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REGULATION OF IMMUNE RESPONSES BY TGF-β*

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ABSTRACT

The transforming growth factor β (TGF-β) family of proteins are a set of pleiotropic secreted signaling molecules with unique and potent immunoregulatory properties. TGF-β1 is produced by every leukocyte lineage, including lymphocytes, macrophages, and dendritic cells, and its expression serves in both autocrine and paracrine modes to control the differentiation, proliferation, and state of activation of these immune cells. TGF-β can modulate expression of adhesion molecules, provide a chemotactic gradient for leukocytes and other cells participating in an inflammatory response, and inhibit them once they have become activated. Increased production and activation of latent TGF-β have been linked to immune defects associated with malignancy and autoimmune disorders, to susceptibility to opportunistic infection, and to the fibrotic complications associated with chronic inflammatory conditions. In addition to these roles in disease pathogenesis, TGF-β is now established as a principal mediator of oral tolerance and can be recognized as the sine qua non of a unique subset of effector cells that are induced in this process. The accumulated knowledge gained through extensive in vitro functional analyses and from in vivo animal models, including newly established TGF-β gene knockout and transgenic mice, supports the concept that clinical therapies based on modulation of this cytokine represent an important new approach to the treatment of disorders of immune function.

INTRODUCTION

Several established cytokine families are recognized for their roles in regulating the immune response (1). These include the tumor necrosis factor (TNF)-related

molecules, the interferons, the chemokines, the interleukins, and the other hematopoietins known to orchestrate the development and function of a variety of immunocompetent cells. Though rarely included under this umbrella of immunoregulatory molecules, the members of the family of transforming growth factors–β (TGF-β) must also be viewed as having a unique and essential role in regulating immune function (2–5). Best known for their roles in development, in epithelial cell growth and differentiation, and in the process of carcinogenesis (6), they are possibly the most pleiotropic, multifunctional secreted peptides known in that they are produced by and act on a wide variety of cell types to regulate endpoints ranging from control of cell growth and differentiation to regulation of cellular function and target gene activity (6).

The three isoforms of TGF-β, i.e., TGF-β 1, 2, and 3, signal through the same serine-threonine kinase type I and type II cell surface receptors and have similar if not identical cellular targets, although each isoform is expressed in a distinct pattern under control of a unique promoter (6, 7). These molecules are now recognized to have profound effects on immune cells, including all classes of lymphocytes, macrophages, and dendritic cells. The action of TGF-β on these cells is dependent not only on the cell type and its state of differentiation, but also on the total milieu of cytokines present (8), suggesting that perturbations of the balance of this cytokine array may also alter effects of TGF-β and contribute to immunopathology. The complex and often context-dependent manner in which TGF-β controls immune responses is gaining greater appreciation, largely through recognition of its roles in experimental models of disease and by evaluation of transgenic and knockout mice with altered expression of these ligands or their receptors (9). In this review we focus on the ability of TGF-β to regulate the function and interaction of cells of the immune system in the development of both humoral and cell-mediated immunity and in immune aspects of disease pathogenesis.

TGF-β REGULATES T CELL DEVELOPMENT AND EFFECTOR FUNCTION

T lymphocytes are clearly influenced by TGF-β at all stages of development, from their differentiation to their activation and proliferation, and can serve as a paradigm for the pleiotropic nature of this cytokine. As in many systems, the effects of TGF-β on T cells are, in part, a function of their state of differentiation, and such effects often are altered by the nature of the activating signals (10, 11). The earliest studies of the effects of TGF-β on human lymphocyte function revealed that activated T cells themselves synthesize and secrete TGF-β and that exogenous TGF-β typically inhibits IL-2-dependent proliferation (12). Several studies have highlighted these inhibitory effects of

TGF-β, particularly its ability to interfere with events that occur subsequent to IL-2 production and receptor binding (13) such as the production of various cytokines (14–16) and cytolytic functions (17, 18).

In contrast to the many inhibitory effects of TGF-β on T lymphocytes delineated by these early studies, a growing body of literature demonstrates that TGF-β can also enhance the growth of T cells, predominantly of the naive phenotype (19), induce T cell expression of specific cytokines and enhance their capacity to respond to subsequent stimulation, and even promote effector expansion through inhibition of T cell apoptosis (20–22). Though these differential activities appear to be contradictory, they exemplify the common association between cellular differentiation or activation status and effect of TGF-β that exists in most responsive cell types. We expand this discussion below in an overview of the roles of TGF-β in thymocyte development and T_{H1}-T_{H2} differentiation.

Autocrine and Paracrine Effects of TGF-β During Normal Thymopoiesis

Early thymocytes progress through a series of differentiation steps largely defined by their expression of specific receptor molecules (23, 24). These cells undergo extensive proliferation promoted by a number of cytokines, including IL-2, IL-4, and IL-7, and their development from the T cell receptor negative (TCR^-), $CD4^-CD8^-$ (triple negative, TN) thymic precursor into either the $CD4^+$ or the $CD8^+$ single positive (SP) populations is controlled by a variety of cellular interactions. Through in vitro studies of thymopoiesis, roles for both autocrine and paracrine TGF-β have now been defined.

TGF-β was first reported to induce CD8 expression in studies designed to determine the influence of various cytokines on the differentiation of murine thymic TN cells to either the $CD4^+$ or the $CD8^+$ SP populations. IL-7 can maintain the viability of IL-2 receptor negative ($CD25^-$) early TN thymocytes, including their capacity to differentiate fully in vitro in a lymphoid-depleted fetal thymus organ culture system (25). TGF-β synergizes with TNF-α to promote or favor the development of thymic precursor cells expressing CD8 (26). Furthermore, these thymic precursors treated with TGF-β and TNF-α retained the capacity to differentiate fully when transferred to fetal thymus organ culture, suggesting that this TGF-β-induced expression of CD8 on $CD25^+$ TN thymocytes represents a normal differentiation step (25).

