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Special Article

Transforming Growth Factor Beta (TGF-β) in Inflammation: A Cause and a Cure

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INTRODUCTION

Of the many polypeptide growth factors, transforming growth factor beta (TGF-β) appears to be relatively unique in its global effects on cell growth and differentiation (1). In addition to its complex growth regulatory activities on essentially all cell types, compelling evidence has accumulated over the past 5 years documenting the role of TGF-β as a potent immunomodulatory molecule (2). Quickly evident were its profound immunosuppressive actions and many diverse inhibitory effects *in vitro*. These observations were promptly followed by therapeutic trials of the peptide in experimental models of inflammation and immune-mediated disease. Although in many cases TGF-β has fulfilled its promise as an immunotherapeutic agent, continuing investigations have revealed the bifunctional nature of the peptide. Thus, as both an immunosuppressive and a potent proinflammatory molecule, TGF-β orchestrates events vital to the initiation, progression, and resolution of immune-mediated inflammatory responses. These opposing effects are likely controlled by the cytokine's selective production and latency, as well as receptor modulation and differential susceptibility of target cells at various stages of development, maturation, and activation (Table I). As the seemingly contradictory nature of TGF-β continues to be unraveled and knowledge of its role in host defense continues to expand, we must remain cognizant that TGF-β is but one mediator, albeit an influential one, in the cytokine network that regulates the immune system.

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TGF-β SYNTHESIS AND ACTIVATION

Synthesis

At least five homodimeric polypeptides, which share 70–80% homology and many biological activities (reviewed in Ref. 1), are represented in the highly conserved TGF-β family. Of these multiple forms, only TGF-β1, -β2, and -β3 have been identified in mammalian species, and differences in cellular origin and actions between these isoforms are now being recognized. The gene for each of the isoforms is located on a different chromosome and differential expression may occur through distinct cell-specific regulatory mechanisms. The peptides are encoded as preproproteins of 390–412 amino acids with signal peptides of 20–23 amino acids at the N terminus (3). Two major transcriptional start sites for TGF-β1 mRNA have been identified, with two promoter regions containing binding sites for the transcription factors, NF-1, SP-1, and AP-1 (4–6).

TGF-β1, -β2, and -β3 have a 4- or 5-amino acid processing site and, after signal peptidase and proteolytic cleavage, are processed to the 112-residue forms (12.5 kD), which have in common 9 cysteine

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Table I. Factors Determining Biological Effects of TGF-β

Latent or active TGF-β
TGF-β isoform
Target-cell type
State of cell differentiation
Resting or activated cells
Receptor type and number
Influence of other growth factors
Binding proteins
Presence of antagonists
Extracellular matrix

residues and the C-terminal sequence, Cys–Lys–Cys–Ser–COOH (3). The processed mature TGF-β homodimer (25 kD) is biologically inactive or latent due to its noncovalent association with the 75-kD glycosylated latency-associated protein (LAP), which is covalently linked to a 135-kD binding protein (1, 7).

Activation

The above complex requires activation to release the biologically active molecule, which then binds to the receptor to initiate signal transduction. The masking of TGF-β activity by its association with the LAP proregion dimer is important in protecting cells that make TGF-β from the molecule's potent regulatory effects and in controlling the pleiotropic paracrine activities. In all forms of TGF-β, except TGF-β2, LAP contains the fibronectin cellular recognition (RGD) sequence (8), raising the possibility that the latent complex or, possibly, free LAP binds to cells through this domain or via an alternative heparin binding site (7). The ability of TGF-β to bind to matrix molecules may be pivotal in localizing, focusing, and concentrating its activities. The dynamic interaction between growth factors such as TGF-β and the extracellular matrix is likely essential to the evolution of inflammatory events (9).

