

## Transforming Growth Factor- $\beta$ : Recent Progress and New Challenges

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It is just 10 years since the peptide, transforming growth factor- $\beta$ 1 (TGF- $\beta$ 1)<sup>1</sup>, was isolated from human platelets, human placenta, and bovine kidney and characterized as a discrete molecular entity, namely a 25-kD homodimer with a unique NH<sub>2</sub>-terminal sequence. Two years later this molecule was cloned, and subsequently four other closely related isoforms have been found in vertebrates; three isoforms (TGF- $\beta$ s 1, 2, and 3) are known in man. TGF- $\beta$  can now be considered the prototype of a multifunctional cytokine, especially after the discovery that it could act both as an inhibitor and stimulator of cell replication, as well as control the synthesis of many of the components of the extracellular matrix (for reviews of the above see Roberts and Sporn, 1990; Massagué, 1990; Moses et al., 1990; Sporn and Roberts, 1990). There has been immense progress in TGF- $\beta$  research in the past two years. This mini-review will highlight some of these accomplishments and indicate a few challenges for the future. We will confine this brief review to the TGF- $\beta$ s themselves and will not consider the extended TGF- $\beta$  family, which includes the inhibins, activins, bone morphogenetic proteins, and related morphogenetic peptides, all of which are of increasing importance in many areas of cell biology, such as reproduction and development.

### *Molecular Structure of TGF- $\beta$*

The molecular structure of the TGF- $\beta$ 2 dimer has been determined at high resolution by x-ray crystallography (Daopin et al., 1992; Schlunegger and Grütter, 1992). Several unique features have emerged from these studies: the dimer has an extended, rather than a compact, globular conformation; eight of the nine cysteine residues in each monomer chain are involved in an unusual, compact pattern of intrachain disulfide bridges, called a "TGF- $\beta$  knot"; and there is only a single interchain disulfide bridge, suggesting that hydrophobic interactions between the two chains are of major importance in stabilizing the dimer. Furthermore, there are two cavities, accessible to water, between the interchain disulfide bridge and the hydrophobic cores in the interface area, although the functional implications of this unusual hydrophilic area in the interior of the molecule are not clear at present. The possibility that the interchain disulfide bond might undergo reversible reduction and reoxidation, as has

been shown for a disulfide bond in the transcription factors, Fos and Jun (Abate et al., 1990), remains to be determined. In addition to the above studies performed on crystalline TGF- $\beta$ 2, NMR analysis of TGF- $\beta$ 1 in solution (Archer et al., 1993) has confirmed many aspects of the above x-ray analysis. Moreover, the NMR studies suggest that in solution TGF- $\beta$  may exist in several conformations. The three-dimensional analysis from these molecular studies will provide a firm basis for future understanding of many aspects of TGF- $\beta$  function, such as its interaction with specific receptors, or the biological specificity of particular isoforms.

### *Receptors and Other Binding Proteins for TGF- $\beta$*

The molecular cloning of a TGF- $\beta$  receptor which appears to function in signal transduction has only been achieved within the past year. Although at least nine different proteins have been reported to bind TGF- $\beta$  specifically, only two major species (receptors I and II) are believed to be signalling molecules (for a review see Massagué, 1992); the type II receptor has been cloned (Lin et al., 1992) and shown to be a member of a new serine/threonine kinase receptor family, which also includes two homologous activin receptors. The details of the signal transduction pathway remain to be established, although it appears that cooperativity between the type I and type II receptors is involved in binding of the ligand and the initiation of signalling (Massagué, 1992; Wrana et al., 1992).