The ability of TGF-β to regulate the expression of CD8 is not restricted to thymic precursors. Inge and colleagues found that TGF-β alone could enhance de novo CD8 expression not only on $CD4^-CD8^-$ thymocytes, but also on the IL-2-dependent CTLL-2 cytotoxic T cell line (27). The induction of CD8 in CTLL-2 cells was dependent on the continuous presence of TGF-β, was not a consequence of growth arrest, and was in contrast to the ability of TGF-β to

inhibit IL-2-dependent increases in IL-2Rα, IL-2Rβ, and Granzyme B mRNA levels in these cells.

TGF-β IN THE CONTEXT OF CELLULAR INTERACTIONS IN THE THYMUS More recent studies examine the role of specific cellular interactions in these events and point toward both autocrine and paracrine TGF-β activity in normal thymopoiesis. One such study focused on the inhibitory effects of TGF-β on the in vitro proliferation of freshly isolated human triple negative thymocytes in response to IL-2, IL-4, and IL-7 (28). The dramatic inhibitory effect of TGF-β on these proliferative responses was mimicked by the coculture of these thymocytes with autologous irradiated $CD8^+$ T cells, but not their cell free supernatants. Cell fixation studies determined that the thymocytes themselves produce a latent TGF-β1 that could only be activated through the interaction of TN cells with the $CD8^+$ cells, thus identifying TGF-β1 as an autocrine inhibitory factor for early human thymocytes.

Studies of murine thymocyte development in fetal organ culture further substantiate the role of TGF-β in early thymopoiesis (29) and also implicate potential paracrine mechanisms involved in this process. The progression of $CD4^-CD8^-$ thymocytes to $CD4^-CD8^{lo}$, and to the subsequent $CD4^+CD8^+$ stages show that differentiation into the double positive state requires progression through at least one cell division, an event that is effectively inhibited by thymic epithelial cells expressing TGF-β (30). The effect is specific for this stage of development in that the generation of $CD4^-CD8^{lo}$ cells is unaffected.

Thus, it is likely that both paracrine and autocrine mechanisms participate in a complex regulatory loop to control thymopoiesis. This may include the regulation of stromal cell production of stimulatory cytokines, such as IL-7, by TGF-β produced by thymocytes (31, 32). As noted above, TGF-β produced either by thymic precursors or thymic epithelial cells can affect the development of thymocytes, particularly their response to stimulatory cytokines such as IL-7.

A Role for TGF-β in the Development of Specific T_H Responses

Our understanding of the process of differentiation from a naive, peripheral $CD4^+$ T cell into distinct T_H subsets with unique cytokine profiles and functions continues to increase. There are multiple pathways for the initiation of this differentiation into any of these functionally polarized T_H subsets, and certain cytokines are clearly established as inducers of either the T_{H1} (IFN-γ and IL-12), or the T_{H2} (IL-4) response (reviewed in 33) (see Figure 1). More recent studies have focused on determining not only the cellular origin of these cytokines, but also the mechanisms controlling their production (34, 35). Even though expression of these cytokines remains the hallmark of their respective T_{H1} or

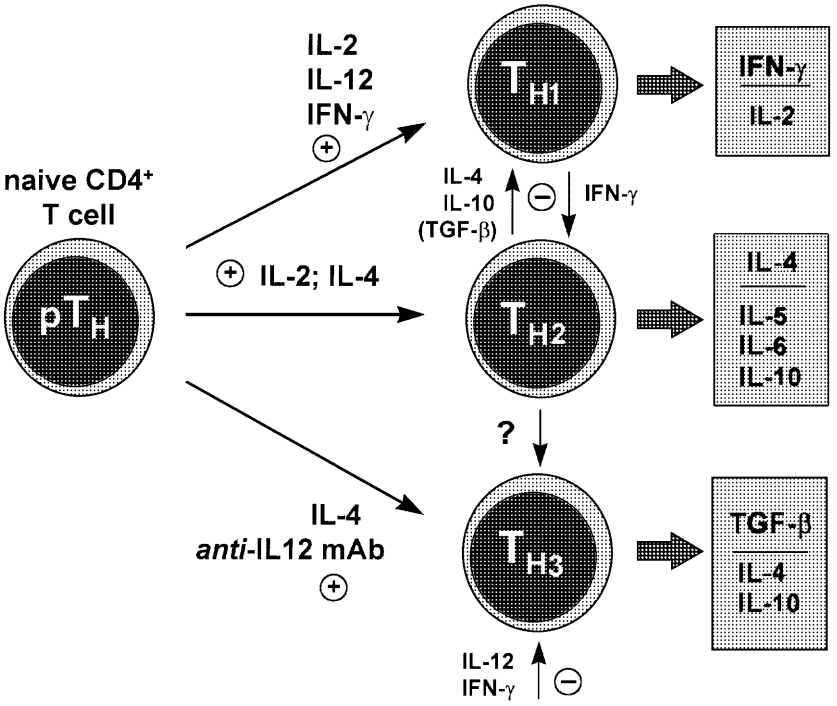

Figure 1 The characteristic production of active TGF-β by a specific subset of T_H cells is now recognized as an important factor in the establishment of oral tolerance. While this subset produces cytokines characteristic of the T_{H2} class of effector cells, the secretion of TGF-β is critical to their ability to suppress an inflammatory response. Although the exact requirements for the establishment of these TGF-β-secreting cells remained to be defined, the progress is clearly antagonized by factors that promote differentiation toward a T_{H1} response.

T_{H2} subset, the recent identification of differential expression of the $\beta 2$ chain of the IL-12 receptor on these two cell populations shows this subunit to be a distinct marker of T_{H1} cells, in both mice (36) and humans (37).

