induces Foxp3+ regulatory T cells via a TGF-beta and retinoic acid-dependent mechanism. *J Exp Med* 2007;204:1757–1764. [PubMed: 17620361] These studies show that oral tolerance is achieved via the production of RA by the CD103+ gut-associated DCs that aid in the conversion of naïve CD4+ T cells into Treg cells.
14. Hill JA, Hall JA, Sun CM, Cai Q, Ghyselinck N, Chambon P, Belkaid Y, Mathis D, Benoist C. Retinoic acid enhances Foxp3 induction indirectly by relieving inhibition from CD4+CD44hi Cells. *Immunity* 2008;29:758–770. [PubMed: 19006694] This study demonstrates that RA functions indirectly by reducing the expression of effector cytokines by activated CD4+ T cells, which in turn releases their inhibitory effect on TGF-beta-driven Foxp3 expression.
15. Korn T, Bettelli E, Oukka M, Kuchroo VK. IL-17 and Th17 Cells. *Annu Rev Immunol*. 2009
16. Zhou L, Lopes JE, Chong MM, Ivanov II, Min R, Victora GD, Shen Y, Du J, Rubtsov YP, Rudensky AY, et al. TGF-beta-induced Foxp3 inhibits T(H)17 cell differentiation by antagonizing RORgamma function. *Nature* 2008;453:236–240. [PubMed: 18368049]
17. Dardalhon V, Awasthi A, Kwon H, Galileos G, Gao W, Sobel RA, Mitsdoerffer M, Strom TB, Elyaman W, Ho IC, et al. IL-4 inhibits TGF-beta-induced Foxp3+ T cells and, together with TGF-beta, generates IL-9+ IL-10+ Foxp3(-) effector T cells. *Nat Immunol* 2008;9:1347–1355. [PubMed: 18997793]
18. Veldhoen M, Uyttenhove C, van Snick J, Helmby H, Westendorf A, Buer J, Martin B, Wilhelm C, Stockinger B. Transforming growth factor-beta 'reprograms' the differentiation of T helper 2 cells

- and promotes an interleukin 9-producing subset. *Nat Immunol* 2008;9:1341–1346. [PubMed: 18931678]
19. Filippi CM, Juedes AE, Oldham JE, Ling E, Togher L, Peng Y, Flavell RA, von Herrath MG. Transforming growth factor-beta suppresses the activation of CD8+ T-cells when naive but promotes their survival and function once antigen experienced: a two-faced impact on autoimmunity. *Diabetes* 2008;57:2684–2692. [PubMed: 18689691]
 20. Laouar Y, Sutterwala FS, Gorelik L, Flavell RA. Transforming growth factor-beta controls T helper type 1 cell development through regulation of natural killer cell interferon-gamma. *Nat Immunol* 2005;6:600–607. [PubMed: 15852008]
 21. Laouar Y, Town T, Jeng D, Tran E, Wan Y, Kuchroo VK, Flavell RA. TGF-beta signaling in dendritic cells is a prerequisite for the control of autoimmune encephalomyelitis. *Proc Natl Acad Sci U S A* 2008;105:10865–10870. [PubMed: 18669656]
 22. Town T, Laouar Y, Pittenger C, Mori T, Szekeley CA, Tan J, Duman RS, Flavell RA. Blocking TGF-beta-Smad2/3 innate immune signaling mitigates Alzheimer-like pathology. *Nat Med* 2008;14:681–687. [PubMed: 18516051] This is the first study showing that TGF- β signaling may play a critical role in the development of Alzheimer's disease.
 23. Massague J. TGFbeta in Cancer. *Cell* 2008;134:215–230. [PubMed: 18662538]