"Receptor" III (also known as betaglycan) and the related molecule, endoglin, bind TGF- $\beta$  with high affinity, but have yet to be shown to be directly involved in signal transduction (Lopez-Casillas et al., 1991; Wang et al., 1991; Cheifetz et al., 1992); it has been suggested that they control the availability of TGF- $\beta$  in the local extracellular microenvironment and thus may control the presentation of active TGF- $\beta$  to the signalling receptor complex. Interestingly, endoglin, which is found in relatively high concentrations in endothelial cells, binds TGF- $\beta$ 1 and - $\beta$ 3 with high affinity, but does not bind TGF- $\beta$ 2 (Cheifetz et al., 1992); this specificity of binding of TGF- $\beta$  isoforms correlates with the biological responsiveness of endothelial cells to the three isoforms (highly responsive to TGF- $\beta$ 1 and - $\beta$ 3, much less so to TGF- $\beta$ 2). The elucidation of the specificity of binding and action of the various isoforms in endothelial cells will be facilitated by the recent synthesis of chimeric TGF-betas which structurally are predominantly TGF- $\beta$ 2, but contain certain amino acid replace-

1. *Abbreviations used in this paper:* NGF, nerve growth factor; TGF- $\beta$ , transforming growth factor- $\beta$ .

ments derived from TGF- $\beta$ 1; some of these chimeric molecules act like TGF- $\beta$ 1 on endothelium (Qian et al., 1992). As described below, the cardioprotective action of TGF- $\beta$  may depend largely on its action on endothelium; thus the problem of isoform specificity in this tissue may be of clinical importance.

### Cellular Actions of TGF- $\beta$

In light of its multifunctional nature, it is not surprising that study of TGF- $\beta$  is germane to almost every cell in the body. Several recent reviews have emphasized the role of TGF- $\beta$  in control of the cell cycle, and in particular the interface between TGF- $\beta$  and suppressor genes such as *Rb*. (Moses et al., 1990). Although in some instances the ability of TGF- $\beta$  to suppress epithelial proliferation is coupled to the functional integrity of the RB protein and its ability, in turn, to suppress the function of the *myc* gene (Pietenpol et al., 1991), there are many instances in which this model does not hold (Roberts et al., 1991). Thus, the question of the mechanisms whereby epithelial cells lose their sensitivity to the regulatory actions of TGF- $\beta$  during the process of carcinogenesis remains of paramount importance. Comparison of human colon carcinoma cell lines of high and low degrees of malignancy has shown a loss of responsiveness to TGF- $\beta$  as carcinogenesis progresses (Manning et al., 1991), while the expression of anti-sense RNA to TGF- $\beta$  can convert a colon carcinoma line of low malignancy to a more highly malignant one (Wu et al., 1992).

A related phenomenon is the ability of TGF- $\beta$  to suppress the appearance of the transformed phenotype in co-cultures of *ras*-transformed keratinocytes and normal dermal fibroblasts; in this case, expression of TGF- $\beta$ 3 by a mesenchymal cell modulates the malignant phenotype in a neighboring epithelial cell (Missero et al., 1991). These data are of obvious relevance to the entire issue of mesenchymal-epithelial interactions, both in normal development as well as in carcinogenesis. In epithelial carcinogenesis, it has generally been assumed that epithelial cells "invade" the underlying mesenchymal stroma, and that the stroma is a relatively passive partner in this interaction. A newer perspective would add the possibility that mesenchymal cells might actively suppress carcinogenesis in adjacent epithelia, and that the loss of such active suppression by mesenchyme might contribute to carcinogenesis. If this is indeed the case, then it will be necessary to study the role of tumor suppressor genes in the mesenchyme, as well as in the epithelium, of any tissue during carcinogenesis.

As has been noted before, tissues, as well as individual cells, need to be regarded as the targets for cytokine action (Nathan and Sporn, 1991), and the function of TGF- $\beta$  as an information channel between cells provides an excellent example of this paradigm. We would suggest that the role of TGF- $\beta$  in regulating mesenchymal-epithelial interactions, as is already known from embryologic studies (Symposium, 1992), will become a central theme in the cell biology of many tissues of the adult animal. The recent demonstration that the steroid analogue, tamoxifen (a useful agent for adjuvant therapy of breast cancer), can induce the formation of TGF- $\beta$  in the stroma of human breast tumors provides an excellent example of the practical importance of this topic (Butta et al., 1992).