While different experimental systems have produced conflicting data regarding the influence of TGF-β on T_H differentiation, clearly TGF-β can inhibit the production of and response to cytokines associated with each subset, and the production of TGF-β by antigen-specific T cells may mark a unique subset already referred to as T_{H3} (38–40). Initial studies of the exposure of naive $CD4^+$ cells to TGF-β during their priming phase suggested that this factor would push differentiation toward a T_{H1} phenotype, with the primary result being an increase in INF-γ-producing cells and a decrease in IL-4-producing cells (41). This effect has also been observed in a murine IL-2-secreting $CD4^+$

T cell clone (42), and in cultures of directly alloresponsive human $CD4^+$ T cells when TGF-β is included in a mixed lymphocyte culture (MLC) performed with purified responder T cells (43). However, the significance of these observations has been questioned, particularly since systemic administration of TGF-β in mice infected with *L. amazonis* or *L. brasiliensis* leads to increased production of the T_{H2} cytokine, IL-4, and decreased production of the T_{H1}-associated IFN-γ, and ultimately results in a greater severity of disease (44).

Further studies appear to have more clearly aligned TGF-β exposure with T_{H2} differentiation by virtue of its reciprocal relationship with both IFN-γ and IL-12 (45). For example, exposure of naive murine $CD4^+$ T cells to IL-12 during activation with anti-CD3 monoclonal antibody (mAb), in the absence of accessory cells, not only leads to autocrine production of IFN-γ, but synergizes with IFN-γ to induce T_{H1} differentiation (46). However, simultaneous exposure to TGF-β strongly inhibits this induction of IFN-γ in response to IL-12 and suppresses IL-12-induced T_{H1} development, an effect that is enhanced by the addition of anti-IFN-γ neutralizing antibodies (46). This functional interaction between TGF-β and IL-12 has also been observed in human primary allogeneic proliferative and cytotoxic T cell responses (47), in which the effects of TGF-β are mediated through mechanisms associated with the abrogation of IL-12 production. This response cannot be reversed even with the addition of exogenous IL-12 or IFN-γ.

This antagonistic relationship between IL-12, IFN-γ, and TGF-β may also be a factor in the induction of peripheral tolerance following the oral administration of antigen (48). In several animal models of autoimmunity, and in their respective disease counterparts in humans, the ability to ameliorate disease severity through this mechanism is linked to the establishment of a T_{H2}-like subset that is further distinguished by its ability to secrete bioactive TGF-β (38). This process has been modeled in ovalbumin (OVA)–T cell receptor (TCR) transgenic mice, in which the oral administration of high doses of OVA leads to local T cell expression of IFN-γ in Peyer's patches, followed by systemic unresponsiveness due primarily to clonal anergy rather than the suppressor cytokine production typically seen with low dose oral antigen (49). This predominance of T_{H1} cytokines, including IL-12, effectively inhibits the establishment of TGF-β-secreting suppressor T cells. More importantly, the concomitant systemic administration of anti-IL-12 blocking antibodies not only significantly enhances TGF-β production in this model, but this induction is completely independent from any effect on IL-4 production, further distinguishing these suppressor cells as a potentially distinct T_H subset (see Figure 1). This conclusion is also supported by models of inflammatory bowel disease in which TGF-β-producing $CD45RB^{lo}$ T cells protect against colitis induced in SCID mice following their reconstitution with $CD45RB^{hi}$ T cells, even when the $CD45RB^{lo}$ cells are from an IL-$4^{-/-}$ donor (50).

TGF-β INFLUENCES DENDRITIC CELL DEVELOPMENT AND FUNCTION

Dendritic cells (DC) are a distinct population of leukocytes that function as the primary antigen-presenting cells in the activation of T lymphocyte responses (51). Several highly specialized populations of dendritic cells, including the Langerhans cells (LC) of the epidermis and the follicular dendritic cell (FDC) of lymph nodes, have been identified, and TGF-β may both regulate their development and mediate their effects.

A Role for TGF-β1 in the Generation of Dendritic Cells

The recent discovery of a requirement for TGF-β1 during the in vitro differentiation of functional dendritic cells identified a new role for TGF-β in regulating these specialized antigen presenting cells (52). Though in vitro differentiation of DC from cultures of $CD34^+$ precursor cells is dependent on the presence of specific cytokines, such as TNF-α, SCF, and GM-CSF, it is greatly enhanced by the addition of exogenous TGF-β1, which can effectively substitute for the serum or plasma requirement in this system. Further studies determined that TGF-β protects DC viability, as demonstrated by a reduction of more than 60% in the number of apoptotic cells at 72 h in culture (53).

The importance of TGF-β1 in DC development is also emphasized by recent studies in the TGF-β1 knockout mouse, where the complete absence of the epidermal LC is a striking feature of the phenotype (54), which also includes the generalized activation of most immune cell populations and widespread tissue inflammation (55, 56). The loss of epidermal LC is a consistent defect in TGF-β1 null mice whether they are bred onto a variety of immunodeficient backgrounds (SCID, athymic nude, RAG2-null) or maintained on the immunosuppressive agent rapamycin, so it appears unlikely that inflammatory cytokines are effecting migration of LC from the TGF-β1-null epidermis (54). This absence of epidermal LC may reflect an absence of precursor populations or perhaps some defect in their homing to the epidermis. However, transplantation of TGF-β1-null marrow into wild-type, lethally irradiated recipients leads to repopulation of recipient skin with donor-derived LC, implying that normal LC progenitors exist in the TGF-β1 null mouse bone marrow, and that paracrine sources of TGF-β1 are sufficient to support normal LC development and perhaps even their migration into the epidermis (57).

