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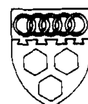

CLINICAL APPLICATIONS OF TGF-β

A Wiley-Interscience Publication

1991

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CLINICAL APPLICATIONS OF TGF-β

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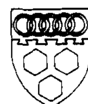

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Introduction: What is TGF-β?

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This symposium deals with clinical applications of transforming growth factor-β (TGF-β) in the control of cell differentiation and proliferation, with special emphasis on its unique role in the formation, remodelling and destruction of extracellular matrix. The molecular and cellular biology of TGF-β has been extensively reviewed in another recent symposium (Piez & Sporn 1990). Here, particular attention is given to the role of TGF-β in the pathogenesis of disease and as a mediator of inflammation and the repair of tissue injury. Moreover, since there are important prospects for the use of TGF-β (or its antagonists) in human therapeutics, this topic is stressed. There are at least three distinct molecular species of TGF-β in humans (Roberts & Sporn 1990); this monograph is therefore concerned with the activities of a set of molecules, rather than one unique peptide.

The fundamental question 'What is TGF-β?' can be answered either simplistically in classical structural terms (which is relatively easy at present, but may become more difficult) or in functional terms, which is a more complex problem. To turn first to the structural definition: the first isoform of TGF-β to be purified to homogeneity, now known as TGF-β1, was defined as a homodimer of M_r 25 000 with a unique N-terminal amino acid sequence (Assoian et al 1983, Frolik et al 1983, Roberts et al 1983). A year later the human gene was cloned (Derynck et al 1985). Subsequently, a second isoform (TGF-β2) was isolated from bovine bone, human glioblastoma cells and porcine platelets and shown to have an amino acid sequence distinct from that of TGF-β1 (Seyedin et al 1985, 1987, Wrann et al 1987, Cheifetz et al 1987). The third known human isoform (TGF-β3) has now been cloned (ten Dijke et al 1988, Derynck et al 1988), and an almost identical homologue has been found in the chicken (Jakowlew et al 1988).

All three human isoforms are believed to exist predominantly as homodimers, but a rigorous structural definition of TGF-β is complicated by its potential to form heterodimers. A biologically active heterodimer, consisting of one chain each of TGF-β1 and β2, has already been found in small amounts in porcine

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platelets (Cheifetz et al 1987). Whether other heterodimers exist is not yet known. Considering the proven ability of other members of the extended TGF-β gene family, particularly the inhibins and activins, to exist in several heterodimeric forms (Vale et al 1990), it would not be surprising if this were also true for TGF-β. Furthermore, considering the similarities in the fundamental molecular architecture of TGF-β and its relatives, it is also conceivable that recombinant DNA techniques could generate chimeras between even more distantly related subunits. What would the receptor specificity be for these chimeric molecules? And what would be the proper nomenclature for a molecule that had one TGF-β chain and one inhibin chain?

The problem of defining TGF-β functionally is much more complicated, perhaps almost impossible. As we have noted before (Sporn & Roberts 1988, 1990), in reality there is no such thing as a peptide 'growth factor'; all such molecules have multiple effects on cells. This is particularly true for TGF-β, which may be considered the prototypic multifunctional signalling molecule. Like all other peptide 'growth factors', it is an element of a complex biological signalling language which provides the basis for intercellular (and perhaps even intracellular) communication in higher organisms. Thus, like a symbol or a letter of the alphabet in a code or language, the meaning of the action of TGF-β can be considered only in a cellular context where it is one member of a set of signals.

