ELSEVIER

Transforming growth factor-β: innately bipolar

Sharon M Wahl

Widely heralded for depressing ongoing immune responses, renewed interest in the proficiency by which transforming growth factor β (TGF-β) not only engages but also might drive an over-reactive innate response highlights its bipolar nature. Although coordination of the development and function of Treg, in addition to direct inhibition of cellular activation, are prominent pathways by which TGF-β controls adaptive immunity, paradoxically TGF-β appears instrumental in initiation of host responses to invasion through recruitment and activation of immune cells and persuasion of Th17 lineage commitment. Nevertheless, true to its manic-depressive behavior, new evidence links TGF-β with depression of innate cells, including NK cells, and by way of a potential bridge between mast cells and Treg. Disruption of the tenuous balance between these opposing actions of TGF-β underlies immunopathogenicity.

Addresses
Oral Infection and Immunity Branch, National Institute of Dental and Craniofacial Research (NIDCR), National Institutes of Health (NIH), Bethesda, MD 20892-4352, USA

Corresponding author: Wahl, Sharon M (smwahl@dir.nidcr.nih.gov)

Current Opinion in Immunology 2007, **19**:55–62

This review comes from a themed issue on
Innate immunity
Edited by Dieter Kabelitz and Ruslan Medzhitov

Available online 28th November 2006

0952-7915/$ – see front matter
Published by Elsevier Ltd.

DOI 10.1016/j.coi.2006.11.008

Introduction

The pioneering discovery of transforming growth factor β (TGF-β) by Roberts, Sporn and co-workers 25 years ago [1] presaged an incredible tale of discovery and insight into control of the immune system. Their initial observations and dogged pursuit of the structure and function of this new molecule provided the springboard from which it became appreciated that TGF-β might not only mediate neoplastic events, as initially described, but also have a role in immunity. On the shoulders of such giants began an epoch devoted to understanding how TGF-β choreographs host defense. Although inevitable that someone would have eventually identified this potent immunoregulatory molecule, it was this early discovery by Roberts and co-workers that ushered in an era of enlightenment, and, even today, TGF-β continues to mystify [2].

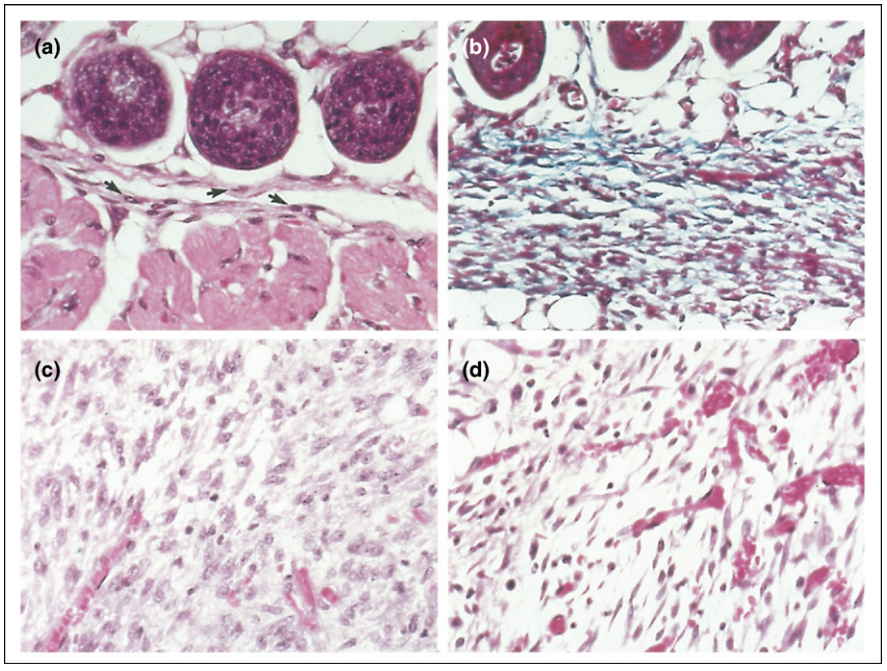
With the recent emphasis on the powerful immune-depressing actions of TGF-β, we might have transiently neglected its equally pivotal influence in engaging and agitating innate immune pathways; however, recent discoveries have brought us back to the future. Following on the heels of the identification and characterization of TGF-β [3], a 25 kD homodimeric cytokine secreted as a latent complex that requires proteolytic cleavage or structural modification to enable high affinity receptor recognition, evidence emerged that this newly described molecule might influence inflammatory cells [4]. More prescient than even Roberts imagined were her observations that local injection of TGF-β induced not only accumulation of fibroblasts and matrix but also appearance of leukocytes at the injection site (Figure 1), precipitating assessment of the possibility that TGF-β might be a recruitment factor for macrophages and neutrophils [5,6]. Endogenous expression of TGF-β at sites of injury or infection is consistent with accumulation of inflammatory cells and with dissuasion of leukocyte congregation pursuant to local delivery of TGF-β-neutralizing antibodies [7–9]. Exploring this same model of synovial inflammation, the first inklings of bipolarity began to materialize with evidence that TGF-β could, inexplicably at the time, both induce and inhibit arthritic lesions [2,8–10]. Exciting recent findings offer insight into this apparent paradox.

This review will emphasize the newly appreciated connections between TGF-β and cellular components of innate immunity, particularly mast cells and NK cells, and the unique ability of TGF-β to direct $CD4^+$ T-cell lineage commitment to both proinflammatory Th17 cells and anti-inflammatory Treg.