TGF-β and Follicular Dendritic Cells

TGF-β may also have an important role in another highly specialized class of antigen-presenting cell, the follicular dendritic cell (FDC). Localization of TGF-β within the FDC of lymphoid follicles (58), combined with its ability to inhibit antigen-induced rescue of germinal center (GC) B cells, suggests

there is specific function for TGF-β in FDC. While these FDC, which have the capacity to take up antigen and display it on their surface for more than a year, express receptors for several cytokines, TGF-β is the only one they are known to produce (58). The surface of the FDC is the site of primary selection events in B cells that have undergone activation, clonal expansion, and somatic hypermutation of Ig v^- region genes (centrocytes). Antigen trapped on the surface of FDC provides an effective survival signal for centrocytes with high-affinity surface membrane immunoglobulin (smIg), and TGF-β is the only factor known to interrupt this signal (59). This effect is specific, in that the survival signals initiated through CD40 are not inhibited by TGF-β; this may represent a regulatory mechanism to prevent the selection of centrocytes with low-affinity receptors bearing autoreactive mutations (60). Finally, the fact that TGF-β1 null mice also lack $gp40^+$ lymph node DC, despite normal numbers of $CD11c^+$ DC, suggests that the loss of TGF-β1 may affect other specialized DC populations participating in extrafollicular immune responses (54).

TGF-β PRODUCTION AND RESPONSIVENESS IN B CELLS

A Link Between Activation and TGF-β Expression in B Cells

As in most immune cell populations, the synthesis, secretion, and response to TGF-β in B cells are often a consequence of their state of differentiation and the activation signals involved. For example, the activation of human tonsillar B cells with the polyclonal mitogen *Staphylococcus aureus* Cowan (SAC) only slightly upregulates TGF-β1 mRNA expression, but results in a sevenfold induction of TGF-β1 protein secretion, over 90% of which remains in the latent or inactive form (61). These effects contrast with those of another polyclonal B cell activator, lipopolysaccharide (LPS), which stimulates mouse splenic B cells to produce bioactive TGF-β (62). This autocrine TGF-β is required for the secretion of IgG by B cells in response to LPS in vitro. Activation through antigen receptor by binding a monoclonal anti-IgM antibody to cell surface IgM induces expression of TGF-β1 mRNA in normal human peripheral blood B cells and stimulates murine B-lymphoma cell lines to secrete large amounts of active TGF-β1 (63, 64). Given that TGF-β typically inhibits B cell proliferation and may induce apoptosis in both B cells (59, 65) and in the fully differentiated plasma cells (S Amoroso, N Huong, A Roberts, M Potter, J Letterio, manuscript in preparation), this induction of TGF-β likely serves as an important regulatory feedback loop to limit expansion of an activated population. This autocrine inhibitory loop remains intact in some B lymphoid malignancies and has been

implicated in their reduced proliferative responses and slow rate of progression (66, 67). However, it is interesting that several murine and human B lymphoid malignancies have been identified that have become insensitive to the growth inhibitory effects of TGF-β and that express substantial amounts of an active form of the molecule (68–70). This association is common in epithelial malignancies and may be important in tumor progression and in suppression of immune surveillance (71).

B Cell Differentiation and Immunoglobulin Production Are Regulated by TGF-β

TGF-β controls several aspects of the normal maturation and differentiated functions of B cells, in addition to its effects on B cell proliferation. This includes the regulation of expression of cell surface molecules, including the inhibition of IgM, IgD, IgA, κ and λ chains, CD23 (FcϵRII) and the transferrin receptor, and the induction of MHC class II expression on both pre-B and mature B cells (reviewed in 72). The ability of TGF-β to direct switch recombination in immunoglobulin isotypes IgA and IgG2b in mouse and IgA in human appears to be due to the induction of transcription from the unrearranged Iα-Cα and Iγ2b-Cγ2b gene segments, but the specific events that lead to this switching and whether TGF-β is even essential are not yet known (72). Indeed, although the loss of endogenous TGF-β1 gene expression in the TGF-β1-null mouse results in the production of autoantibodies (predominantly IgG), it does not lead to a diffuse polyclonal hypergammaglobulinemia nor to a predominance of an Ig clonotype (73).

While TGF-β is noted for this ability to inhibit immunoglobulin synthesis and the secretion Igs of all classes, here too there can be a dichotomy in the response to TGF-β. Most studies demonstrating this inhibitory effect have investigated the effects of addition of exogenous TGF-β to cultures of activated B cells. In a revealing study by Snapper et al (62), the addition of an anti-TGF-β blocking antibody to LPS-activated cultures led to a significant decrease in the secretion of IgG1, IgG2a, IgG3, and IgE, without an effect on IgM, suggesting that low levels of autocrine TGF-β may actually serve to enhance production and secretion of immunoglobulins under certain conditions.

CONTROL OF MONOCYTE/MACROPHAGE FUNCTION BY TGF-β

Cells of the mononuclear phagocyte system function both as accessory antigen-presenting cells in the induction of immune responses and as effector cells in mediation of certain responses. Monocytes/macrophages secrete TGF-β, which can also regulate a broad spectrum of their activities from chemotaxis, to host

defense, to activation and deactivation as mediated by expression of cytokines and their receptors and small effector molecules such as reactive oxygen species and nitric oxide (for a review, see 74). As such, these cells mediate many of the effects of TGF-β on inflammation (reviewed in 4, 75) and, indirectly, on pathological fibrosis associated with chronic inflammation (reviewed in 76).

Expression and Activation of TGF-β by Monocytes/Macrophages

As is the case for all cell lineages derived from the bone marrow, both circulating monocytes and tissue macrophages secrete TGF-β, predominantly as the type 1 isoform (77, 78). In macrophages, as in lymphocytes, the primary level of control is not in the regulation of expression of TGF-β mRNAs, but in the regulation of both the secretion and activation of latent forms of TGF-β. Thus, treatment of peripheral blood monocytes with bacterial lipopolysaccharide (LPS) or of alveolar macrophages with concanavalin A results in significant increases in secreted active TGF-β1 without changes in the level of TGF-β1 mRNA (77, 78).