The role of TGF- $\beta$  in mediating the response of both cells

and tissues to injury is another area of intensive investigation, with particular relevance to the problem of protecting the heart and brain from damage caused by anoxia or reperfusion. TGF- $\beta$  has an intrinsic role in the physiology of isolated cardiac myocytes, as shown by its ability to maintain the rhythmic beating of these cells in culture and to protect them from the deleterious action of interleukin-1 (Roberts et al., 1992a). This appears to be mediated by the suppressive action of TGF- $\beta$  on the synthesis of nitric oxide induced by IL-1 (Roberts et al., 1992b). Other cardioprotective actions are mediated by the ability of TGF- $\beta$  to prevent the adhesiveness of neutrophils to endothelium; this is a first and critical step in reperfusion injury caused by neutrophils. In isolated human fibroblasts, hypoxia upregulates synthesis of TGF- $\beta$ 1 (Falanga et al., 1991); this has not yet been shown in isolated myocytes. It will be important to determine the mechanism whereby low oxygen tension activates the synthesis of TGF- $\beta$ .

The brain is a new organ for studying the role of TGF- $\beta$  in modifying the response of cells to injury. Recent studies have shown that TGF- $\beta$  stimulates the synthesis of nerve growth factor (NGF) in cultured astrocytes and in neonatal brain in vivo, and further, that mRNA for TGF- $\beta$ 1 is increased in cerebral cortex after a penetrating brain injury (Lindholm et al., 1992). Additionally, in cell culture experiments, TGF- $\beta$ 1, but not TGF- $\beta$ 2, protected isolated neurons from degeneration and death induced by hypoxia or excess glutamate (Prehn et al., 1993). In co-cultures of neurons and non-neuronal cells from dorsal root ganglia, TGF- $\beta$  markedly increased neuronal survival, particularly when non-neuronal cells were present (Chalazonitis et al., 1992); these neurotrophic actions are believed to depend on mediation by molecules immunologically related to NGF. Clearly, further studies will be required to define the neuroprotective actions of TGF- $\beta$  and to determine if it might have a useful neurotrophic action in vivo, either directly or by its ability to induce other neurotrophic cytokines such as NGF. Whether TGF- $\beta$  mediates any trophic action of glial cells upon neurons under normal physiological conditions remains to be determined. With the intense current interest in the role of cytokines in the pathogenesis and treatment of many degenerative diseases of the nervous system, it will be of critical importance to define the role of TGF- $\beta$  in any neuronal-glial cytokine network.

Thus, with respect to the generic problem of injury to both cells and tissues, as well as its repair, data from many studies indicate that TGF- $\beta$  exerts a homeostatic action, either by lessening the extent of injury or increasing repair itself. Injury and repair are clearly coupled to each other at both the cell and tissue level; we would suggest that TGF- $\beta$  is a critical molecule for transmission of the information that enables cells and tissues to make appropriate responses to injury.

### Physiologic and Therapeutic Roles

Striking advances have occurred in studies conducted with intact animals. The technique of homologous recombination has created mice with a non-functional gene for TGF- $\beta$ 1 (Kulkarni et al., 1993; Shull et al., 1992). These "knockout" mice are born without any apparent gross developmental defect; however, within two to three weeks they develop a diffuse inflammatory syndrome, characterized by massive infiltrates of mononuclear cells in vital organs such as the heart and lungs, leading uniformly to death. These animals

will be an invaluable resource in study of the role of TGF- $\beta$  in inflammation and immunity, with a host of applications in many human diseases, particularly those of an autoimmune nature. The creation of mice that over-express TGF- $\beta$  has also been achieved, although many attempts to make such transgenic mice have led to embryonic lethality. With restriction of expression of TGF- $\beta$  to the mammary gland, using various promoters, one can obtain mice with defective development of mammary ducts and glandular end-buds, or with impaired ability to lactate (D. Pierce and H. Moses, personal communication; G. Merlino, C. Jhappan, and G. Smith, personal communication).