Intense interest in the activation of the latent complex, a crucial regulatory step in TGF-β bioactivity (1, 10), has led to new insights into the physiologic mechanisms of activation of TGF-β. The LAP carbohydrates appear to be essential to maintaining latency since sialidase and glycosidases are effective activators of TGF-β (11). The latent complex reportedly binds, at least on bovine aortic endothelial and smooth muscle cells, to mannose 6-phosphate/insulin-like growth factor II receptors, where, once bound, it becomes susceptible to protease activation (12). These proteases, including plasmin and cathepsin, and transient exposure to acidic conditions (6) partially activate the latent molecule (13). The activation of TGF-β is likely a multistep process, involving cells such as gamma interferon (γIFN)-treated monocytes (14) and inflammatory macrophages (15) which generate sialidases and proteases within an acidic microenvironment (16, 17) to activate the latent complex. Once activated, TGF-β binds either to cellular receptors or to one of several binding proteins. Although the latent form of the molecule circulates with a half-life >90 min, active TGF-β is cleared from the circulation within minutes (18). Thus, latency confers stability, target-cell specificity, and a means for extracellular control of the broad spectrum activities of this ubiquitous molecule.

PROINFLAMMATORY ACTIVITIES OF TGF-β

Inflammatory-Cell Recruitment

The nearly immediate release of TGF-β by platelets (19) at sites of injury or immunologic challenge provides an important clue that TGF-β has an agonist role in the sequence of events constituting an inflammatory response. Platelets represent the most concentrated natural source of TGF-β, producing nearly 20 mg/kg (20). It is difficult to fathom that a molecule with only immunosuppressive potential would be one of the first mediators released in an inflammatory cascade. In fact, the notion of TGF-β as a promoter of the inflammatory process has been borne out in subsequent studies. Originally identified as the most potent chemoattractant for monocytes with activity in the femtomolar concentration range (21), TGF-β has more recently been found to have chemotactic activity for both neutrophils (22–24) and T lymphocytes (25). Moreover, the recruitment potential of TGF-β has been confirmed *in situ* following direct injection of the native or recombinant molecule intradermally (26) and intraarticularly (23, 27). Whereas locally applied TGF-β initiates leukocyte infiltration (28), this response is absent in monocytopenic animals (29).

In defining the underlying molecular signals responsible for this proinflammatory activity of TGF-β, evidence now indicates that TGF-β influences mononuclear phagocyte recruitment by four interrelated pathways: modulation of integrin expression, increased monocyte–matrix adhesion, enhancement of matrix-specific collagenase secretion, and chemotaxis (21, 30) (Table II). In order for circulating monocytes to exit the vascular bed at

Table II. Immunoregulation by TGF-β

Proinflammatory	Immunosuppressive
↑ Integrins	↓ ROI, NOI
↑ Collagenase	⇵ TGF-β receptors
↑ Chemotaxis	↓ IL-1 receptor
↑ Th 1 lymphocytes	↑ IL-1ra
↑ Cytokines	↓ Hematopoiesis
↑ Matrix	↓ Proliferation
	↓ LAK, NK, CTL

sites of injury, they become enmeshed in a clot or adhere to the vascular wall, subsequently encountering the basement membrane, a barrier of type IV collagen, laminin, and fibronectin, which they must traverse. The signals regulating these events are not well delineated, although the cytokines interleukin 1 (IL-1), tumor necrosis factor (TNF), and γIFN appear to be influential in certain aspects of this process (31–33). Considering that TGF-β has been shown to inhibit adhesion of leukocytes to endothelial cells *in vitro* (34), it is unclear how TGF-β induces leukocyte accumulation at injection sites *in vivo*. However, one mechanism whereby TGF-β may initiate the requisite cell–cell and cell–matrix interactions is by virtue of its ability to enhance cell surface integrin expression (30).

Integrins are heterodimers composed of an α and a β subunit (32, 33) which form transmembrane links between the extracellular matrix and the cellular cytoskeletal elements. Functionally, the β_2 heterodimers mediate cell–cell interactions, whereas the β_1 integrins (VLA) foster cell–matrix binding. In response to picomolar levels of TGF-β, monocyte $\alpha_3\beta_1$ receptors, which interact with cell binding domains of laminin, collagen, and fibronectin, increase. Besides $\alpha_3\beta_1$, monocyte fibronectin receptors ($\alpha_5\beta_1$) also increase as previously shown for mesenchymal cells (35). Augmentation of integrin receptor expression is reflected by increased mononuclear phagocyte adhesiveness to structural matrix substrates.