We suggest that it is most appropriate to regard TGF-β as an important component in the transmission of biological information, where it often acts as a switch. It does not have an intrinsic action, but serves as a mechanism for coupling a cell's behaviour to its environment, giving the cell the plasticity to respond appropriately to external changes or to changes in its own state. There are many differential biological responses in which TGF-β acts as a switch. When some process is in an inactive state, TGF-β facilitates its activation; conversely, in the same cells, once the process has been activated, TGF-β functions as a stop signal. For example, TGF-β induces a substantial increase in production of IgA in B lymphocytes that do not express IgA on their cell surface; however, it is a potent inhibitor of IgA production in these same cells once they have expressed IgA on their surface (Coffman et al 1989). In embryonic rat mesenchymal cells, TGF-β induces the cartilaginous phenotype by stimulating the synthesis of type II collagen and specific proteoglycans (Seyedin et al 1986); when these primitive mesenchymal cells have differentiated into chrondroblasts, TGF-β strongly inhibits production of the same molecules (Rosen et al 1988). Another example is the ability of TGF-β both to activate and to deactivate macrophages: it stimulates mRNA synthesis or the secretion of several other growth factors in inactive macrophages (Wahl et al 1990), but suppresses hydrogen peroxide formation in active macrophages (Tsunawaki et al 1988).

If TGF-β acts as as biological switch, many questions remain about how it functions. In particular, we know very little about the mechanisms that operate this switch. Most of the phenomena described for TGF-β occur over relatively

long periods; one may now ask if there are more rapid, even cyclical, changes that are mediated by TGF-β. If TGF-β is a switch, how quickly can it be turned on and off? What is the most rapid oscillation that could be mediated by TGF-β? Might one conceive of cyclical processes with periods as short as one second or less that might involve TGF-β? We have suggested that TGF-β might have a switching function in the heart (Thompson et al 1988). Can TGF-β be post-translationally modified (by phosphorylation, acylation, alkylation or polyisoprenylation, followed by dephosphorylation, deacylation, dealkylation or depolyisoprenylation) and its function thereby altered in a cyclical manner? Mature TGF-β can be reversibly dissociated from and reassociated with its latency protein, LAP (Wakefield et al 1989), resulting in gain and then loss of biological activity; whether such a process occurs cyclically is unknown. Although most of these questions and problems are speculative, they can be approached in the laboratory and could lead to further understanding of the multifunctionality of TGF-β.

We do not imply that because of its diverse activities TGF-β cannot be useful as a therapeutic agent. The context of an early wound *in vivo* appears to be sufficiently constrained for TGF-β to be used to promote healing. Again, the actions of TGF-β in isolated cell systems *in vitro* may differ markedly from its actions *in vivo*. Thus TGF-β inhibits the growth of endothelial cells in monolayer cell culture (Baird & Durkin 1986) but is a potent angiogenic agent *in vivo* (Roberts et al 1986). Similarly, there are marked differences between the actions of TGF-β on isolated keratinocytes and its action on intact epidermis. Although it was felt several years ago that the inhibitory effect of TGF-β on growth of isolated keratinocytes (Shipley et al 1986) would prevent its use *in vivo* for wound healing, TGF-β has actually enhanced the epithelial covering of exposed dermis in healing wounds (Hebda 1988, Beck et al 1990).

Many observations indicate that the action of TGF-β depends on the presence or absence of specific molecules in the extracellular matrix and on the presence or absence of other cells that may modify its actions. In the original work describing the isolation and characterization of TGF-β, it was noted that while TGF-β enhanced the growth of NRK cells in agar suspension, it inhibited their growth in monolayer (Assoian et al 1983, Roberts et al 1985). More recently the effects of TGF-β on isolated endothelial cells have been shown to be influenced by the extracellular milieu (Madri et al 1988). In view of the multifunctional nature of TGF-β, it is dangerous to extrapolate from one context to another. Ultimately, evaluation of the therapeutic usefulness of the various isoforms will require comprehensive *in vivo* experimentation. It would not be surprising if desirable results were obtained only with appropriate dosage, scheduling and route of administration. Failure to investigate these parameters carefully could lead to erroneous conclusions about potential clinical applications.

Yet another way to modify the context in which TGF-β might be used therapeutically is to alter local cellular concentrations of the various isoforms by pharmacological agents that regulate their synthesis, secretion or activation. It has already been shown that retinoids and steroids selectively increase the expression of specific isoforms of TGF-β, either *in vitro* or *in vivo* (Glick et al 1989, Colletta et al 1990); this action can target specific cell types *in vivo* (Glick et al 1989). These new observations open up an entire new area of pharmacology, which will deal with the cell- and tissue-specific regulation of TGF-β. There is good reason to expect that many ligands of the steroid receptor superfamily (Green & Chambon 1986, Evans 1988) will have relevant actions here.