TGF-β as an instigator

The initial recruitment of inflammatory cells that recognize, process and present antigen to lymphocytes is key to innate immunity and provides the bridge over which adaptive immunity is launched. An innate response to trauma or infection engages a sequence of events that might include clot formation, platelet aggregation and rapid mediator release, which forward-drive inflammation, remodeling and re-epithelialization [11]. Among the platelet secretome is TGF-β — one of the first agents on the scene — which jump-starts the ensuing response. Intuitively, release of a molecule at the onset of an innate response would not be anticipated to depress, but rather to excite, host defense. As sentinels, resident and recruited monocytes, macrophages and dendritic cells not only detect and combat invaders, apoptotic cells and cellular debri but also, through a complex interactive process, alert other immune cells to guide the repertoire

Figure 1



Local delivery of TGF-β initiates recruitment of inflammatory cells. The first evidence that TGF-β influenced cells of the immune system was revealed following local injection of TGF-β into newborn mouse skin. Mice (1 day old) were injected each day in the nape of the neck with 20 μl of a solution of saline alone or with 20 μl of a saline solution of TGF-β (800 ng). **(a)** Control injection (72 h). This section shows the interface below the reticular dermis, between the subcutaneous adipose tissue (containing hair follicles, top) and the underlying skeletal muscle (bottom). Only a small number of fibroblasts are usually found at this interface, as indicated by arrows (hematoxylin and eosin [H&E] ×430). **(b)** TGF-β injection (48 h). The subcutaneous interface is expanded by fibroblasts, macrophages, granulocytes and newly formed collagen bundles (blue). (Masson trichrome; ×260.) **(c)** TGF-β injection showing cellular infiltrate (72 h). The subcutaneous space is now further expanded by sheets of fibroblasts, endothelial cells and macrophages, surrounded by a collagenous network (H&E ×430). **(d)** TGF-β injection showing new blood vessels (72 h). This section shows pronounced neovascularization, with newly formed capillary loops, surrounded by fibroblasts and occasional macrophages. Extravasated erythrocytes are also present (H&E ×430). Reproduced with permission from [4].

of tolerance, ignorance or immune responsiveness. Initially, TGF-β amplifies the reaction via autocrine and paracrine upregulation of TGF-β and TGF-β receptors (TGFβRs), and overexuberant release of TGF-β fuels manic inflammatory responses [2,7].

Following extravasation and emigration from blood vessels, TGFβR-bearing leukocytes respond to TGF-β ligands, which coordinate multiple and diverse cell autonomous and cell–cell interactions [12], through induction of kinase activity of the intracellular domain of TGFβRII. This oligomerizes with and phosphorylates TGFβRI kinase [13] to activate transcriptional co-regulators — Smad2 and Smad3 — and crosstalk with Smad-independent pathways. Receptor-associated Smad3 has been linked to leukocyte chemotaxis [12], and Smad2 or Smad3 partnering with common Smad4 enables nuclear translocation and transcriptional regulation of gene expression. Transduction of noncanonical pathways adds to the diversity of cell type-dependent and context-dependent molecular programs, related to activation status and a myriad of other contextual cues. In this regard, TGF-β increases FcR expression [14] and triggers cytokines in immature or newly recruited monocytes [15], whereas, to activated leukocytes, TGF-β becomes inhibitory [16]. This bipolarity was once likened to a light switch: if it is off TGF-β will turn it on, whereas if it is on TGF-β will turn it off [17]; for the most part, this paradigm still holds.

TGF-β as an innate depressant

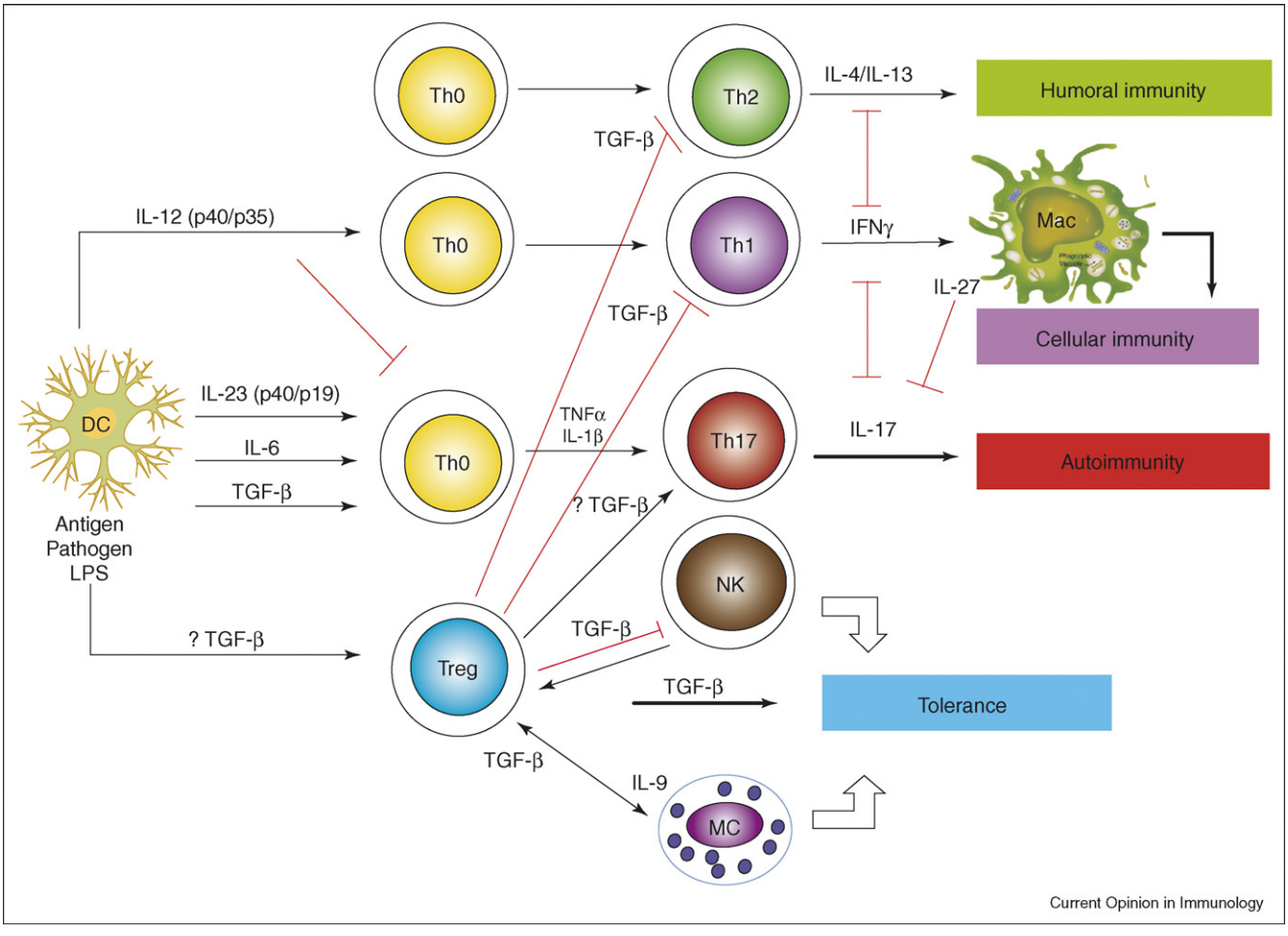
In addition to neutrophils and macrophages, an innate response, depending on the inciting agent, typically involves mast cells and natural killer cells (NK cells); evolving data provide insight into previously unrecognized links between TGF-β and these populations. Mast cells, which are responsive to injury and pathogens and were once thought to have a relatively predictable role in innate immunity, are perhaps no longer so predictable. Considered primary responders in allergy and asthma and also agents of autoimmune pathogenesis, these cells have, of late, become appreciated for their plasticity in producing both pro- and anti-inflammatory mediators. Whereas TGF-β stimulates early mast cell recruitment [18], it characteristically has differentiation status-dependent effects on IgE-mediated release of effector molecules,