Potential therapeutic applications for TGF- $\beta$  have been explored in many laboratories (reviewed in Roberts and Sporn, 1992a), with the repair of injury a fundamental theme in many tissues. The anoxic, ischemic, and infarcted heart or brain all may be regarded as unique wounds of varying degrees of severity; thus, data obtained from studies of the role of TGF- $\beta$  in more conventional aspects of wound healing will be useful in these newer areas. In the infarcted heart there is a strong induction of the 1.9-kb transcript of TGF- $\beta$ 1, which is believed to have an important role in the response to injury (Qian et al., 1991). The possibility of using exogenous TGF- $\beta$  to protect the heart from reperfusion injury, previously demonstrated in the rat (Lefer, 1991), has received further support from studies in the cat, in which TGF- $\beta$  exerts a potent effect on endothelium, antagonizing the effects of both IL-1 and TNF- $\alpha$  on emigration of neutrophils from the vascular compartment (Lefer et al., 1993). With respect to cerebrovascular injury, there has been the first report of the ability of TGF- $\beta$  to protect the brain from ischemic cell death *in vivo*; these studies have used a rabbit model of thrombotic stroke (C. Gross, personal communication).

The potential for using TGF- $\beta$  for surgical wound healing has been increased by the demonstration that a single dose of TGF- $\beta$ , administered systemically to animals before wounding, enhances subsequent healing (Amento et al., 1991). The mechanism of this phenomenon is not understood, but may involve a priming of macrophages so that they respond more effectively at the wound site. In repair of bone, major advances have also occurred; a single topical administration of less than a microgram of TGF- $\beta$  can cause complete closure of a skull defect that otherwise would not heal (Beck et al., 1991). The ability of TGF- $\beta$  to have such a profound and prolonged effect on bone healing is undoubtedly a reflection of its potent mitogenic effect on osteoblasts, as well as its known capacity for auto-induction.

The full clinical potential for use of TGF- $\beta$  to prevent tissue injury and enhance repair is yet to be realized. However, the first definitive clinical report has appeared with the publication of the successful use of TGF- $\beta$ 2 to treat holes in the macular portion of the retina; these holes represent localized areas of retinal detachment and result in severe loss of vision. In a large series of patients with macular holes, direct application of TGF- $\beta$  to the macula caused a significant improvement in vision (Glaser et al., 1992). TGF- $\beta$  is believed to induce microscopic fibrosis at the edge of macular holes, thereby preventing further retinal detachment. This study is the first report of the use of a cytokine to alter the outcome of a retinal disease in man. Since retinal epithelium is of neural origin, it also opens the possibility for further evaluation of any potential neurotrophic action of TGF- $\beta$ , whether it be

in the retina for a condition such as diffuse degeneration of macular photoreceptors, or in the brain itself. Conceivably, such activity might be mediated by glial cells, such as the Müller cells of the retina, which correspond to the radial glial cells of the central nervous system (both Müller cells and radial glial cells are known to stain intensely for TGF- $\beta$ ) (Unsicker et al., 1991; Flanders et al., 1991).

While there are many instances in which TGF- $\beta$  may enhance a beneficial healing response, there are clearly situations in which persistent over-expression or dysregulated activation of TGF- $\beta$  elicits an over-response which may lead to serious fibrotic or proliferative disease, such as glomerulonephritis or pulmonary fibrosis. In a similar manner, TGF- $\beta$  may enhance the desmoplastic response to malignant cells and potentiate their metastatic capacity. The topic of maladaptive responses to TGF- $\beta$  and their role in disease has been reviewed recently (Border and Ruoslahti, 1992) and will not be considered further here.

### *New Challenges*

The impact of TGF- $\beta$  research on cell biology, physiology, pathology, and clinical medicine has created a new set of problems and challenges, ranging from basic scientific to applied clinical considerations. We will mention only a few. At the molecular level, structure and function must be rationalized in the most basic terms. What is the molecular fit between the various isoforms and the various receptor and binding molecules? How is the signal first transduced from ligand to receptor, and then what are the intracellular targets for the kinase activity of the receptor? What is the role of TGF- $\beta$  in the regulation of intracellular calcium homeostasis, as shown by the recent cloning of a new ryanodine receptor-calcium channel that is regulated by TGF- $\beta$  (Gianini et al., 1992)? What are the molecular pathways whereby TGF- $\beta$  participates in the regulation of the cell cycle, especially the suppression of DNA synthesis in epithelial cells? The advances of the past year now open these problems to more direct experimentation.