Activation of the latent forms of TGF-β, which are blocked from receptor binding, is an important posttranscriptional control point in both physiological and pathological actions of TGF-β (79, 80). Specific mechanisms of activation of TGF-β by LPS-treated thioglycollate-elicited peritoneal macrophages include complex cooperativity between the serine proteases plasmin and urokinase (uPA), the uPA receptor, tissue type II transglutaminase, and the mannose-6-phosphate receptor (81). Expression of active TGF-β1 by macrophages and other leukocytes plays a central role not only in inflammation, but also in accompanying fibrosis by paracrine stimulation of resident mesenchymal cells to produce excessive extracellular matrix. Thus, in bleomycin-induced pulmonary fibrosis in mice, nearly all of the active TGF-β implicated in pathologic matrix deposition is secreted by alveolar macrophages and is temporally concordant with the deposition of collagen in the lung (82). In these cells, activation of latent TGF-β is dependent both on plasmin and on binding of the latent complex to the cell membrane via thrombospondin-1 (TSP-1) and its receptor, CD36, expressed on the surface of monocytes and macrophages (82–84) (Figure 2). These findings suggest that the mannose-6-phosphate receptor and transglutaminase might also serve to anchor latent TGF-β to the cell surface to optimize proteolysis and activation by plasmin. Studies with human THP-1 macrophage-like cells show that TGF-β itself may contribute to its activation by macrophages. Adherent THP-1 cells respond to TGF-β by upregulation of uPA and its receptors, essential components in the regulation of macrophage plasminogen activation, resulting in a threefold increase in membrane-bound plasmin activity (85). Parasitic infection of macrophages

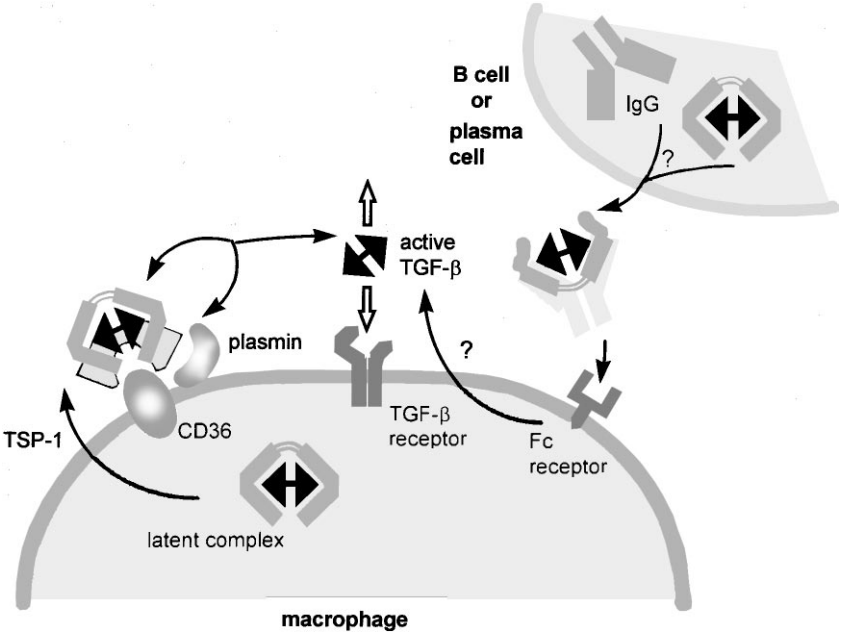

Figure 2 Activation of latent TGF-β by macrophages is key to both its physiological and pathological actions on immune cells. Following processing of pre-pro TGF-β to its latent complex, the secreted latent TGF-β is anchored to the macrophage membrane via TSP-1 bound to its receptor, CD36, and activated by cell membrane bound plasmin (84). Macrophages can also activate IgG-TGF-β complexes secreted by plasma cells by binding these complexes to cell membrane Fc receptors (119, 121). Alternatively, certain IgG-TGF-β complexes may be intrinsically active (120). Active TGF-β then signals through its cell surface receptor in an autocrine or paracrine mode.

also results in activation of secreted TGF-β, as discussed in greater detail below, although the particular mechanisms involved have not yet been identified.

Activation of Macrophage Function by TGF-β

Similar to the pleiotropic effects of TGF-β on lymphocyte subsets, effects of TGF-β on proliferation of macrophages can be either stimulatory or inhibitory, depending on the other cytokines present and the state of differentiation or tissue origin of the cells (86–88). Femtomolar concentrations of TGF-β induce a chemotactic response in human peripheral blood monocytes, a response that likely plays a critical role in the recruitment of mononuclear cells into sites of injury or inflammation (89–91). Picomolar concentrations of TGF-β activate resting human blood monocytes to express increased levels of mRNAs encoding

a variety of cytokines including IL-1α and β, TNF-α, PDGF-BB, and bFGF, which indirectly affect various immune processes as well as non-immune targets as in angiogenesis (89–91). Other proinflammatory effects of TGF-β on leukocytes include its ability to increase expression of several integrin receptors on monocytes including LFA-1, which binds ICAM-1 expressed on the surface of endothelial cells; VLA-3 ($\alpha_3\beta_1$), which binds collagen, fibronectin, and laminin; and VLA-5 ($\alpha_5\beta_1$), which binds fibronectin, thereby increasing both cell-cell and cell-matrix interactions (92, 93). This enhanced expression of adhesion receptors as well as of type IV collagenase by activated monocytes likely enhances their penetration of the basement membrane of vascular endothelium and their subsequent transmigration into tissues (93). As in other responses, IFNγ can antagonize certain of these effects of TGF-β on cellular adhesion, suggesting that the cytokine balance may regulate leukocyte motility within sites of inflammation (94).

The phagocytic activity of monocytes/macrophages is also activated by TGF-β. One such mechanism involves its ability to stimulate circulating monocytes to upregulate expression of cell surface FcγRIII, which recognizes bound IgG and is thought to play a key role in immunophagocytosis (95). A second mechanism involves a TGF-β–dependent increase in macrophage recognition of phosphatidyl serine, which, though normally localized to the inner membrane, is expressed on the outer membrane leaflet of apoptotic cells (96). Thus TGF-β contributes both to the formation of inflammatory foci by direct effects on chemotaxis and adhesion and to the resolution of acute inflammatory reactions and restoration of homeostasis by increasing the phagocytic activity of macrophages toward inflammatory cells and damaged parenchymal cells.