In summary, it is clear that the three human TGF-β isoforms are a versatile set of molecules, and that their general structure has been used as a modular component for many different purposes during evolution. The specific involvement of TGF-β in the processes whereby many different tissues respond to injury and initiate their repair presages a host of useful therapeutic applications. Since certain disease processes, particularly those involving fibrosis, may be characterized by excessive and inappropriate actions of TGF-β, there are also clear indications for the development of antagonists of TGF-β for therapeutic purposes.

Many new data were presented for the first time at the Ciba Foundation Symposium and are recorded as such in this monograph. The volume should provide the reader with abundant evidence, obtained from molecular, cellular and *in vivo* studies, to support the prediction of important clinical applications of TGF-β in medicine and surgery in the years to come.

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Multiple forms of TGF-β: distinct promoters and differential expression

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Abstract. There are now five known distinct isoforms of TGF-β with 64–82% identity. Of these, only TGF-β1, 2 and 3 thus far have been demonstrated to be expressed in mammalian tissues; TGF-β4 has been described only in chicken and TGF-β5 only in frog. Although the biological activities of these five isoforms of TGF-β are indistinguishable in most *in vitro* assays their sites of synthesis and localization *in vivo* are often distinct. Expression of the various isoforms is differentially controlled both *in vivo*, as in development, and *in vitro* after treatment of cells with steroids, such as oestrogen or tamoxifen, or with retinoids. To investigate the basis of these observations we have cloned and characterized the promoters for the human TGF-β1, 2 and 3 genes. Significant differences have been found: whereas the TGF-β1 promoter has no TATAA box and is regulated principally by AP-1 sites, both the TGF-β2 and 3 promoters have TATAA boxes as well as AP-2 sites and cAMP-responsive elements. Accordingly, TGF-β1 gene expression is induced strongly by phorbol esters whereas that of TGF-β2 and 3 is induced by forskolin, an activator of adenylate cyclase. Expression of TGF-β2 and 3 is often coordinately regulated *in vivo* in a pattern distinct from that of TGF-β1.

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Like several other growth factors, TGF-β has been found to exist in multiple isoforms, approximately 64–82% homologous to each other (for review see Roberts & Sporn 1990). Three of these, called TGF-β1, 2 and 3, are expressed in mammalian cells and tissues; their amino acid sequences are greater than 98% conserved between different species examined. In many *in vitro* assays using established cell lines as well as primary cell cultures, these isoforms are interchangeable, indicating either that cells co-express multiple TGF-β receptors or that a particular receptor can bind the various isoforms with nearly equal affinity. In contrast, relative levels of expression of the different isoforms vary from cell to cell; they are regulated differentially both in embryogenesis and in adults by a variety of growth factors and hormones, including epidermal

growth factor, retinoic acid, dexamethasone, tamoxifen, phorbol esters and the TGF-βs themselves. In this brief review, we shall summarize the evidence on differential regulation of expression of TGF-β1, 2 and 3 and demonstrate that the basis for these differences lies, in part, in the different character of the 5′ flanking promoter sequences of each of these genes.

Differential expression of TGF-β1, 2 and 3 *in vivo*

Recent studies comparing developmental expression of TGF-β1, 2 and 3 by either immunohistochemical or *in situ* hybridization analyses indicate that the three isoforms are often co-expressed but the temporal pattern or specific cell types involved are sometimes distinct. For example, in the developing palate (Fitzpatrick et al 1990) mRNA for TGF-β3 is expressed very early in the presumptive medial edge epithelial cells and its expression increases in intensity as these cells migrate prior to fusion: TGF-β1 mRNA is expressed in the same cells at a slightly later stage. In contrast, expression of TGF-β2 mRNA is confined to mesenchymal cells underlying the fusing epithelia. Expression of all three factors is dramatically reduced after disruption of the epithelial seam and mesenchymal transformation of the medial edge epithelial cells. Immunohistochemical analysis of TGF-β1 expression showed intense staining in palatal mesenchyme at the time of fusion, suggesting that in this tissue, as well as in many others including the hair follicles, tooth buds and submandibular gland, TGF-β1 synthesized by epithelial cells is localized to and probably acts on adjacent mesenchymal cells (Heine et al 1987, Lehnert & Akhurst 1988).