such as histamine, proteases and tumor necrosis factor α (TNFα). In turn, mast cells produce TGF-β, amplifiable by the Th2 cytokine interleukin (IL)-9 [19], and, because TGF-β and chymase are co-stored in secretory granules, degranulation might coordinately trigger secretion and activation of TGF-β [20].

Of considerable intrigue is whether this TGF-β forges the newly uncovered partnership between mast cells and regulatory T cells (Treg), which are instrumental in controlling peripheral tolerance (Figures 2 and 3). Antigen-stimulated thymic-derived and peripheral TGF-β-converted $CD4^+CD25^+Foxp3^+$ Treg [21] —bastions of suppression of effector T-cell activation, proliferation and function to curtail an immune response [22,23] — also produce high levels of IL-9, which influences not only mast cell growth but also their generation of cytokines, proteases and IgE receptors [24••]. Co-localizing mast cells, in turn, have the power to impact Treg-mediated allograft tolerance, possibly through TGF-β. Confirming the novel contribution of this dynamic duo, tolerance mechanisms abrogated in mast cell-deficient mice were restored following injection of mast cells [24••]. While unique in identifying a connection between mast cells and Treg, these studies also implicate Treg much earlier in an immune response than usually appreciated. Such a concept is in keeping with recent demonstrations that Treg block innate NK cell activity [25••]. NK cells vehemently respond to viruses, other pathogens and tumor cells with secretion of cytolytic molecules and interferon (IFN)γ, and Treg intervene in an unanticipated contact-dependent way [25••,26•].

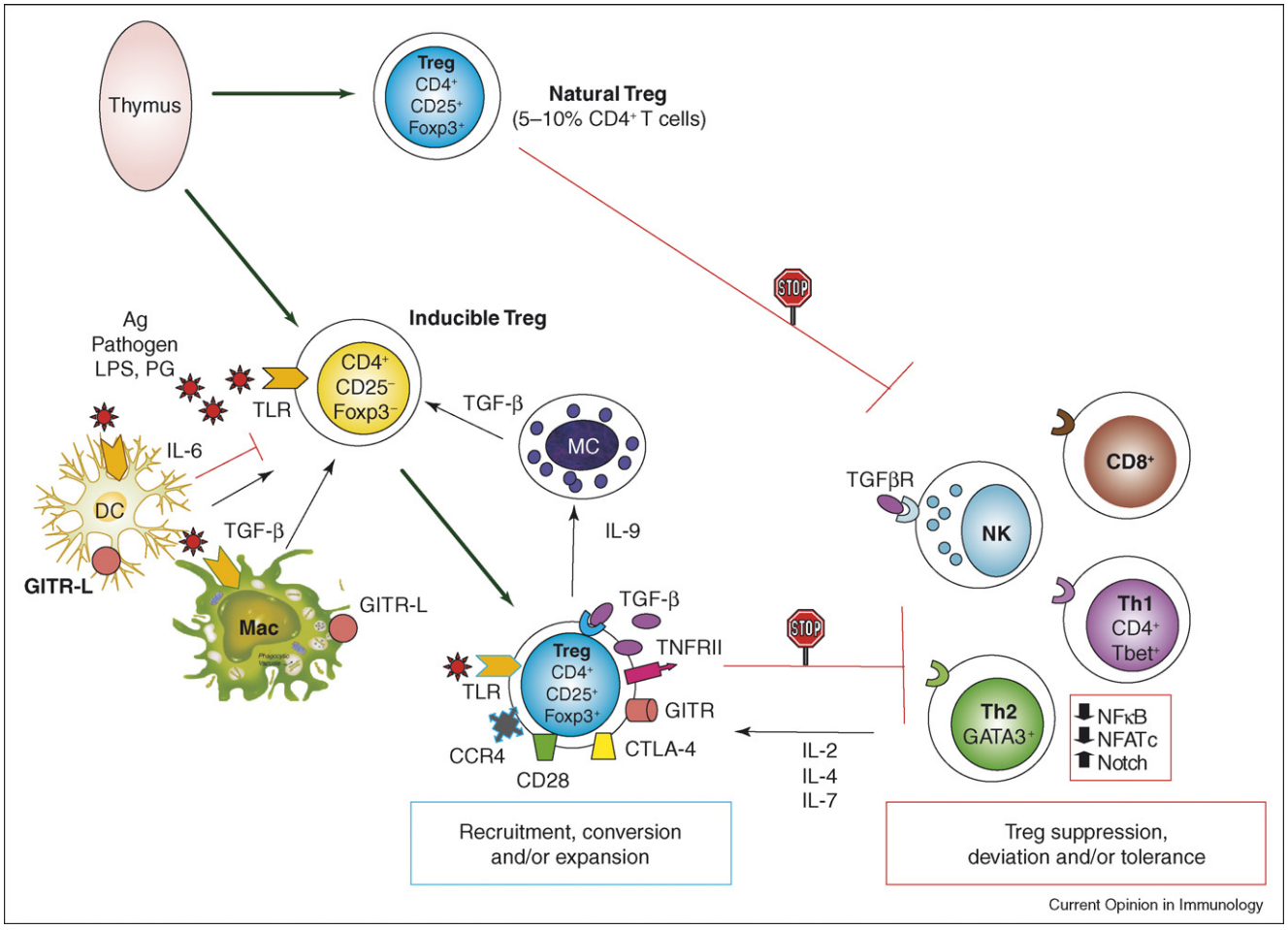
Certainly, involvement of innate immune mechanisms in regulation of acquired immunity has been demonstrated in the generation and function of Treg [27•,28••], but the