Deactivation of Monocytes/Macrophages by TGF-β

In contrast to the activating effects of TGF-β on peripheral blood monocytes, its actions on tissue macrophages are generally suppressive and contribute to the resolution of an inflammatory response. This has been attributed, in part, to a dramatic difference in the expression pattern of receptors for TGF-β on these two populations of the mononuclear phagocyte system. Resting monocytes express high levels of TGF-β type I and II receptors, whereas receptor levels decline as cells mature and then become activated by agents such as LPS or IFNγ (4). Examples of suppressive effects of TGF-β on activated macrophages are its ability to modulate the profile of activating cyokines as by limiting production of IFNγ (15) or increasing expression of the IL-1 receptor antagonist (97) similar to effects of IL-4, IL-10, and macrophage deactivating factor (MDF) (74). This also parallels the role of TGF-β in resolution of inflammation in models of inflammatory bowel disease, where there is suggested to be a deficiency in elaboration of suppressive cytokines such as IL-1 receptor antagonist, IL-4,

IL-10, and TGF-β to counterbalance the pro-inflammatory cytokines such as IFNγ and TNFα, which are produced in response to the constant luminal exposure to antigens (45, 98).

SUPPRESSION OF NITRIC OXIDE AND REACTIVE OXYGEN INTERMEDIATES Possibly the most important deactivating effect of TGF-β on macrophages is its ability to limit the production of cytotoxic reactive oxygen and nitrogen intermediates by cells activated by either IFNγ or LPS (for reviews, see 74, 99). The enzyme responsible for production of nitric oxide by activated macrophages is an inducible form of nitric oxide synthase (iNOS). Regulation of this enzyme by cytokines including TGF-β is now known to underlie control of the antimicrobial and tumoricidal pathways of macrophages and of immune responses in general (74). TGF-β regulates iNOS at both transcriptional and posttranscriptional levels, resulting in downregulation of iNOS mRNA levels and suppression of both the expression and activity of iNOS protein. The latter effects are still observed even when TGF-β is added to cultures after expression of iNOS is maximal (99). TGF-β also suppresses expression of reactive oxygen intermediates and respiratory burst capacity by both resting blood monocytes (100) and activated macrophages (101), but the mechanisms involved are still unknown.

SUPPRESSION OF MACROPHAGE FUNCTION IN PARASITIC INFECTION TGF-β is now recognized as an important immunoregulator and parasite escape mechanism in all forms of human and murine leishmaniasis, a parasitic disease with both tegumentary and visceral effects, depending on the strain. Protozoan parasites such as *Trypanosoma cruzi*, which infect all nucleated cells (102), and *Leishmania*, for which macrophages serve as the exclusive cellular host (44, 103), have evolved mechanisms to induce the infected host cell to secrete active TGF-β, which then suppresses the killing activity of macrophages and enhances intracellular proliferation of the pathogen. Fascinating studies with *T. cruzi* suggest that autocrine signaling of TGF-β is required for parasite entry into cells: Epithelial cells lacking TGF-β receptors are resistant to trypanozome infection and infectivity is restored following transfection of functional TGF-β receptors (104). In vitro experiments demonstrate that the amount of active TGF-β produced by macrophages upon infection with various strains of *Leishmania* promastigotes correlates roughly with both the strain virulence and the proliferation of parasites within the macrophage (105). Moreover, studies in mice demonstrate a role for endogenous TGF-β in susceptibility to infection in that systemic administration of TGF-β increases the infectivity of relatively avirulent strains of *Leishmania* and reverts the genetic resistance of certain strains of mice to infection, whereas systemic administration of antibodies to

TGF-β arrests development of lesions in susceptible mice (44). Similar results are found with *T. cruzi* infection in mice (106).

Both resistance to and recovery from leishmaniasis are related to cell-mediated immune responses. Two mechanisms are proposed to explain the disease-enhancing effects of TGF-β in leishmaniasis, and each depends on the balance of the cytokine milieu. One involves the ability of TGF-β to decrease cell-mediated immunity by suppressing effects of IFNγ or IL-4 on expression of MHC class II antigen on antigen presenting cells (107, 108). The second mechanism is related to the ability of TGF-β to modulate the activity and differentiation of $CD4^+$ and $CD8^+$ T cell subsets. Thus in patients with the destructive mucosal form of leishmaniasis, TGF-β inhibits cytotoxic $CD8^+$ T cells, which are thought to play a role in elimination of infected cells (109). And in mice, a T_{H2}-like response with accompanying increased production of IL-4 and IL-10 is found in regional lymph nodes of susceptible strains, whereas in resistant mice, a T_{H1}-type response predominates with production of IL-2 and IFNγ, which promotes leishmanicidal activity by enhanced production of cytotoxic oxygen and nitrogen radicals (103). As discussed above, TGF-β appears to play a direct role in modulation of these T_H cell subsets (see Figure 1).

Infection of macrophages with the intracellular bacteria *Mycobacterium avium* also increases macrophage production of active TGF-β and suppresses their antibacterial activity, suggesting that a variety of infectious agents may have developed similar mechanisms for their survival and proliferation in a mammalian host (110).