Negotiation of a path through the matrix to the site of inflammation requires leukocyte release of matrix-degrading enzymes. To this end, monocyte production of the 92- and 72-kDa gelatinase/type IV collagenases is augmented by TGF-β (30). This response is quite specific since circulating monocytes require an appropriate stimulus, such as TGF-β, to synthesize and secrete this enzymatic activity. Consequently, the rapid release of TGF-β, first by platelets and then by inflammatory cells themselves, can serve as a powerful recruitment factor, mediating integrin expression, attachment, and collagenolytic activity in a finely tuned scenario enabling the leukocytes to marginate, undergo diapedesis, traverse the basement membrane, and commence extravascular directed migration.

Mononuclear-Cell Activation

Once at the site of injury or inflammation, the recruited leukocytes are subject to regulation by higher concentrations of a plethora of potential mediators and cytokines, including TGF-β. For example, levels in excess of 20 ng/ml of active TGF-β have been reported in inflamed synovial fluids (15, 36, 37). Understanding the mechanisms regulating the exuberant production of this peptide may elucidate the immunopathology of diseases such as rheumatoid arthritis. Within inflammatory sites, several types of cells are capable of producing TGF-β, including activated lymphocytes, macrophages, neutrophils, and synovial fibroblasts (15, 37–43). Moreover, TGF-β has been shown to augment its own secretion in many cells, setting in motion both autocrine and paracrine circuits of potentially cyclic release. The autoinduction of TGF-β1 transcription is mediated by AP-1 (Jun-Fos) binding sites in the TGF-β1 promoter (6). Monocytes, responding to picomolar concentrations of TGF-β, demonstrate increased mRNA expression and secretion of both TGF-β1 and β2 peptides (40). Activated T cells also transcribe and translate both TGF-β1 and TGF-β2 (42). Accordingly, expression of TGF-β mRNA appears most abundant during active inflammation and cell-mediated immune reactions (44).

Apart from its capacity to promote cell recruitment, TGF-β is a potent regulator of other critical cell functions. At picomolar concentrations, TGF-β increases resting monocyte mRNA levels for IL-1, TNF, platelet-derived growth factor (PDGF), basic fibroblast growth factor (bFGF), and IL-6 (21, 27, 39, 40–47). The secretion of IL-1, TNF, and IL-6 (40, 45, 47) can trigger a cytokine cascade in which each of these molecules plays a part. For example, induction of IL-1 stimulates T cells posttranscriptionally to produce elevated levels of active TGF-β (48), which can further increase IL-1 synthesis (40) in a positive feedback loop. In addition to cytokines, TGF-β induces FcγRIII (CD16) on newly recruited monocytes (49, 50), which together with increased adhesion molecules, promote phagocytic activity and the release of toxic oxygen radicals *in*

vitro (49) and *in vivo* (51, 52). Thus, one of the principal actions of TGF-β in monocytes may be to control gene transcription, albeit transiently (53), for a series of biologically active molecules that control many of the subsequent events in the inflammatory process.

The effect of TGF-β on T lymphocytes, originally considered to be exclusively inhibitory, now appears to be more complex. TGF-β has recently been shown to function in a costimulatory capacity to regulate T-cell proliferation triggered through the T-cell receptor/CD3 complex (54). Moreover, exposure of $CD4^+$ murine T cells to TGF-β results in the rapid conversion of putative precursors (CD45RB high, Pgp-1 low) to mature memory CD4 cells as characterized by loss of CD45RB, increased expression of Pgp-1 (CD44), increased proliferative capacity, and secretion of lymphokines (IL-2), all traits consistent with the T-helper cell type I phenotype (54, 55). The differentiation state of the cell appears to be particularly critical in determining how the cell will respond to TGF-β, with naive T cells induced to proliferate and the growth of mature T cells (CD45RB low, CD44 high) subject to inhibition. At sites of immunologic challenge the initial rapid release and accumulation of TGF-β would be conducive to the generation of TH_1 cells, which are essential to the evolution of delayed-type hypersensitivity and antigenic memory. Localization of this subset of cells within the lesion may be facilitated by the TGF-β regulated increase in CD44 (33).

The identification of TGF-β at sites of inflammation (15, 36, 37, 43, 56), coupled with the consequences observed following local injection of TGF-β (23, 26, 27), implicates the peptide as playing a critical role in the early phases of an inflammatory response (Table II). While functioning to promote inflammatory events, aberrant expression of TGF-β may have pathologic consequences. In this context, locally administered TGF-β exacerbates an ongoing inflammatory lesion as demonstrated in an experimental model of synovial inflammation and tissue destruction (57).