Figure 2



TGF-β biases T-cell lineage commitment. Multiple cell–cell interactions, soluble factors and cross-regulatory mediators impact the lineage specification of T lymphocytes; one of these is TGF-β, which might inhibit IL-12-mediated Th1 and IL-4-dependent Th2 lineages while driving $CD4^+CD25^+$ Treg conversion of $CD4^+CD25^-$ T cells through upregulation of the transcription factor Foxp3. TGF-β is also essential in commitment of naïve T cells to the IL-17-generating Th17 lineage, in collaboration with additional APC-derived cytokines. TGF-β and Treg, through TGF-β-linked pathways, cross-regulate Th1 and Th2 lymphocyte activation, proliferation and function.

Figure 3



TGF-β influences $CD4^+CD25^+Foxp3^+$ regulatory T cell development and function. $CD4^+CD25^+Foxp3^+$ Treg, once thought to be derived only from the thymus in limited quantities (5–10% of $CD4^+$ T cells), are now recognized as a peripherally inducible population in which $CD4^+CD25^-$ T cells can, under the correct influences, including products of dendritic cells and macrophages, be recruited, converted and/or expanded into a functionally suppressive population. The identification of an ever-expanding repertoire of cell surface receptors reveals vulnerability to a plethora of ligands, including TGF-β. Recognition of TLRs on Treg links this population to innate immunity, in addition to their complement of co-regulatory molecules (GITR, CTLA-4, CD28), TNFRII and other cytokine receptors. Expression of CCR4 probably favors targeting and recruitment of Treg to further increase their numbers at sites of immune reactivity. Functional suppression of NK cells and effector T cell functions might be related to altered NFκB, NFATc and/or Notch1 signaling pathways.

reverse — that is, Treg targeting of innate cells — is striking. Cell-bound active TGF-β of autocrine/paracrine origin provides a molecular switch central to the complex physiology by which Treg mediate depression of $TGF\beta R^+$ T effector and NK cells [21,25••,26•,29], potentially entwined with nuclear transcription factor-κB (NF-κB), nuclear factor of activated T cells (NFATc) and/or Notch1–hairy and enhancer of split 1 (Notch1–HES1) signaling [30••]. Whereas recipient effector T cells augment their TGFβR upon antigen activation to become more receptive to Treg–TGF-β inhibitory signals, NK cells constitutively express a full complement of TGFβR and are vulnerable to TGF-β-mediated suppression even in the innate or nonactivated state, as reflected by Smad phosphorylation and functional inability to eliminate tumor cells [25••,26•]. Thus, TGF-β is not only inhibitory in adaptive immune sequelae but can also run roughshod over innate immunity.

Innately satisfying then was the recognition that Treg express Toll-like receptors (TLRs) for pathogen-associated molecular patterns, enabling them to sense and to be influenced by infectious and environmental TLR ligands. The TLR repertoire of Treg minimally includes TLR1, TLR2 and TLRs 4–8 [27•,31]. TLR2 ligands might transiently promote Treg expansion while abrogating their suppressive powers to unhinder an anti-microbial response, and at the same time hold the expanded Treg in reserve to restore homeostasis post-containment [28••]. Another Treg-dampening mechanism inherent in

innate, as well as in adaptive, responses includes TNF–TNFRII interactions, recently linked to downmodulation of Foxp3 [32]. Consequently, beyond TGF-β, a myriad of influential innate forces impact recruitment, activation, survival, conversion and/or expansion of Treg, including local cooperative actions of pathogens, cytokines and antigen-presenting cells (APCs) (Figure 3). Whether locally derived and/or rapidly recruited by way of targeting molecules, such as CCR4 [33••], Treg suppressive manoeuvres become necessary and, usually, sufficient to limit inflammatory responses once the inciting agent is subdued, thereby enabling leukocytes to channel the response toward repair, restoring homeostasis and integrity of afflicted tissues. However, persistence of antigen tilts the balance away from resolution towards an adaptive response.