ROLE OF TGF-β IN AUTOIMMUNE DISEASES

In autoimmune disease, the normal ability to distinguish between self and non-self is disturbed. Many lines of evidence implicate TGF-β in the pathogenesis of autoimmune diseases. Studies of experimental allergic encephalomyelitis (EAE) and collagen-induced arthritis (CIA) in mice and rats demonstrated that systemic administration of TGF-β suppressed the symptoms of the disease, whereas antibodies to TGF-β enhanced the disease process, demonstrating that endogenous TGF-β has a suppressive effect on disease progression (111–114). TGF-β also mediates certain aspects of peripheral immune tolerance induced by oral administration of antigens. Both $CD4^+$ and $CD8^+$ suppressive T cell subsets that secrete active TGF-β1 have been identified following induction of oral tolerance (115), and the protective function of these cells both in vitro and in vivo can be blocked by antibodies to TGF-β (38, 114). Consistent with these observations, mice in which the TGF-β1 gene has been deleted by homologous recombination develop a phenotype characterized by numerous hallmarks of autoimmune disease (for a review, see 116). All of these data support a model

in which TGF-β actively participates in the development of self-recognition and plays an essential role in the maintenance of self-tolerance, as discussed in greater detail below.

Mechanisms of Action of TGF-β in Autoimmune Disease

STUDIES IN THE MRL/LPR MOUSE Several new insights into pathologic TGF-β activation and trafficking and its regulation of immune cell activity have come from the study of the MRL/lpr mouse, a murine model of systemic autoimmunity. These mice, which uniformly develop systemic autoimmune disease resembling human systemic lupus erythematosis (SLE), have a mutation in the gene encoding Fas that results in defective apoptosis, one of the key mechanisms responsible for deletion of self-reactive lymphocytes (117).

Study of the MRL/lpr mice has demonstrated that these mice have elevated levels of circulating plasma TGF-β1 bound to IgG autoantibodies (118–120). Unrelated studies show that mice repeatedly immunized with an antigen carry TGF-β1 on a small fraction of the specific IgG induced, and that this complex suppresses $CD8^+$ CTL responses in mixed lymphocyte cultures, but only in the presence of macrophages (121). The demonstration that this suppression could be blocked by an antibody to Fc receptors suggests a novel regulatory circuit in which antigen-specific IgGs are processed by macrophages to suppress CTL responses to unrelated antigens (see Figure 2). Given the highly pleiotropic nature of TGF-β, it has been proposed that its binding to IgG could restrict or direct its activity to antigenic sites where it plays important roles in the homeostasis of immunity and suppression of autoimmune disease (121).

Elevated circulating levels of IgG-bound TGF-β1 are found not only in the MRL/lpr mice, but also in pristane-induced systemic autoimmunity in Balb/c mice and, importantly, in the plasma of some patients with SLE (118–120). Two consequences directly attributable to these increased levels of IgG-bound TGF-β1 in plasma are increased susceptibility to infection by gram-negative and gram-positive bacteria and defects in polymorphonuclear leukocyte (PMN) function as assayed by their inability to extravasate into the thioglycollate-inflamed peritoneum and failure to amplify phagocytic function in response to stimulation by phorbol ester or the chemotactic peptide FMLP (118, 120, 122). Importantly, the IgG bound form of TGF-β1 is consistently 10–500-fold more active than uncomplexed recombinant TGF-β1 in mediation of effects on immune cells both in vitro and in vivo, suggesting that targeting of this TGF-β complex to the cell surface, possibly via Fc receptors, may increase its propensity to interact with signaling receptors on immune cells and possibly even modulate its receptor binding in such a way as to augment its effects (120, 121).

Whereas total plasma levels of TGF-β1 in MRL/lpr mice are only about double that of congenic control mice, plasma levels of IgG-bound TGF-β1 are

nearly 150-fold higher than the controls, with about 75% of the total plasma TGF-β1 in MRL/lpr mice bound to IgG (120). Both immunohistochemical data and in vitro assay of culture supernatants of cells purified from MRL/lpr mice identify B cells and plasma cells as the source of the circulating IgG-bound TGF-β1 (120). Notably, greater than 80% of the secreted TGF-β1 complexed to IgG is in the active form, suggesting that the pathogenicity of this complex may derive not only from the specific targeting imparted by the bound IgG, but also from loss of regulation of the activation of latent TGF-β.

Many aspects of this important mechanism are still unknown (see Figure 2). For example, whether TGF-β can associate with any other immunoglobulin isotypes is not known, and whether mature or latent TGF-β1 associates with specific IgGs intracellularly prior to secretion (119, 120) or associates with IgG extracellularly (121, 123) is still controversial. Moreover, since TGF-β appears to associate only with IgG autoantibodies and antibodies raised in response to specific immunization (120, 121), it is important to identify the precise determinants of IgGs that mediate the interaction with TGF-β and whether the binding is isoform specific. Thus, the demonstration that glomerulopathic but not tubulopathic monoclonal κ light chains are associated with TGF-β-like effects on mesangial cells, implicating TGF-β in the pathogenesis of light chain deposition disease (124), does not exclude the interpretation that glomerulopathic light chains might actually bind TGF-β and that the variable region of the light chain might be the site of TGF-β binding to IgG. However, studies demonstrating the ability of TGF-β to mimic an IgG-binding factor, thought to be important in negative-feedback inhibition of IgG and IgM antibody production by B cells (123), show that TGF-β can bind insolubilized IgG but not $F(ab')_2$ (123). Based on these observations, the possibility that TGF-β might bind to both the variable region and the Fc domain of IgG cannot be excluded. Interesting in this regard, activation of latent TGF-β by binding to TSP-1 involves the bimodal interaction of a WSXW motif with mature TGF-β followed by interaction of another sequence, RFK, with the amino terminus of the latency-associated protein (LAP) (125). Binding of this TSP-1/TGF-β1 complex to macrophages via the CD36 receptor represents another mechanism for targeting TGF-β complexes, though with an additional level of control by plasmin (84).

In summary, active TGF-β1/IgG complexes secreted by B cells and plasma cells and interacting, in certain cases, with cellular Fcγ receptors may be found to modulate B cell responses, to mediate immunosuppressive effects on both T cells and neutrophils in a broad spectrum of diseases, and possibly to participate in physiological trafficking of TGF-β1 as proposed for the transfer of maternal TGF-β1 to fetuses and neonates (126).