Inhibition of TGF-β-Mediated Recruitment and Activation

If TGF-β is, in fact, contributing to the chronicity of arthritis and other lesions, then specific antagonists of TGF-β should be able to block the progression of disease or, possibly, induce regression. In animals receiving an arthropathic dose of bacterial cell walls, a single injection of anti-TGF-β intraarticularly reversed the sequelae of events culminating in joint destruction. Whereas the isotype control antibody had no appreciable effect, anti-TGF-β administered to the contralateral joint suppressed both the acute neutrophil-mediated synovitis and the chronic T cell-dependent and monocyte-mediated destructive phase of the arthritis. These data establish a very strong association between the enhanced TGF-β identified in synovial fluids and tissues of arthritis patients (15, 36, 37, 43) and the pathophysiology of this disease. In another disease model, antibodies to TGF-β also suppressed the progression of experimental glomerulonephritis (59), indicating that abnormal expression of this otherwise normal chemotactic and growth factor can be pathologic.

TISSUE REPAIR AND FIBROSIS

Wound Healing

After the initial inflammatory reaction, TGF-β plays a critical role in resolution of the response and in tissue repair and fibrosis. The response initiated solely by TGF-β is self-limited and reversible (26, 27), likely due to the short half-life of TGF-β (18), down-regulation of TGF-β receptors (49, 60), and the fact that TGF-β cannot prevent leukocyte programmed cell death (PCD) (61). However, in concert with other inflammatory mediators as well as with matrix components, TGF-β regulates many processes including angiogenesis, chemotaxis, fibroblast proliferation and controlled synthesis and degradation of the extracellular matrix, necessary for tissue repair (1). Fibroblasts recruited by TGF-β (27, 62) are indirectly stimulated by this cytokine to proliferate through the induction of PDGF (63) and PDGF receptors (64). Subsequently, the production of fibroblast collagen, fibronectin, and glycosaminoglycan is directly upregulated by TGF-β (1, 26, 65). Moreover, many of the effects of TGF-β on connective tissue regeneration and neovascularization (26, 27, 66) may well be secondary to the induction of mediators by the infiltrating mononuclear phagocytes. Compounding the TGF-β-mediated matrix regeneration is the TGF-β enhanced production of protease inhibitors and suppression of proteolytic enzymes (67, 68). The net effect of this differential regulation of matrix proteins, enzymes, and enzyme inhibitors is the accumulation of matrix proteins

Table III. Potential Therapeutic Applications of TGF-β

Local administration
Soft tissue repair
Wound healing
Decubitus, diabetic, vascular ulcers
Burns
Periodontal lesions
Retinal tears
Hard tissue repair: fractures
Psoriasis
Systemic administration
Antiinflammatory agent
Autoimmune diseases
Organ transplants
Osteoporosis
Malignancies

and scar formation (26), which can be taken advantage of clinically to promote deficient wound healing (69, 70) (Table III).

Fibroproliferative Disorders

Beyond the normal regulation of inflammation and scar formation, aberrations in persistence, production, or regulation of TGF-β may be important in excessive matrix generation. TGF-β apparently must be present in a regulated amount, since insufficient levels of the peptide may impede inflammatory and healing processes, and excess TGF-β potentiates chronic inflammatory and fibrotic lesions. In this regard, TGF-β is a potent upregulator of collagen gene expression (71–73), indicating its potential role in fibrotic disorders of the liver (56, 74, 75), lung (76, 77), and skin (78). Moreover, a spectrum of connective tissue disorders including rheumatoid arthritis, sceleroderma, myelofibrosis, and hepatic, intraocular, and pulmonary fibrosis exhibits substantial TGF-β mRNA and peptide synthesis (15, 36, 76, 78–82). These observations showing an increase in TGF-β, especially TGF-β_2 (78, 81), are consistent with a role for overexpression of the molecule in pathologic processes. Whether this abundance of TGF-β is the consequence of increased synthesis or absence of regulation is unclear.