TGF-β in the transition from innate to adaptive immunity

Through cell–cell interactions and generation of a plethora of regulatory factors, such as TGF-β, innate cells coordinate protection of the host from infection, neoplastic cells and other foreign antigens. Although programmed to recognize pathogen-associated molecular patterns, initiating APC phagocytosis and degradation of microbes, TLRs integrate signaling pathways [34] embedded in a contextual framework structured around kinetics, costimulatory molecules and other factors, and their differential cytokine production foments subsequent responses. Two early cytokines, heterodimeric IL-12 and IL-23, which share an IL-12p40 subunit, orchestrate separate as well as cross-regulatory pathways in NK and T lymphocyte responses [35]. Although not mutually exclusive, Gram-positive microbial recognition by APCs through TLR2 typically favors production of IL-23 [36], whereas Gram-negative bacteria evoke IL-12 by way of TLR4 [35]. However, local macrophage IL-10 production [37] or TGF-β might subvert the transition to adaptive immunity. Once released, IL-12 drives naïve T cells into Th1 IFNγ-producing cells (Figure 2), which are essential to controlling infections, and APC-derived IL-23 facilitates survival of a population of $CD4^+$ T cells that express proinflammatory IL-17 (Th17) [38••].

Not to be ignored, new evidence identifies TGF-β as the protagonist in Th17 lineage commitment; it does this with support from IL-6 [38••,39••,40••], but with IL-27 as a counterbalance and adversary to TGF-β [41••,42••]. Th17-secreted IL-17A and IL-17F induce rapid recruitment of polymorphonuclear leukocytes in acute infections or wounds and promote further release of proinflammatory and neutrophil-mobilizing cytokines (GM-CSF, IL-6 and TNF), which sustain Th17 and might eventuate in chronic autoimmune and inflammatory disease; this is all consistent with early studies [5–10], but with a new Th17 mod look. Although activated and/or uncontrolled Th1 cells were typically blamed for evolution of autoimmune lesions, Th17 cells have emerged as the culprits, partially exonerating Th1 cells. This is consistent with prior studies in which exogenous IFNγ, which cross-regulates IL-17, actually decreased chronic inflammatory (arthritic) disease [43]. Nonetheless, an either/or situation does not bode well and, whereas excess IL-17 drives autoimmunity, an overabundance of IFNγ is also pathogenic; this is evident in TGF-β1 null mice, in which the paucity of Th17 cells [39••] enables lethal overproduction of IFNγ [44]. Unraveling this link between TGF-β and Th17 lineage specification provides a long-sought explanation of the mechanism by which TGF-β can drive inflammation and how TGF-β mania provokes chronic inflammatory and autoimmune pathology.

B cells and T cells, in the context of co-stimulatory molecules and cytokines, recognize antigen by distinct routes, with T cells typically dependent upon APCs to present antigenic peptides to T cells within organized lymphoid tissues. During this close encounter in an immunological synapse [45], APCs might proffer TGF-β as a controller along with antigen. Although TGF-β supports T-cell differentiation, survival and expansion [21,38••,39••,46], during a lull in TGF-β, stimulated T cells might be unleashed to differentiate into Th1, Th2 and/or NKT cells. As recent evidence [47] reveals a related activation-inducing encounter between B cells and antigen-bearing dendritic cells, such an encounter can enhance receptivity to T cell help for antibody production and might provide a brief taste of TGF-β. Early work connected TGF-β with generation of antibody-secreting B cells, particularly isotype switching to IgA [48]. The combined actions of TGF-β on T and B cells, whether cell autonomous and/or dependent upon cell–cell interactions, might shape the outcome of immune homeostasis and/or immunopathogenesis.

TGF-β-dependent adaptive depression

The adaptive immune response is a powerful tool for defense against invading pathogens, yet can get mired in molecular mimicry and/or response to self-antigens. Although multiple peripheral tolerance mechanisms (Treg, Tr1, Th3 and NKT), many of which are dependent on TGF-β, exist to protect against such deviant behavior, the existence of autoimmune and chronic infectious diseases indicates that such mechanisms are not infallible. Regulation of early onset autoimmune lesions is dependent upon TGF-β, cytotoxic T lymphocyte antigen 4 (CTLA-4) and $Foxp3^+$ Treg, because mice that lack these molecules develop aggressive and fatal autoreactive immunity [23,49]. Transgenic mice that express a dominant negative form of the TGFβRII under a T cell specific promoter exhibit a milder delayed development of autoimmune pathology [50,51], but all these models point to TGF-β as the mastermind of immune homeostasis.

Conclusions

Although much has been learned, TGF-β continues to confound our understanding of immune regulation

through its influential diversity in the development, survival and homeostasis of APCs, mast cells, NK, $CD4^+$, $CD8^+$ and NKT cells. How can one molecule do so much? The recent identification of a role for TGF-β in driving Th17 lineage commitment provides a new piece of the puzzle, but it is likely that this is not the final one. Clearly not acting in solitaire, TGF-β is crucial to development of immunity and is often associated with exaggerated immune excitability. Conversely, TGF-β is key in restraining essentially all innate and adaptive immune cells, particularly self-reactive T cells, to restore immune homeostasis and to prevent autoimmunity. Augmentation of TGF-β and/or increasing Treg might facilitate a beneficial outcome in chronic and autoimmune diseases. Overly depressive TGF-β in neoplastic and infectious diseases has prompted exploration of immunotherapies targeting TGF-β that have achieved success in animal models and are being considered in humans [52]. Nonetheless, one must not lose sight of the bipolar nature of TGF-β in the design and/or implementation of such strategies. Clearly, when it comes to TGF-β, one size does not fit all and tailored therapies will be required. The current renaissance in deciphering pro-inflammatory actions of TGF-β coupled with its already appreciated inhibitory repertoire will enable meaningful pursuit of these goals. (See Box 1.)