Is there an entire domain of intracellular physiology mediated by TGF- $\beta$ , independent of the known cell-surface receptors (Sporn and Roberts, 1990)? The presence of significant amounts of TGF- $\beta$  localized in mitochondria of cardiac myocytes and hepatocytes (Heine et al., 1991) strongly suggests that this may be the case. Although we speak of each of the three mammalian isoforms as single, discrete structures, can they in turn be modified by post-translational mechanisms (such as acylation, alkylation, phosphorylation, or prenylation), thus adding to the informational content of the cytokine networks in which TGF- $\beta$  participates? Since aberrant expression of TGF- $\beta$  may be pathogenic (Border and Ruoslahti, 1992), can new antagonists, such as soluble receptors or other peptides with high binding affinity, be developed for treatment? Conversely, can the potent immunosuppressive activity of TGF- $\beta$  be used in a practical manner for control of autoimmune disease, without incurring undesirable side effects (Johns et al., 1991; Kuruvilla et al., 1991; Miller et al., 1992; Racke et al., 1991)? Is it better to use agents such as retinoids, vitamin D analogues, and other ligands of the steroid-receptor superfamily to induce TGF- $\beta$ , rather than to use TGF- $\beta$  itself as a therapeutic agent (Roberts and Sporn, 1992b)? What is the tissue-specific and

isoform-specific pattern of induction of TGF- $\beta$  by these agents? It is already known that they can induce the expression and activation of latent TGF- $\beta$ . What are the overall mechanisms for control of the activation of latent TGF- $\beta$  by proteolytic enzymes, and how are the activities of other cytokines, such as TNF, gamma-interferon, and basic FGF, in turn regulated by active TGF- $\beta$  in a homeostatic loop (Flaumenhaft et al., 1992)?

The control of synthesis of TGF- $\beta$ , particularly by steroids and related molecules, is often at the posttranscriptional level; what are the mechanisms, and how are the unusually long 5'- and 3'-untranslated regions of the genes for TGF- $\beta$  1, 2, and 3 involved in the regulation of the synthesis of the respective peptides (Kim et al., 1992)? How much function is regulated transcriptionally, posttranscriptionally, or by alteration of latency? How can the new mice in which the TGF- $\beta$ 1 gene has been inactivated best be used to study selective aspects of TGF- $\beta$  function, including the respective functions of each of the three isoforms? Can tissue-specific gene replacement therapy be designed that would allow study of the physiology and pathology of TGF- $\beta$  in specific organs of mice with the null genotype?

The role of TGF- $\beta$  in mediating homeostatic responses to cell and tissue injury will undoubtedly receive much more attention, particularly in view of the potential of using TGF- $\beta$  for therapeutic purposes. Although some homeostatic actions of TGF- $\beta$  can be analyzed at the single cell level, we would suggest that the study of the role of TGF- $\beta$  in mediating cooperative interactions between pairs of cells will be of increasing importance. As examples, one may cite the interactions between pericytes and capillary endothelial cells, glia and neurons, satellite cells and skeletal muscle cells, or myoepithelial cells and mammary epithelial cells. In some of these pairs, TGF- $\beta$  has already been shown to be a critical regulator of tissue response. One might consider this to be a local tissue decision network, with TGF- $\beta$  acting as a mediator to couple the appropriate response of a pair of cells to their environment.

The above is only a partial list of the many challenges that lie ahead in TGF- $\beta$  research. The past two years have yielded an abundance of important information and new leads, which now make these challenges much more accessible for investigation at the laboratory bench or in the clinic. Studies of the cell biology of TGF- $\beta$  will continue to be a key component in linking basic molecular studies with clinical applications.

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