Unregulated TGF-β may result from inadequate levels of binding proteins and clearance (83, 84) or lack of antagonists such as IL-4 (85). *In vitro*, both γIFN and TNF counteract the profibrotic activity of TGF-β (86), and importantly, patients with chronic hepatitis C show decreases in both liver TGF-β1 mRNA and procollagen type 1 mRNA following αIFN therapy (79). Thus, regulation of production and bioactivity of this cytokine influence the outcome of an inflammatory response.

REGULATION OF RECEPTORS

The ability of cells and the local milieu to generate TGF-β in a form that binds to cellular TGF-β receptors is crucial to the progression of inflammatory events. Once dissociated from the latency-associated peptide, the active form of the TGF-β interacts with its specific cell surface receptors to mediate its multiple biologic activities. With few exceptions, cells express TGF-β receptors, and coupled with the diversity of its effects, this peptide appears somewhat unique in its broad spectrum of effects on normal as well as pathologic immune responses. Thus, the study of TGF-β receptors and their modulation is of prime importance for understanding the mechanisms by which TGF-β elicits its biologic effects and for identifying mechanisms to control the effects of this pleiotropic peptide.

Receptor Characterization

Based on size, three classes of TGF-β receptors have been characterized: type I 53-kDa binding glycoprotein, type II 85- to 110-kDa glycoprotein, and type III dimer composed of 250- to 350-kDa proteoglycan subunits (87, 88). Of these different forms, types I and II appear to have signal transducing capabilities (89–91), whereas type III receptors, which consist of the glycosaminoglycan residues heparan sulfate and chondroitin sulfate attached to a 100- to 140-kDa core binding protein, appear to function in a ligand storage capacity and/or to interact with extracellular matrix constituents (88). Soluble forms of the type III receptor have been detected which bind TGF-β similarly to the membrane bound form (92). Although not identified on hematopoietic cells, a type IV (60-kD) and a type V (400-kD) glycoprotein receptor have recently been reported (93, 94) but their functional characteristics have not been established. No signaling occurs following ligand binding to the type III proteoglycan (89). However, signal transduction pathways initiated via ligand binding to the types I and II receptors, which may number only a few hundred, have not been fully elucidated, with no demonstrated tyrosine kinase activity (95, 96), but possible involvement of G proteins (97). After cloning of the genes for these TGF-β receptors has been accomplished, a better understanding of receptor

function in transmitting signals to the cell interior will be forthcoming.

Receptor Modulation

The number of TGF-β receptors on cell surfaces varies with the cell type and with the level of maturation or activation, providing a basis for differential sensitivity and regulation of cellular functions in response to TGF-β (Table I). This differential regulation may provide a crucial mechanism for reversing the potentially cyclic protagonist activities of TGF-β. For example, resting human monocytes freshly isolated from peripheral blood possess ~400 TGF-β binding sites (binding constant, <10 pM), all type I receptors. In these circumstances, the monocytes are exquisitely sensitive to stimulation by TGF-β (21, 53). As these cells become activated by inflammatory stimuli including γIFN or bacterial-derived lipopolysaccharide, the expression of TGF-β receptors is down-regulated. Consequently, these cells lose their sensitivity to TGF-β stimulation (21, 53), even though they are not globally suppressed since they may remain responsive to stimulation by other agents.

In addition to activation-induced loss of TGF-β receptors, the type I receptors are acutely down-regulated or internalized ($T_{1/2}$ = 5 min) following exposure to TGF-β as the receptor–ligand complexes are internalized (60). This measurable ligand-induced loss of TGF-β receptors is somewhat unique to monocytes, probably because these cells express only one class of receptors, whereas many other types of cells express two or three receptor classes, masking receptor fluctuation. Subsequent receptor regeneration in monocytes is slow (60), and the incomplete regeneration of receptors, together with the loss of TGF-β receptors induced by inflammatory stimuli, is responsible for the reduced sensitivity of activated monocytes to TGF-β. This loss of sensitivity to TGF-β by activated monocytes may be a turning point in reversing an inflammatory response.