Box 1 In memoriam

This review is dedicated to the memory of Anita Bauer Roberts (1942–2006) without whose discovery of TGF-β in 1981 and continued insightful exploration of its functions in health and disease throughout her career, this field would not have advanced nor would TGF-β have been recognized for its potential as a therapeutic agent and target. Her ground-breaking scientific endeavors, always at the forefront, coupled with her myriad roles as colleague, mentor, collaborator, friend, wife, mother and grandmother have left a huge void. Before her recent death, Roberts remained awestruck by the incredible extent to which TGF-β impacts so many fundamental biological processes, from embryogenesis, morphogenesis and tissue repair to neoplasia and, especially, in the control of physiologic and aberrant immune pathways. What a legacy she leaves!

Acknowledgements

This research was supported by the Intramural Research Program of the NIH, NIDCR. The author is grateful for the editorial assistance of Nina Schneider and the thoughtful review and comments of Nancy Vazquez, Nancy McCartney Francis, and Ashok Kulkarni (NIDCR, NIH), and of Michael B Sporn (Dartmouth Medical School, Hanover, NH).

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Roberts AB, Anzano MA, Lamb LC, Smith JM, Sporn MB: **New class of transforming growth factors potentiated by epidermal growth factor: isolation from non-neoplastic tissues**. *Proc Natl Acad Sci USA* 1981, **78**:5339-5343.

2. Wahl SM: **Transforming growth factor beta: the good, the bad, and the ugly**. *J Exp Med* 1994, **180**:1587-1590.

3. Assoian RK, Komoriya A, Meyers CA, Miller DM, Sporn MB: **Transforming growth factor-beta in human platelets. Identification of a major storage site, purification, and characterization**. *J Biol Chem* 1983, **258**:7155-7160.

4. Roberts AB, Sporn MB, Assoian RK, Smith JM, Roche NS, Wakefield LM, Heine UI, Liotta LA, Falanga V, Kehrl JH *et al.*: **Transforming growth factor type beta: rapid induction of fibrosis and angiogenesis *in vivo* and stimulation of collagen formation *in vitro***. *Proc Natl Acad Sci USA* 1986, **83**:4167-4171.

5. Wahl SM, Hunt DA, Wakefield LM, McCartney-Francis N, Wahl LM, Roberts AB, Sporn MB: **Transforming growth factor type beta induces monocyte chemotaxis and growth factor production**. *Proc Natl Acad Sci USA* 1987, **84**:5788-5792.

6. Brandes ME, Mai UE, Ohura K, Wahl SM: **Type I transforming growth factor-beta receptors on neutrophils mediate chemotaxis to transforming growth factor-beta**. *J Immunol* 1991, **147**:1600-1606.

7. Wahl SM, Allen JB, Costa GL, Wong HL, Dasch JR: **Reversal of acute and chronic synovial inflammation by anti-transforming growth factor beta**. *J Exp Med* 1993, **177**:225-230.

8. Allen JB, Manthey CL, Hand AR, Ohura K, Ellingsworth L, Wahl SM: **Rapid onset synovial inflammation and hyperplasia induced by transforming growth factor beta**. *J Exp Med* 1990, **171**:231-247.

9. Fava RA, Olsen NJ, Postlethwaite AE, Broadley KN, Davidson JM, Nanney LB, Lucas C, Townes AS: **Transforming growth factor beta 1 (TGF-β 1) induced neutrophil recruitment to synovial tissues: implications for TGF-β-driven synovial inflammation and hyperplasia**. *J Exp Med* 1991, **173**:1121-1132.

10. Brandes ME, Allen JB, Ogawa Y, Wahl SM: **Transforming growth factor beta 1 suppresses acute and chronic arthritis in experimental animals**. *J Clin Invest* 1991, **87**:1108-1113.

11. Singer AJ, Clark RA: **Cutaneous wound healing**. *N Engl J Med* 1999, **341**:738-746.

12. Ashcroft GS, Yang X, Glick AB, Weinstein M, Letterio JL, Mizel DE, Anzano M, Greenwell-Wild T, Wahl SM, Deng C *et al.*: **Mice lacking Smad3 show accelerated wound healing and an impaired local inflammatory response**. *Nat Cell Biol* 1999, **1**:260-266.

13. Derynck R, Zhang YE: **Smad-dependent and Smad-independent pathways in TGF-β family signalling**. *Nature* 2003, **425**:577-584.

14. Welch GR, Wong HL, Wahl SM: **Selective induction of FcγRIII on human monocytes by transforming growth factor-beta**. *J Immunol* 1990, **144**:3444-3448.

15. McCartney-Francis N, Mizel D, Wong H, Wahl L, Wahl S: **TGF-β regulates production of growth factors and TGF-β by human peripheral blood monocytes**. *Growth Factors* 1990, **4**:27-35.

16. Tsunawaki S, Sporn M, Ding A, Nathan C: **Deactivation of macrophages by transforming growth factor-beta**. *Nature* 1988, **334**:260-262.

17. Sporn MB, Roberts AB: **TGF-β: problems and prospects**. *Cell Regul* 1990, **1**:875-882.

18. Gruber BL, Marchese MJ, Kew RR: **Transforming growth factor-beta 1 mediates mast cell chemotaxis**. *J Immunol* 1994, **152**:5860-5867.

19. Mesples B, Fontaine RH, Lelievre V, Launay JM, Gressens P: **Neuronal TGF-β1 mediates IL-9/mast cell interaction and exacerbates excitotoxicity in newborn mice**. *Neurobiol Dis* 2005, **18**:193-205.