In other tissues, one or more isoforms of TGF-β is selectively expressed. The best studied example is in the nervous system where expression of TGF-β1 is confined to the meninges and choroid plexus, whereas TGF-β2 and 3 are co-expressed in glial cells, and neuronal perikarya and axons (Heine et al 1987, Flanders et al 1990). Other examples of selective expression include human platelets which contain only TGF-β1 (Assoian et al 1983) and the aqueous and vitreous humours of the eye which contain no TGF-β1 but high levels of TGF-β2 (Connor et al 1989, Jampel et al 1990).

Regulation of TGF-β expression *in vivo* and *in vitro*

Examination of the relative expression of TGF-β1 and 2 in a variety of cell lines reveals that some cells, such as human lung WI-38 and normal rat kidney fibroblasts, secrete predominantly TGF-β1, whereas others, such as monkey kidney BSC-1 cells and human adenocarcinoma PC-3 cells, secrete principally TGF-β2 (Danielpour et al 1989). This regulation of isoform expression can be modulated by retinoic acid, which increases TGF-β2 synthesis, or by epidermal growth factor, which increases synthesis of TGF-β1 (Danielpour et al 1990). In primary cell cultures, retinoic acid increases TGF-β2 synthesis by mouse

keratinocytes over 100-fold (Glick et al 1989), and tamoxifen selectively increases synthesis of TGF-β1 in human fetal fibroblasts (Colletta et al 1990).

Expression of TGF-β1, 2 and 3 is also regulated by the TGF-βs themselves. Van Obberghen-Schilling et al (1988) were the first to describe up-regulation of TGF-β1 mRNA and protein synthesis by TGF-β1. More recently, Bascom et al (1989) have described complex interregulation of expression of the TGF-β isoforms; in murine AKR-2B cells TGF-β1 up-regulates its own expression and down-regulates expression of the mRNAs for TGF-β2 and 3, whereas TGF-β2 up-regulates expression of mRNA for TGF-β1, 2 and 3.

Differential regulation of expression of TGF-β isoforms has also been observed *in vivo*. Treatment of mouse skin with phorbol ester results in increased TGF-β1 expression (Akhurst et al 1988), whereas treatment with retinoic acid results in enhanced expression of TGF-β2 (Glick et al 1989). Response of tissue to injury often involves increased expression of TGF-β1: examples include myocardial infarction (Thompson et al 1988), healing of fractures in bone (Joyce et al 1990), liver regeneration (Braun et al 1988) and the response of the liver to schistosome infection or to treatment with carbon tetrachloride (Czaja et al 1989).

Characterization of the promoters for TGF-β1, 2 and 3

To investigate the mechanistic basis for the differential regulation of expression of the TGF-β isoforms, the 5′ flanking regions of the human genes for TGF-β1, 2 and 3 were analysed (Kim et al 1989a,b,c, 1990a, Noma et al 1991, Lafyatis et al 1990). A summary of the main features of each of these promoter sequences is presented in Fig. 1. In the TGF-β2 and 3 genes classic TATAA boxes are found 20 to 30 nucleotides upstream of the transcriptional start sites. The TGF-β1 gene lacks a TATAA box and is characterized by the presence of a very GC-rich region containing several Sp1 binding sites just upstream of the first transcriptional start site. A variety of experimental approaches have identified AP-1 sites, which bind the Jun/Fos protein complex and were originally identified as targets for phorbol ester control of gene expression, as the major positive regulatory sequences involved in up-regulation of TGF-β1 expression (Kim et al 1989b,c, 1990a,b). In contrast, the TGF-β2 promoter has only one consensus AP-1 site which does not confer phorbol ester responsiveness (Noma et al 1991), and the TGF-β3 promoter has no such sites (Lafyatis et al 1990). Rather, control of the TGF-β2 and TGF-β3 promoters appears to be mediated by cAMP-responsive elements (CRE) and possibly by AP-2 binding sites, as demonstrated by the induction of activity of these promoter constructs by forskolin, an activator of adenylate cyclase. These observations demonstrate that one basis for the differential regulation of expression of the TGF-β isoforms is the very different character of their 5′ flanking sequences. The long 5′ untranslated regions of each of these TGF-βs, ranging from 841 nucleotides