Conversely, but with similar immunosuppressive consequences, T lymphocytes increase their expression of types I and II TGF-β receptors and also express the proteoglycan binding sites for TGF-β as they become activated (42, 89, 98). This dichotomy in receptor expression by these two cells central in an immune response may subserve the same function, namely, down-regulation of the response. Once T cells become activated, expressing increased TGF-β receptors, exposure to picomolar levels of TGF-β may compromise the ability of these cells to proliferate and secrete certain cytokines (42, 55, 98–100). It is remarkable that TGF-β stimulates naive CD4 cells to become memory cells and then ultimately blocks their further clonal expansion in a negative feedback loop. Escape from suppression has been associated with loss of TGF-β receptors (101). Thus, whether the effects of TGF-β on a cell are inhibitory or stimulatory depends in large part on the context (9).

IMMUNE SUPPRESSION BY TGF-β

Growth Inhibition

In several models of inflammatory disease, including adjuvant and bacterial cell wall-induced arthritis (102, 103), experimental allergic encephalomyelitis (103–105), graft rejection (106), and reperfusion-induced injury (107), systemically administered TGF-β down-modulates organ inflammation. How TGF-β promotes or inhibits immune processes remains elusive, yet the evidence appears solid that systemic, in contrast to local, administration favors suppression (Table II). Significantly, a striking absence of generalized immune suppression was noted in experimental animals receiving TGF-β systemically (102, 105), despite pronounced suppression of the localized immune responses. These observations are consistent with the resistance of resting lymphocytes, but increased susceptibility of activated T lymphocytes, to suppression. Probably the single most important determinant of the biological consequences of TGF-β on a particular target cell is related to its state of differentiation (Table I). Such opposing effects of TGF-β on cells at different times in their life cycle suggests the following sequence of events: TGF-β is released early and functions in many capacities to promote inflammation based on its stimulating effects on immature monocytes and T cells. Once these cells differentiate and become activated, they become susceptible to phenotypic modulation and functional inhibition by TGF-β. Under normal conditions, the transition from pro- to antiinflammatory responsiveness occurs with minimal disruption of host cell and tissue homeostasis.

In T-lymphocyte populations susceptible to growth arrest, TGF-β directly inhibits mitosis in the late G_1 phase of the cell cycle (99, 109) by an ill-defined mechanism. Inhibition involves regula-

tion of transcription of the early growth factor-dependent *c-myc* gene and of the transferrin receptor (110, 111). Moreover, in addition to these critical regulatory targets, growth inhibition may involve accumulation of the underphosphorylated retinoblastoma (*Rb*) gene product (111, 112). In addition to direct suppression of clonal expansion, TGF-β may also suppress these events indirectly by inhibiting IL-1 receptor expression (113, 114) and by inducing synthesis of IL-1 receptor antagonist (IL-1ra) (115, 116). IL-1ra, a 22-kDa protein which binds to the IL-1 receptor, has no agonist properties and blocks IL-1 binding and signal transduction, thereby aborting IL-1-dependent responses (117).

The reported differential susceptibility of $CD4^+$ and $CD8^+$ lymphocytes to TGF-β inhibition of proliferation may further contribute to resolution of an inflammatory response, since inhibition of mature $CD4^+$ cells responsible for cell-mediated immune functions (44, 118) is near-complete, whereas TGF-β is considerably less inhibitory (119) and may even promote (54) $CD8^+$ lymphocyte growth. This resistant $CD8^+$ population synthesizes products including IL-4, IL-5, and IL-10 which have additional immunosuppressive actions (118). Under the appropriate conditions, TGF-β appears to foster the generation of inhibitory feedback molecules and apparently works in concert with them to reverse immune processes.

Inhibition of Hematopoiesis

Another contributing factor to the immunosuppression observed following systemic administration of TGF-β may be related to suppression of leukocytosis (102). A number of studies have shown that TGF-β is an effective inhibitor of hematopoietic events, acting primarily on immature precursor populations (120–124), consistent, once again, with the concept that the effects of TGF-β are linked to differentiation. While these *in vitro* studies have been useful for evaluating the direct effects of TGF-β in defined systems where, dependent on dose and duration, inhibition of essentially all lineages has been observed, the *in vivo* administration of TGF-β more closely reflects the physiologic consequences of this peptide based on multisystem responses (102, 125). In animal models, exogenously delivered TGF-β may not influence normal hematopoietic events to the same extent as that observed in animals undergoing intense hematopoietic activity. This conclusion is based on studies showing that the peripheral white blood-cell count of animals receiving only TGF-β was not markedly suppressed, whereas animals with active inflammation and exhibiting leukocytosis had markedly reduced leukocyte numbers after systemic TGF-β treatment (102). Similarly, in animals subjected to a lethal dose of ionizing radiation, in which induction of IL-1 and TNF is radioprotective, TGF-β, albeit at very high doses (10 μg/mouse), reversed the host's ability to recover (126). Thus, TGF-β's ability to inhibit early progenitor bone marrow cell proliferation may include direct suppression of growth, but also reflect its capacity to antagonize IL-1.