20. Lindstedt KA, Wang Y, Shiota N, Saarinen J, Hyytiainen M, Kokkonen JO, Keski-Oja J, Kovanen PT: **Activation of paracrine TGF-β1 signaling upon stimulation and degranulation of rat serosal mast cells: a novel function for chymase**. *FASEB J* 2001, **15**:1377-1388.

21. Chen W, Jin W, Hardegen N, Lei KJ, Li L, Marinos N, McGrady G, Wahl SM: **Conversion of peripheral CD4$^+$CD25$^-$ naïve T cells to CD4$^+$CD25$^+$ regulatory T cells by TGF-β induction of transcription factor Foxp3**. *J Exp Med* 2003, **198**:1875-1886.

22. Shevach EM, Dipaolo RA, Andersson J, Zhao DM, Stephens GL, Thornton AM: **The lifestyle of naturally occurring CD4CD25Foxp3 regulatory T cells**. *Immunol Rev* 2006, **212**:60-73.

23. Sakaguchi S, Ono M, Setoguchi R, Yagi H, Hori S, Fehervari Z, Shimizu J, Takahashi T, Nomura T: **Foxp3CD25CD4 natural regulatory T cells in dominant self-tolerance and autoimmune disease**. *Immunol Rev* 2006, **212**:8-27.

24. •• Lu LF, Lind EF, Gondek DC, Bennett KA, Gleeson MW, Pino-Lagos K, Scott ZA, Coyle AJ, Reed JL, Van Snick J *et al.*: **Mast cells are essential intermediaries in regulatory T-cell tolerance**. *Nature* 2006, **442**:997-1002.

In contrast to prior concepts of restricted mast cell involvement in triggering inflammatory events, this study demonstrates that mast cells are essential in mediation of peripheral tolerance through a collaborative arrangement with CD4$^+$CD25$^+$Foxp3$^+$ Treg. Treg production of IL-9 fosters recruitment and activation of mast cells within tolerant tissue sites.

25. •• Ghiringhelli F, Menard C, Terme M, Flament C, Taieb J, Chaput N, Puig PE, Novault S, Escudier B, Vivier E *et al.*: **CD4$^+$CD25$^+$ regulatory T cells inhibit natural killer cell functions in a transforming growth factor-beta-dependent manner**. *J Exp Med* 2005, **202**:1075-1085.

The authors demonstrate the first evidence that Treg can impose functional restrictions on innate NK cell functions through a contact-dependent mechanism that involves Treg membrane associated TGF-β and constitutive expression of cognate receptors on unactivated NK cells.

26. • Wahl SM, Wen J: **Moutsopoulos NM: The kiss of death: interrupted by NK-cell close encounters of another kind**. *Trends Immunol* 2006, **27**:161-164.

This article provides evidence that NK cell–Treg interactions result in phosphorylation of intracellular Smad3 TGF-β signaling molecules. This is indicative of engagement of a TGF-β–TGFβR signaling cascade.

27. • Peng G, Guo Z, Kiniwa Y, Voo KS, Peng W, Fu T, Wang DY, Li Y, Wang HY, Wang RF: **Toll-like receptor 8-mediated reversal of CD4$^+$ regulatory T cell function**. *Science* 2005, **309**:1380-1384.

Interaction of TLR8 ligands with Treg, which highly express membrane TLR8, results in direct antagonism of their ability to function as suppressors of effector T-cell proliferation and activity.

28. •• Sutmuller RP, den Brok MH, Kramer M, Bennink EJ, Toonen LW, Kullberg BJ, Joosten LA, Akira S, Netea MG, Adema GJ: **Toll-like receptor 2 controls expansion and function of regulatory T cells**. *J Clin Invest* 2006, **116**:485-494.

In an interesting twist, these authors define the consequences of TLR2 engagement on Treg as an increase in proliferation, concurrent with blockade of their suppressor function. These data suggest that marginalizing the inhibitory actions of this population might enable a more effective antimicrobial response, whereas expansion of the Treg population will allow for subsequent control of the host response.

29. Chen W, Wahl SM: **TGF-β: the missing link in CD4$^+$CD25$^+$ regulatory T cell-mediated immunosuppression**. *Cytokine Growth Factor Rev* 2003, **14**:85-89.

30. •• Ostroukhova M, Qi Z, Oriss TB, Dixon-McCarthy B, Ray P, Ray A: **Treg-mediated immunosuppression involves activation of the Notch–HES1 axis by membrane-bound TGF-β**. *J Clin Invest* 2006, **116**:996-1004.

On the basis of evidence that membrane TGF-β on Treg activates the Notch1–HES1 transcriptional repressor axis in Treg target cells following cell–cell contact, the authors conclude that this signaling pathway is fundamental to the mechanism by which Treg suppress effector T cells in the induction of tolerance.

31. Caramalho I, Lopes-Carvalho T, Ostler D, Zelenay S, Haury M, Demengeot J: **Regulatory T cells selectively express Toll-like receptors and are activated by lipopolysaccharide**. *J Exp Med* 2003, **197**:403-411.

32. Valencia X, Stephens G, Goldbach-Mansky R, Wilson M, Shevach EM, Lipsky PE: **TNF downmodulates the function of human CD4$^+$CD25^{hi} T-regulatory cells**. *Blood* 2006, **108**:253-261.

33. •• Lee I, Wang L, Wells AD, Dorf ME, Ozkaynak E, Hancock WW: **Recruitment of Foxp3$^+$ T regulatory cells mediating allograft tolerance depends on the CCR4 chemokine receptor**. *J Exp Med* 2005, **201**:1037-1044.