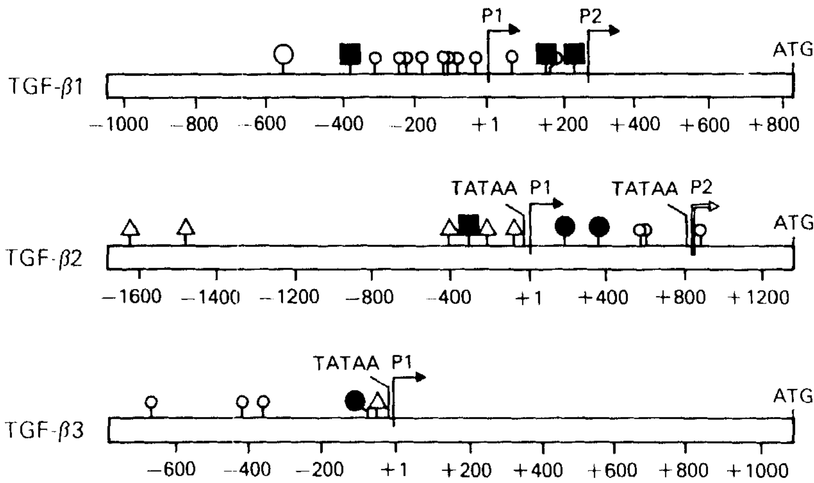

FIG. 1. Comparison of the major features of the 5′ flanking regions of the human TGF-β1, 2 and 3 genes. Transcriptional start sites are designated as P1 and P2. The consensus transcription factor binding sites are indicated as follows: ○, FSE2 (fat-specific elements); ■ , AP-1 binding sites; ●, AP-2 binding sites; △ , cAMP-responsive elements; ○, Sp1 binding sites; positions of TATAA boxes in the TGF-β2 and 3 genes are indicated. For more details, refer to Kim et al 1989a,b,c for TGF-β1; Noma et al 1991 for TGF-β2 and Lafyatis et al 1990 for TGF-β3.

for TGF-β1 to 1357 nucleotides for TGF-β2, suggest that contributions of secondary structure and the presence of other open reading frames to translational regulation will also be important.

Regulation of TGF-β1 transcription by AP-1 sites

Because its promoter was characterized first, more is known about regulation of TGF-β1 transcription than about that of TGF-β2 and 3. The TGF-β1 gene has two distinct transcriptional start sites separated by 271 nucleotides and each of these is preceded by one or more AP-1 binding sites (Fig. 1); these regions have been designated as the first and second promoters, respectively. AP-1 binding sites, which bind the Jun/Fos complex, have been implicated in transcriptional control of TGF-β1 expression by the following lines of evidence: (1) activity of chimeric TGF-β1–chloramphenicol acetyltransferase (CAT) constructs induced by either TGF-β1 or phorbol ester treatment of cells is lost when AP-1 sites are deleted; (2) expression of both c-*fos* and c-*jun* is required for activity of TGF-β1 promoter constructs in F9 embryonal carcinoma cells; (3) co-transfection with either antisense c-*fos* or antisense c-*jun* ablates the effects of TGF-β1 on expression of both first and second TGF-β1 promoter constructs (Kim et al 1989b,c, 1990a).

Yet another level of control is exerted via the effects of TGF-β1 on expression of *jun*. Both Pertovaara et al (1989) and Li et al (1990) have demonstrated that TGF-β1 treatment of a variety of cells results in increased expression of c-*jun*, *junB* and *junD*. Kim et al (1990a) have shown that this effect, like regulation by TGF-β1 and Jun of their own gene promoters, is mediated by AP-1 sites in the 5′ regulatory regions of the c-*jun* gene. These interactions between TGF-β1 and c-*jun* autoinduction suggest that the ability of growth factors, such as TGF-β1, and of transcription factors, such as Jun, to control cellular growth and differentiation may depend, in part, on their ability to regulate each other's expression.