AUTOIMMUNE DISEASE IN THE TGF-β1 NULL MOUSE Mice null for the TGF-β1 gene clearly illustrate that this isoform is a critical regulator of immune cell

differentiation and function, and further, that loss of this gene is sufficient for development of an autoimmune-like phenotype including enhanced expression of MHC class I and II antigens, circulating SLE-like IgG antibodies to nuclear antigens, pathogenic glomerular IgG deposits, and a progressive infiltration of lymphocytes into multiple organs similar to that seen in human autoimmune syndromes such as Sjögren's disease (73, 127, 128; for a review, see 116). The inflammatory infiltrates compromise organ function and contribute to the death of these mice at about 3 weeks. Although tissue infiltration can be blocked by systemic administration of fibronectin peptides, which block adhesion of TGF-β1-null leukocytes to endothelium, this treatment does not block the primary autoimmune response (129). In contrast, aberrant expression of the MHC class I and II antigens clearly represents an important mechanism in development of this autoimmune phenotype because backcrosses of the TGF-β1 null mice onto either an MHC class I- or class II–deficient background develop neither circulating autoantibodies nor immune complex deposits (130). TGF-β1 is known to suppress expression of MHC class II antigen (107, 131), consistent with its overexpression in TGF-β1 deficiency. Since MHC class II molecules play a role in the selection and activation of $CD4^+$ T cells, which regulate both humoral and cell-mediated immune responses to antigens, the suppression of the autoimmune phenotype in TGF-β1(−/−)/MHC-II (−/−) mice also derives, in part, from the absence of this T cell subset in the MHC-II-null background (130).

Role of TGF-β in Immunologic Tolerance

The term tolerance describes the process whereby the immune system distinguishes self from nonself to prevent pathologic reactivity against self-antigens. Development of autoimmunity is normally prevented by selection processes operative during lymphocyte maturation that result in apoptosis of self-reactive clones and by mechanisms that maintain or establish tolerance in peripheral tissues. Data suggest that TGF-β can contribute to both of these processes. In the thymus, negative selection ordinarily takes place at the $CD4^+CD8^+$ "double positive" stage of T cell development and involves recognition of MHC class II molecules (30). Since TGF-β regulates the maturation of these doubly positive cells from their $CD4^-CD8^{lo}$ precursors, it is possible that, in the absence of TGF-β1, doubly positive thymocytes are generated too rapidly for their appropriate elimination (30). Dysregulated production of $CD4^+CD8^+$ T cells in these mice may be exacerbated by defects in apoptosis of T cell subsets, as suggested by preliminary data (JJ Letterio, unpublished).

Mechanisms involved in the maintenance of peripheral tolerance include clonal anergy and a delicate balance of reactive and suppressor T cells and their respective cytokines (see Figure 1). Thus in the EAE model, oral tolerization with myelin basic protein induces peripheral tolerance by generating both a

population of $CD8^+$ T cells that secretes active TGF-β1 and a regulatory population of $CD4^+$ T_{H2}-like cells producing IL-4, IL-10, and secreting TGF-β1 in an antigen-specific manner (38, 114, 115). Since only a subset of the T_{H2} clones isolated produced TGF-β1, and since the cytokine profile of these cells is stable on prolonged culture, these cells may constitute a novel T cell subset (T_{H3}) with mucosal T helper function and suppressive activity for T_H3 cells (38). The suppression of disease is proposed to be mediated by inhibition of the autoimmune responses by TGF-β1 secreted by these mucosally derived cells at the target organ. Importantly, a similar distinct subset of T_{H3} cells has been identified in multiple sclerosis patients treated for 2 years with oral antigen (39). Some evidence suggests that the cytokine profile can drive the preponderance of specific T_H cell subsets. As shown in Figure 1, antibodies to IL-12 or IFNγ enhance expression of TGF-β, presumably by a T_{H2}-like or possibly T_{H3} T cell subset (45, 48). Disruption of this tightly controlled cytokine network, as by the loss of TGF-β1, can potentially upset the normal regulation of self-reactivity and contribute to the development of systemic autoimmunity by disturbing the delicate balance between T_{H1} and T_{H2} cells (116). Although the direct involvement of such a mechanism remains to be demonstrated for autoimmune disease in humans, in murine models of inflammatory bowel disease there is now substantial data to support antagonistic effects of pro-inflammatory (T_{H1}) and anti-inflammatory (T_{H2}) T_H subsets characterized by secretion of IFNγ and IL-12 or IL-4 and TGF-β, respectively (45, 50).

CONCLUSION

The multifunctional, context-dependent activities of TGF-β described in this article are by no measure unique to its actions in the immune system, but rather, they exemplify the basic tenet that has defined the function of this molecule, as stated succinctly by MB Sporn: "The function of TGF-β... is not to have an intrinsic action, but to serve as a mechanism for coupling a cell to its environment, so that the cell has the plasticity to respond appropriately to changes in its environment or changes in its own state" (132). Thus, the ability of TGF-β to enhance the induction of an immune response is often accompanied by the increased expression of TGF-β itself, which then often serves to dampen the response or inhibit the activated cell populations.

It is significant that each member of the various hematopoietic lineages can be included in the long list of cells responsive to TGF-β. Though we chose to focus on the differentiation and function of just a few highly specialized leukocytes, the expression and function of TGF-β in NK cells, neutrophils and other myeloid lineages, and various hematopoietic progenitors must also be recognized. In each instance, TGF-β clearly provides regulatory signals that

are shaped by ongoing cellular interactions, by the cytokine context, and by the relative state of differentiation of the responsive cell.

As we continue to advance our understanding of the important and essential functions of this cytokine, it is critical that we begin to consider practical and clinically useful approaches to manipulate both the expression of and the response to this cytokine in those disorders where a role for TGF-β has been implicated.

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