Targeted recruitment of Treg to sites of peripheral tolerance might be a function of their enhanced expression of the CCR4 chemokine receptor to respond to CCL22 observed within tolerant allografts. Recognition of such a specific mechanism might enable enhancement or inhibition of Treg accumulation in sites of immune deviation.

34. Doyle SL, O'Neill LA: **Toll-like receptors: from the discovery of NFκB to new insights into transcriptional regulations in innate immunity**. *Biochem Pharmacol* 2006, **72**:1102-1113.

35. Langrish CL, McKenzie BS, Wilson NJ, de Waal Malefyt R, Kastelein RA, Cua DJ: **IL-12 and IL-23: master regulators of innate and adaptive immunity**. *Immunol Rev* 2004, **202**:96-105.

36. Smits HH, van Beelen AJ, Hessle C, Westland R, de Jong E, Soeteman E, Wold A, Wierenga EA, Kapsenberg ML: **Commensal Gram-negative bacteria prime human dendritic cells for enhanced IL-23 and IL-27 expression and enhanced Th1 development**. *Eur J Immunol* 2004, **34**:1371-1380.

37. Verreck FA, de Boer T, Langenberg DM, Hoeve MA, Kramer M, Vaisberg E, Kastelein R, Kolk A, de Waal-Malefyt R, Ottenhoff TH: **Human IL-23-producing type 1 macrophages promote but IL-10-producing type 2 macrophages subvert immunity to (myco)bacteria**. *Proc Natl Acad Sci USA* 2004, **101**:4560-4565.

38. •• Veldhoen M, Hocking RJ, Atkins CJ, Locksley RM, Stockinger B: **TGFβ in the context of an inflammatory cytokine milieu supports *de novo* differentiation of IL-17-producing T cells**. *Immunity* 2006, **24**:179-189.

This article, [39••] and [40••] define an essential role for TGF-β in directing Th17 lineage specification, associated with production of IL-17 and other neutrophil-mobilizing cytokines instrumental in host defense to infections and wounds and in driving pathogenic evolution of autoimmunity.

39. •• Mangan PR, Harrington LE, O'Quinn DB, Helms WS, Bullard DC, Elson CO, Hatton RD, Wahl SM, Schoeb TR, Weaver CT: **Transforming growth factor-β induces development of the T$_H$17 lineage**. *Nature* 2006, **441**:231-234.

See annotation to [38••].

40. •• Bettelli E, Carrier Y, Gao W, Korn T, Strom TB, Oukka M, Weiner HL, Kuchroo VK: **Reciprocal developmental pathways for the generation of pathogenic effector T$_H$17 and regulatory T cells**. *Nature* 2006, **441**:235-238.

See annotation to [38••].

41. •• Batten M, Li J, Yi S, Kljavin NM, Danilenko DM, Lucas S, Lee J, de Sauvage FJ, Ghilardi N: **Interleukin 27 limits autoimmune encephalomyelitis by suppressing the development of interleukin 17-producing T cells**. *Nat Immunol* 2006, **7**:929-936.

In this study and in [42••], new evidence is presented to demonstrate that IL-27 represents a counterbalance to TGF-β in the induction of Th17 cells and might be an important TGF-β antagonist in chronic inflammatory diseases.

42. •• Stumhofer JS, Laurence A, Wilson EH, Huang E, Tato CM, Johnson LM, Villarino AV, Huang Q, Yoshimura A, Sehy D *et al.*: **Interleukin 27 negatively regulates the development of interleukin 17-producing T helper cells during chronic inflammation of the central nervous system**. *Nat Immunol* 2006, **7**:937-945.

See annotation to [41••].

43. Wahl SM, Allen JB, Ohura K, Chenoweth DE, Hand AR: **IFN-γ inhibits inflammatory cell recruitment and the evolution of bacterial cell wall-induced arthritis**. *J Immunol* 1991, **146**:95-100.

44. McCartney-Francis NL, Wahl SM: **Dysregulation of IFN-γ signaling pathways in the absence of TGF-beta 1**. *J Immunol* 2002, **169**:5941-5947.

45. Dustin ML, Tseng SY, Varma R, Campi G: **T cell–dendritic cell immunological synapses**. *Curr Opin Immunol* 2006, **18**:512-516.

46. Cerwenka A, Bevec D, Majdic O, Knapp W, Holter W: **TGF-β1 is a potent inducer of human effector T cells**. *J Immunol* 1994, **153**:4367-4377.

47. Qi H, Egen JG, Huang AY, Germain RN: **Extrafollicular activation of lymph node B cells by antigen-bearing dendritic cells**. *Science* 2006, **312**:1672-1676.

48. Lebman DA, Edmiston JS: **The role of TGF-β in growth, differentiation, and maturation of B lymphocytes**. *Microbes Infect* 1999, **1**:1297-1304.

49. Kulkarni AB, Huh CG, Becker D, Geiser A, Lyght M, Flanders KC, Roberts AB, Sporn MB, Ward JM, Karlsson S: **Transforming growth factor beta 1 null mutation in mice causes excessive inflammatory response and early death**. *Proc Natl Acad Sci USA* 1993, **90**:770-774.

50. Gorelik L, Flavell RA: **Abrogation of TGF-β signaling in T cells leads to spontaneous T cell differentiation and autoimmune disease**. *Immunity* 2000, **12**:171-181.

51. Lucas PJ, Kim SJ, Melby SJ, Gress RE: **Disruption of T cell homeostasis in mice expressing a T cell-specific dominant negative transforming growth factor beta II receptor**. *J Exp Med* 2000, **191**:1187-1196.

52. Yingling JM, Blanchard KL, Sawyer JS: **Development of TGF-β signalling inhibitors for cancer therapy**. *Nat Rev Drug Discov* 2004, **3**:1011-1022.