 24. Wrzesinski SH, Wan YY, Flavell RA. Transforming growth factor-beta and the immune response: implications for anticancer therapy. *Clin Cancer Res* 2007;13:5262–5270. [PubMed: 17875754]
 25. Sellon RK, Tonkonogy S, Schultz M, Dieleman LA, Grenther W, Balish E, Rennick DM, Sartor RB. Resident enteric bacteria are necessary for development of spontaneous colitis and immune system activation in interleukin-10-deficient mice. *Infect Immun* 1998;66:5224–5231. [PubMed: 9784526]
 26. Kang SS, Bloom SM, Norian LA, Geske MJ, Flavell RA, Stappenbeck TS, Allen PM. An antibiotic-responsive mouse model of fulminant ulcerative colitis. *PLoS Med* 2008;5:e41. [PubMed: 18318596]
 27. Couper KN, Blount DG, Riley EM. IL-10: the master regulator of immunity to infection. *J Immunol* 2008;180:5771–5777. [PubMed: 18424693]
 28. Moore KW, de Waal Malefyt R, Coffman RL, O'Garra A. Interleukin-10 and the interleukin-10 receptor. *Annu Rev Immunol* 2001;19:683–765. [PubMed: 11244051]
 29. O'Shea JJ, Murray PJ. Cytokine signaling modules in inflammatory responses. *Immunity* 2008;28:477–487. [PubMed: 18400190]
 30. Mosser DM, Zhang X. Interleukin-10: new perspectives on an old cytokine. *Immunol Rev* 2008;226:205–218. [PubMed: 19161426]
 31. Calado DP, Paixao T, Holmberg D, Haury M. Stochastic monoallelic expression of IL-10 in T cells. *J Immunol* 2006;177:5358–5364. [PubMed: 17015721]
 32. Kamanaka M, Kim ST, Wan YY, Sutterwala FS, Lara-Tejero M, Galan JE, Harhaj E, Flavell RA. Expression of interleukin-10 in intestinal lymphocytes detected by an interleukin-10 reporter knockin tiger mouse. *Immunity* 2006;25:941–952. [PubMed: 17137799]
 33. Maynard CL, Harrington LE, Janowski KM, Oliver JR, Zindl CL, Rudensky AY, Weaver CT. Regulatory T cells expressing interleukin 10 develop from Foxp3+ and Foxp3- precursor cells in the absence of interleukin 10. *Nat Immunol* 2007;8:931–941. [PubMed: 17694059]
 34. Denning TL, Wang YC, Patel SR, Williams IR, Pulendran B. Lamina propria macrophages and dendritic cells differentially induce regulatory and interleukin 17-producing T cell responses. *Nat Immunol* 2007;8:1086–1094. [PubMed: 17873879]
 35. Awasthi A, Carrier Y, Peron JP, Bettelli E, Kamanaka M, Flavell RA, Kuchroo VK, Oukka M, Weiner HL. A dominant function for interleukin 27 in generating interleukin 10-producing anti-inflammatory T cells. *Nat Immunol* 2007;8:1380–1389. [PubMed: 17994022]
 36. Fitzgerald DC, Zhang GX, El-Behi M, Fonseca-Kelly Z, Li H, Yu S, Saris CJ, Gran B, Ciric B, Rostami A. Suppression of autoimmune inflammation of the central nervous system by interleukin 10 secreted by interleukin 27-stimulated T cells. *Nat Immunol* 2007;8:1372–1379. [PubMed: 17994023]
 37. Stumhofer JS, Silver JS, Laurence A, Porrett PM, Harris TH, Turka LA, Ernst M, Saris CJ, O'Shea JJ, Hunter CA. Interleukins 27 and 6 induce STAT3-mediated T cell production of interleukin 10. *Nat Immunol* 2007;8:1363–1371. [PubMed: 17994025] This paper shows that IL-27 and IL-6