Recent evidence indicates that AP-1 sites may also be the targets for activation of TGF-β1 gene expression and TGF-β1 protein secretion by retroviruses such as the human T lymphotropic virus type 1 (HTLV-1; Kim et al 1990b). This virus has been identified as the responsible infectious agent in adult T cell leukaemia (ATL), an aggressive, usually fatal T cell malignancy characterized by immunosuppression and a high incidence of opportunistic infections. Freshly isolated ATL cells and HTLV-1-infected T cell lines show increased expression of TGF-β1 mRNA and secrete increased levels of TGF-β1 protein. The Tax protein encoded by HTLV-1, a potent transcriptional activator of the viral long terminal repeat and of other cellular genes, has been shown to activate transcription from both the first and second promoters of TGF-β1 through their AP-1 sites (Table 1, Kim et al 1990b). These results suggest that the increased production of TGF-β1 by ATL cells may be related to *trans*-activation of the TGF-β1 promoters by the HTLV-1 Tax protein and that TGF-β1 may be important in the immunosuppression characteristic of this disease. Immunosuppression associated with other malignancies or induced by treatment

TABLE 1 Activation of TGF-β1 promoter constructs by the HTLV-1 Tax protein

	Cell line		
Construct	*K-562 Human chronic myelogenous leukaemia*	*Jurkat Human T cell leukaemia*	*A549 Human lung carcinoma*
phTG5	0.4	0.6	0.8
phTG5 + Tax	2.8 (7.1)	4.9 (8.2)	5.7 (7.1)
phTG16	0.8	1.4	0.6
phTG16 + Tax	7.8 (9.7)	11.6 (8.3)	11.4 (19)

Each of the plasmids was transfected into the cells either with or without the pHTLV-1-Tax expression plasmid. 40 hours later cells were harvested and CAT (chloramphenicol acetyl transferase) enzyme activity was determined. phTG5 is a construct of the first promoter of TGF-β1 from −453 to +11 and phTG16 is a construct of the second promoter of TGF-β1 from +102 to +432. Numbers represent the percentage acetylation of chloramphenicol. The numbers in parentheses represent the fold-induction in the presence of Tax. From Kim et al 1990b.

with agents such as cyclosporin might also result from activation of the promoter for one of the TGF-β isoforms (Sporn & Roberts 1989). The model of Tax activation of the TGF-β1 promoter in ATL provides a framework for further investigations into the mechanisms operative in other diseases.

Further evidence for the activation of TGF-β1 expression by HTLV-1 Tax comes from studies of the patterns of TGF-β1 expression in transgenic mice containing the HTLV-1 *tax* gene under control of the viral long terminal repeat (Nerenberg et al 1987, Kim et al 1991). These mice show selective expression of the *tax* gene in nerves, muscle, bone and salivary glands, and eventually develop mesenchymal tumours identified as neurofibromas on the ears, tail and legs (Nerenberg et al 1987). High levels of the TGF-β1 mRNA, but not the TGF-β2 and TGF-β3 mRNAs, are expressed in tissues of these mice which express high levels of *tax* mRNA, including the submaxillary glands and muscle as well as tail and ear tumours (Kim et al 1991). The increased levels of TGF-β1 mRNA in these tissues are paralleled by increased levels of TGF-β1 protein, as determined by a specific sandwich ELISA. These results support earlier observations in patients with ATL and strongly suggest that Tax is able to stimulate TGF-β1 production *in vivo*.

Are there biological consequences of isoform switching?