At a more physiologic level, recent evidence indicates that basal autocrine production of TGF-β by a subpopulation of early progenitors negatively regulates their cycling status and this may occur through interaction with the *Rb* gene product (127). In these studies, the addition of antisense, but not sense, TGF-β oligonucleotides enhanced colony formation of all lineages of early progenitors without any effect on late erythroid progenitors or macrophage colonies. Not only did antisense TGF-β release hematopoietic progenitors from a quiescent growth factor-unresponsive state, but exogenously added TGF-β completely blocked early multipotential progenitor colony formation (127). The control of hematopoiesis by basal autocrine production of TGF-β may, in turn, be subject to retinoid control, since normal systemic levels of retinoids are believed to regulate functional TGF-β expression in responsive cells (128). Any augmentation of the basal TGF-β levels or exogenous administration of TGF-β may then become immunosuppressive, at least in part, by limiting the number of available leukocytes.

Additional Mechanisms of Immune Suppression

In addition to inhibition of T-lymphocyte proliferation and hematopoiesis, other pathways may contribute to the impressive immunosuppression induced by TGF-β in certain disease conditions. One of the earliest effects of elevated systemic TGF-β might be elimination of lesion-centered chemotactic concentration gradients required for directed extravascular migration. In animal models of inflammation, exogenously administered systemic TGF-β impairs inflammatory cell movement into the site of immunologic challenge (102). Moreover, elevations in circulating levels of TGF-β in

the autoimmune mouse strain MRL/lpr are associated with defective neutrophil migratory responses and phagocytic function (129). Another contributing factor to the suppressed host response may be the ability of TGF-β to diminish rapidly and completely IL-1- or TNF-induced hepatocyte acute-phase protein synthesis (130).

In other *in vitro* systems, TGF-β antagonizes the activity of IL-1 and TNF, and this antagonism extends to other cytokines including IL-2, IL-3, and γIFN (42, 99, 131–134). Besides antagonizing cytokine activity, TGF-β may also inhibit the production of IL-1 and TNF (46, 135). Inhibition requires pretreatment of mononuclear phagocytes with TGF-β (37, 136), and when TGF-β is administered after stimulation, TGF-β is less or no longer inhibitory (137), which may reflect the modulation of TGF-β receptors (53). Although the inhibition of IL-1 was initially reported to occur posttranscriptionally (46), it appears that IL-1 production is not inhibited but that TGF-β coinduces IL-1 receptor antagonist (IL-1ra), thereby masking IL-1 bioactivity (115, 116). The induction of IL-1ra, by virtue of its ability to block the plethora of IL-1-dependent inflammatory and immune phenomena, may be a key event in TGF-β-mediated immune suppression.

Whether by cytokine blockade or by other molecular mechanisms, TGF-β also suppresses the development, activity, and/or differentiation of cytotoxic T, LAK, and natural killer cells (108, 133, 138–143), effectively compromising cytolytic functions. The ability of TGF-β to inhibit expression of the cytolytic pore-forming protein by $CD8^+$ T cells likely contributes mechanistically to the overall decreased cytolytic activity (144).