promote IL-10 production from T cells. Using several Stat-knockout mice, they showed the requirement of Stat3 for the expression of IL-10.

38. Anderson CF, Oukka M, Kuchroo VJ, Sacks D. CD4(+)CD25(-)Foxp3(-) Th1 cells are the source of IL-10-mediated immune suppression in chronic cutaneous leishmaniasis. *J Exp Med* 2007;204:285–297. [PubMed: 17283207]
39. Jankovic D, Kullberg MC, Feng CG, Goldszmid RS, Collazo CM, Wilson M, Wynn TA, Kamanaka M, Flavell RA, Sher A. Conventional T-bet(+)Foxp3(-) Th1 cells are the major source of host-protective regulatory IL-10 during intracellular protozoan infection. *J Exp Med* 2007;204:273–283.283 [PubMed: 17283209] This paper shows that Th1 cells that produce IL-10 are generated during Toxoplasma infection.
40. McGeachy MJ, Bak-Jensen KS, Chen Y, Tato CM, Blumenschein W, McClanahan T, Cua DJ. TGF-beta and IL-6 drive the production of IL-17 and IL-10 by T cells and restrain T(H)-17 cell-mediated pathology. *Nat Immunol* 2007;8:1390–1397. [PubMed: 17994024]
41. O'Garra A, Barrat FJ, Castro AG, Vicari A, Hawrylowicz C. Strategies for use of IL-10 or its antagonists in human disease. *Immunol Rev* 2008;223:114–131. [PubMed: 18613832]
42. Dumoutier L, Louahed J, Renauld JC. Cloning and characterization of IL-10-related T cell-derived inducible factor (IL-TIF), a novel cytokine structurally related to IL-10 and inducible by IL-9. *J Immunol* 2000;164:1814–1819. [PubMed: 10657629]
43. Dumoutier L, Van Roost E, Colau D, Renauld JC. Human interleukin-10-related T cell-derived inducible factor: molecular cloning and functional characterization as an hepatocyte-stimulating factor. *Proc Natl Acad Sci U S A* 2000;97:10144–10149. [PubMed: 10954742]
44. Zenewicz LA, Yancopoulos GD, Valenzuela DM, Murphy AJ, Stevens S, Flavell RA. Innate and adaptive interleukin-22 protects mice from inflammatory bowel disease. *Immunity* 2008;29:947–957.957 [PubMed: 19100701] This study shows that IL-22 secreted from NK cells, as well as from CD4⁺ T cells, is protective during inflammatory bowel disease (IBD).
45. Wolk K, Witte E, Reineke U, Witte K, Friedrich M, Sterry W, Asadullah K, Volk HD, Sabat R. Is there an interaction between interleukin-10 and interleukin-22? *Genes Immun* 2005;6:8–18. [PubMed: 15526001]
46. Zenewicz LA, Flavell RA. IL-22 and inflammation: Leukin' through a glass onion. *Eur J Immunol* 2008;38:3265–3268. [PubMed: 19016525]
47. Xie MH, Aggarwal S, Ho WH, Foster J, Zhang Z, Stinson J, Wood WI, Goddard AD, Gurney AL. Interleukin (IL)-22, a novel human cytokine that signals through the interferon receptor-related proteins CRF2-4 and IL-22R. *J Biol Chem* 2000;275:31335–31339. [PubMed: 10875937]
48. Boniface K, Bernard FX, Garcia M, Gurney AL, Lecron JC, Morel F. IL-22 inhibits epidermal differentiation and induces proinflammatory gene expression and migration of human keratinocytes. *J Immunol* 2005;174:3695–3702. [PubMed: 15749908]
49. Pan H, Hong F, Radaeva S, Gao B. Hydrodynamic gene delivery of interleukin-22 protects the mouse liver from concanavalin A-, carbon tetrachloride-, and Fas ligand-induced injury via activation of STAT3. *Cell Mol Immunol* 2004;1:43–49. [PubMed: 16212920]
50. Radaeva S, Sun R, Pan HN, Hong F, Gao B. Interleukin 22 (IL-22) plays a protective role in T cell-mediated murine hepatitis: IL-22 is a survival factor for hepatocytes via STAT3 activation. *Hepatology* 2004;39:1332–1342. [PubMed: 15122762]
51. Liang SC, Tan XY, Luxenberg DP, Karim R, Dunussi-Joannopoulos K, Collins M, Fouser LA. Interleukin (IL)-22 and IL-17 are coexpressed by Th17 cells and cooperatively enhance expression of antimicrobial peptides. *J Exp Med* 2006;203:2271–2279. [PubMed: 16982811]
52. Sugimoto K, Ogawa A, Mizoguchi E, Shimomura Y, Andoh A, Bhan AK, Blumberg RS, Xavier RJ, Mizoguchi A. IL-22 ameliorates intestinal inflammation in a mouse model of ulcerative colitis. *J Clin Invest* 2008;118:534–544. [PubMed: 18172556]
53. Wolk K, Kunz S, Witte E, Friedrich M, Asadullah K, Sabat R. IL-22 increases the innate immunity of tissues. *Immunity* 2004;21:241–254. [PubMed: 15308104]
54. Dong C. TH17 cells in development: an updated view of their molecular identity and genetic programming. *Nat Rev Immunol* 2008;8:337–348. [PubMed: 18408735]
55. Veldhoen M, Hirota K, Westendorf AM, Buer J, Dumoutier L, Renauld JC, Stockinger B. The aryl hydrocarbon receptor links TH17-cell-mediated autoimmunity to environmental toxins. *Nature*

2008;453:106–109.109 [PubMed: 18362914] This paper shows that the transcription factor aryl hydrocarbon receptor (AHR) is essential for IL-22 expression, but not the expression of other Th17 cytokines. In addition, these data show that environmental toxins in combination with TGF- β may contribute to the differentiation of Th17 cells.