Thus far we have discussed differential control of expression of TGF-β1, 2 and 3 at the transcriptional and possibly the translational level. However, the greater than 98% amino acid sequence conservation of each of the TGF-β isoforms suggests that there also will be biological consequences of isoform switching. What happens, for example, when a cell such as the A549 human lung carcinoma cell is induced by retinoic acid to alter the composition of secreted TGF-β isoforms from approximately 41% TGF-β2 to 68% TGF-β2 (Danielpour et al 1990)? What are the biological consequences for the epidermis of increased TGF-β1 expression, as after treatment with phorbol esters (Akhurst et al 1988), or increased TGF-β2 expression, as after treatment with retinoic acid (Glick et al 1989)? Although most *in vitro* studies show nearly equivalent activity of the three TGF-β isoforms in a variety of assays (Roberts et al 1990), experiments such as those of Jennings et al (1988), in which inhibition of the growth of bovine aortic endothelial cells by TGF-β1 was 100-fold greater than that by TGF-β2, suggest that there might be significant consequences in certain systems. In addition to possible intrinsic differences in biological activity of the various isoforms, other characteristics, for example differential binding to and inactivation by proteins such as α_2-macroglobulin, can also contribute to distinct patterns of activity *in vivo* (Danielpour & Sporn 1990).

We have recently demonstrated that a more complex biological assay, namely that of mesoderm induction in explanted ectodermal fragments of *Xenopus* embryos, is able to discriminate between TGF-β1, 2 and 3. The minimum

concentrations required for mesoderm induction are 12 and 1 ng/ml of TGF-β2 and 3, respectively; TGF-β1 is inactive in the assay (Roberts et al 1990). While nothing is known about TGF-β receptor expression in *Xenopus* embryos, the effects on mesoderm induction, in which the cells acquire different developmental fates during the assay, suggest that differential activity of the TGF-β isoforms may be observed in more complex assays involving interactions between different cell types. Epithelial–mesenchymal interactions, in which the TGF-βs have been proposed to play a role (Heine et al 1987, Akhurst et al 1988), are one place to look for such differential activities.

Summary

There is now a wealth of evidence both *in vivo* and *in vitro* that demonstrates differential regulation of expression of TGF-β1, 2 and 3. This and the high degree of sequence conservation of each isoform between species suggest that the isoforms might have different biological activities, but this has not yet been demonstrated conclusively. However, recent characterization of the promoters for the human TGF-β1, 2 and 3 genes provides a basis for understanding the selective regulation of expression of the TGF-βs by a variety of factors including the TGF-βs themselves. Moreover, the finding that the Tax protein encoded by the HTLV-1 virus activates the TGF-β1 promoter suggests that understanding of the mechanisms which contribute to the pathogenesis of certain diseases may come from studies of the TGF-β promoters.

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DISCUSSION

Derynck: With respect to the HTLV-1 Tax–TGF-β1 link, do the *in situ* hybridizations really show localization of TGF-β1 mRNA to the cells? Is it the fibroblasts that are starting to make a lot of β1 or are they haemopoietic cells?

Roberts: Those are very important questions but we have not yet done the *in situ* hybridizations. Our results are based on immunohistochemistry and the Northern blot experiments.

Derynck: Do you have any news about the HIV Tat–TGF-β1 link?

Roberts: Bill Wachsman had an abstract at the Immunology Meeting in New Orleans (June 3rd 1990) (Abstract 1861) which showed data parallel to what we find for HTLV-1, namely that TGF-β1 mRNA and protein are highly expressed in peripheral blood mononuclear cells of HIV-infected individuals. He also shows that HIV-1 Tat-mediated inhibition of lymphocyte activation can be blocked by anti-TGF-β1 antibodies, suggesting that Tat activates expression of TGF-β1, which, in turn, mediates the immunosuppression. However, in experiments we have done in collaboration with John Kehrl in Tony Fauci's lab we have not been able to see those effects (J. Kehrl, personal communication). We have not seen any activation of TGF-β1 expression by the Tat protein, neither do we see elevated levels of TGF-β1 mRNA or protein in peripheral blood mononuclear preparations from HIV-infected patients.

Ruscetti: Following the same theme of relationship to disease, are the transgenic mice that carry the Tax gene immunosuppressed?

Roberts: I don't know, that's a very good question. However, I am not sure that there is high level expression of HTLV-1 Tax in the haemopoietic cells of those transgenic mice, so there might not be immunosuppression in that particular model. In contrast, ATL patients are often reported to be immunosuppressed.