Another susceptible lymphoid target for TGF-β is represented by cells of B lymphocyte lineage. Besides blocking their proliferative response, TGF-β inhibits IgG and IgM mRNA synthesis and protein secretion (145, 146). Inhibition of the switch from the membrane forms of μ and γ-H chain mRNA to the secreted forms in stimulated B-cell cultures occurs without altering expression of Oct-2 or NF-kB (146). As a consequence of this decrease in membrane Ig expression and the capacity to secrete Ig in response to stimulation, B-cell responsiveness to antigens and antigen presentation are compromised. Related to its ability to inhibit B-cell growth, TGF-β promotes switching of IgM- or IgD-bearing B cells to cells bearing the IgA isotype (147–149). By inhibiting enhancement of $FceR_2$ (CD23) expression, TGF-β can also be considered a negative regulatory molecule in IgE-mediated immune responses (150). Thus, by a series of interconnecting pathways, TGF-β suppresses immune cell functions, pivotal in termination of the immune response but with the potential, if unchecked, to cause pathology.

ACQUIRED IMMUNODEFICIENCY SYNDROME

Because of the association of TGF-β with immune suppression, it was not surprising that augmented production of this cytokine was reported in patients with acquired immune deficiency syndrome (AIDS), the prototypic disease of immune suppression. Moreover, *in vitro* infection of mononuclear cells with HIV-1 causes the cells to express and secrete a peptide identified as TGF-β (52, 151–153). Mechanistically, the HIV-1 tat protein appears to up-regulate TGF-β production (152), and typical of the self-perpetuating cyclic nature of cytokine regulation, TGF-β induced by HIV-1 stimulates IL-6 production, and then IL-6 up-regulates TGF-β (154, 155). Crucial to the manifestations of HIV-1 infection, TGF-β enhances HIV-1 replication in cells of monocytic lineage and in PHA-stimulated peripheral blood mononuclear cells (156–158), although decreased viral replication occurs in the chronically infected U1 cell line (156). These data provide fairly compelling reasons to suspect that TGF-β may play an important role in the spread of infection and/or disease progression by amplifying the existing viral load. Such an hypothesis is compatible with the recent observations documenting increased TGF-β in the central nervous system of AIDS patients, a tissue highly susceptible to HIV-1 infection (153).

In addition to modulating virus expression, the increased production of TGF-β likely contributes to impaired lymphocyte proliferative responses characteristic of AIDS; this impaired proliferation can be restored in the presence of antibodies to TGF-β (152). Increased levels of TGF-β may contribute to the HIV-induced CD4/CD8 inversion by promoting naive $CD8^+$ T-cell growth and suppressing mature $CD4^+$ T-cell proliferation. The inability to confine TGF-β to localized areas of infection (53) may influence cellular functions elsewhere and accelerate the spread of opportunistic pathogens.

That TGF-β can suppress the expression of activated macrophage effector functions, including the generation of toxic oxygen and nitrogen species (136, 137, 159), may facilitate pathogen survival in

the tissues. In experimental models, systemically administered TGF-β facilitates parasite survival and the development of fatal infections. Not only did TGF-β exacerbate infection in susceptible animals, but TGF-β treatment of animals otherwise resistant to *Trypanosoma cruzi* caused a striking increase in parasitemia and mortality (160). Elevated levels of endogenous TGF-β have similar consequences since spleen cells of rodents infected with *Trypanosoma cruzi*, lymphocyte choriomeningitis virus (LCMV) or exposed to bacterial products produce sharply elevated quantities of TGF-β which progressively suppress lymphocyte growth and macrophage oxidative metabolism (100, 160, 161). Thus, if bacterium- or virus-infected cells manifest increased generation of TGF-β as has been shown not only for HIV-1 and LCMV (108), but also for CMV (162), EBV (163), and hepatitis C (79), this cytokine may contribute to diminished phagocyte functions, as well as other immune-dependent sequelae associated with such infections. These observations support the hypothesis that augmented TGF-β levels may exert global negative regulatory effects in conditions such as AIDS and provide impetus for identifying relevant antagonists which might alter the course of infections and immune function in these patients.

SUMMARY

As we continue to explore the biology of TGF-β in the network of cells and mediators contributing to host defense, the mechanisms controlling whether the pro- or antiinflammatory effects of this peptide prevail will be unraveled. Understanding these basic mechanisms may offer new approaches for identifying agonists and/or antagonists and in which circumstances their use might be appropriate. The striking differences between local and systemic administration of this cytokine reaffirm that the functional consequences of any biologic mediator must be considered in context (9) and, furthermore, suggest avenues of therapeutic application (Table III). In summary, the central role of TGF-β in normal and aberrant host defense has become indisputable.

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