56. Cella M, Fuchs A, Vermi W, Facchetti F, Otero K, Lennerz JK, Doherty JM, Mills JC, Colonna M. A human natural killer cell subset provides an innate source of IL-22 for mucosal immunity. *Nature* 2009;457:722–725.725 [PubMed: 18978771] This paper presents the characterization of a subset of NK cells that highly express IL-22. Unlike conventional NKs, these cells express low levels of IFN γ and have low cytotoxicity.
57. Luci C, Reynders A, Ivanov II, Cognet C, Chiche L, Chasson L, Hardwigsen J, Anguiano E, Banchereau J, Chaussabel D, et al. Influence of the transcription factor ROR γ on the development of NKp46+ cell populations in gut and skin. *Nat Immunol* 2009;10:75–82. [PubMed: 19029904]
58. Sanos SL, Bui VL, Mortha A, Oberle K, Heners C, Johner C, Diefenbach A. ROR γ and commensal microflora are required for the differentiation of mucosal interleukin 22-producing NKp46+ cells. *Nat Immunol* 2009;10:83–91. [PubMed: 19029903]
59. Satoh-Takayama N, Vosschenrich CA, Lesjean-Pottier S, Sawa S, Lochner M, Rattis F, Mention JJ, Thiam K, Cerf-Bensussan N, Mandelboim O, et al. Microbial Flora Drives Interleukin 22 Production in Intestinal NKp46(+) Cells that Provide Innate Mucosal Immune Defense. *Immunity* 2008;29:958–970. [PubMed: 19084435]
60. Zheng Y, Danilenko DM, Valdez P, Kasman I, Eastham-Anderson J, Wu J, Ouyang W. Interleukin-22, a T(H)17 cytokine, mediates IL-23-induced dermal inflammation and acanthosis. *Nature* 2007;445:648–651. [PubMed: 17187052]
61. Zenewicz LA, Yancopoulos GD, Valenzuela DM, Murphy AJ, Karow M, Flavell RA. Interleukin-22 but not interleukin-17 provides protection to hepatocytes during acute liver inflammation. *Immunity* 2007;27:647–659. [PubMed: 17919941]
62. Aujla SJ, Chan YR, Zheng M, Fei M, Askew DJ, Pociask DA, Reinhart TA, McAllister F, Edeal J, Gaus K, et al. IL-22 mediates mucosal host defense against Gram-negative bacterial pneumonia. *Nat Med* 2008;14:275–281. [PubMed: 18264110]
63. Zheng Y, Valdez PA, Danilenko DM, Hu Y, Sa SM, Gong Q, Abbas AR, Modrusan Z, Ghilardi N, de Sauvage FJ, et al. Interleukin-22 mediates early host defense against attaching and effacing bacterial pathogens. *Nat Med* 2008;14:282–289. [PubMed: 18264109]
64. Chang H, Hanawa H, Liu H, Yoshida T, Hayashi M, Watanabe R, Abe S, Toba K, Yoshida K, Elnaggar R, et al. Hydrodynamic-based delivery of an interleukin-22-Ig fusion gene ameliorates experimental autoimmune myocarditis in rats. *J Immunol* 2006;177:3635–3643. [PubMed: 16951323]

A. Periphery

B. Gut

Figure 1. Under steady-state conditions, TGF- β and IL-10 maintain peripheral and gut tolerance

A) In the periphery, T cells with low affinity for self antigens undergo homeostatic turn-over in response to self antigen and IL-7. TGF- β inhibits T cell activation and effector differentiation in response to self antigens, allowing a slow homeostatic proliferation of naïve T cells. B) In the gut, commensal bacteria/flora and food antigens are constantly sampled by intestinal DCs. Tolerogenic CD103⁺ DCs produce RA which inhibits effector cytokine production by CD4⁺CD44⁺ T cells. In the absence of effector cytokines and in the presence of high concentrations of TGF- β , naïve CD4⁺ T cells are converted into Foxp3⁺ iTregs that produce TGF- β and IL-10. IL-10 produced by IELs and iTregs regulates activation of intestinal lymphocytes, and reduces the expression of IL-12 and downregulates costimulatory molecules on macrophages.

Figure 2. The role of TGF-β, IL-10 and IL-22 during inflammation

Upon recognition of pathogen-associated molecular patterns (PAMPs), such as LPS, APCs become activated and express IL-10. This innate-derived IL-10 in turn down-modulates effector T cell responses, as well as the pro-inflammatory responses of APCs themselves. Naive CD4⁺ T cells when activated in the presence of low concentrations of TGF-β and inflammatory cytokines, such as IL-6, leads to the generation of Th17 cells. Th17 cells comprise a heterogeneous population of cells that express different types and amounts of the prototypical cytokines, which include IL-10, IL-22, and IL-17. Although depicted here as single producers, co-expression of these cytokines has been observed. In the absence of IL-23, this differentiation leads to a population of Th17 cells that express IL-10. IL-10 is important for down-modulating the responses of effector T cells, as well as their proliferation, thereby preventing excessive tissue damage from harmful IFNγ and granzymes, as well as Fas-FasL-mediated apoptosis. In a more direct manner, IL-22 also protects tissues from damage during inflammation. IL-22 directly stimulates anti-apoptotic and proliferative programs in many

kinds of epithelial cells, such as hepatocytes or those cells lining the colon, allowing for tissue maintenance and repair during inflammation. IL-22 is also expressed by NK cells upon stimulation with IL-23. By contrast, IL-23 stimulation combined with low concentrations of TGF- β and IL-6 leads to a population of Th17 cells that express greater levels of IL-17. In some inflammatory settings, these pro-inflammatory cells appear to cause tissue damage, perhaps through recruitment of PMNs.