TNF-induced genes and Proteins

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As a step toward a more complete understanding of the molecular actions of TNF, we prepared a cDNA library from TNF-treated human FS-4 fibroblasts and used differential hybridization to identify cDNA clones corresponding to mRNAs enriched in TNF-treated cells. In quiescent FS-4 cells TNF induces an increase in the level of some mRNAs within 20 to 30 min. Some of these immediate-early response mRNAs are elevated only transiently for about 30 to 120 min, e.g., c-fos and c-myc (Lin and Vilcek,1987) or the transcription factor IRF-1(Fujita et al.1989). Such immediate-early gene products may be important for the activation of other genes, but their transient induction suggests that they are not the actual effector molecules responsible for the phenotypic changes induced by TNF. We chose a 3-h incubation with TNF because we were seeking cDNAs corresponding to messages that are more stably elevated after TNF treatment. Indeed, the results shown in Figure 8 and 9 indicate that all of the mRNAs corresponding to the eight TSG cDNAs isolated remained significantly elevated after 16h of continuous treatment with TNF, and their kinetics of induction were clearly different from those of the immediate-early response mRNAs such as c-fos,c-myc or IRF-1. Nevertheless, only the induction of TSG-21 (collagenase) and TSG-27 (stromelysin) mRNAs was completely inhibited by cycloheximide and the induction of TSG-37 (metallothionein-II) was reduced in the presence of this inhibitor of protein synthesis. Induction of the other five TSG mRNAs by TNF was completelyresistant to cycloheximide, suggesting that no protein intermediate is needed for the upregulation of these mRNAs.

The fact that the responses to IL-1 most closely resembled TNF actions is not surprising. Although TNF and IL-1 are structurally unrelated and they bind to different receptors(Beutler and Cerami, 1985; Matsushima et al, 1986) they appear to produce the activation of similar second messangers and transcription

factors (Fujita et al, 1989; Zhang et al 1988b; Vilcek and Lee, 1991). All of the TNF-inducible mRNAs were also reponsive to TPA, an activator of protein kinase C. Although TNF was recently reported to cause activation of protein kinase C in some cell lines (Brenner et al,1989), it is unlikely that the stimulatory actions of TNF on the eight TSG mRNA species can be ascribed to protein kinase C activation alone, because TPA was more efficient than TNF in inducing collagenase and stromelysin mRNAs but less efficient in stimulating all other TSG mRNAs. However, the possibility cannot be ruled out that TPA and TNF activate different isozymic forms of protein kinase C in FS-4cells. The finding that poly(1)-poly(C) induced all eight TSG mRNA species(albeit mostly with an efficiency lower than that of TNF) was unexpected. Poly(1)-poly(C) is known to cause activation of double-stranded RNA-dependent p68 protein kinase (Hovanessian and Galabru, 1987) and was recently also shown to cause activation of an NF-kB-like protein(Visanathan and Goodbourn, 1989) and to induce IRF-1(Fujita et al, 1989). Which if any, of these actions contribute to the inducing activity of poly(1)-poly(C) on TSG mRNAs is not known. The phosphodiesterase inhibitor isobutymethylxanthine and the cyclic AMP analog N^6-2'-O-dibutyladenosine cyclic 3',5'-monophosphate were at best weakly active in elevating levels of some of the mRNAs. This result contrasts with the very pronounced ability of these agents to induce IL-6, another TNF-inducible gene(Zhang et al, 1988a). The results summarized in Table 4 suggest that the stimulation of the eight TSG mRNAs species by TNF is probably not due to the activation of a single known major second messenger pathway.

Induction of IL-8 and MCAF by TNF (and IL-1) was recently observed by others (Larsen et al, 1989b; Matsushima et al. 1989).IL-8 has been identified as a neutrophil and T-cell chemotactic factor structurally related to several members of a family of inflammatory cytokines that include platelet factor 4, the IFN-γ-inducible protein IP-10, and a growth-regulated gene in transformed cells termed GRO (Kawahara and Deuel, 1989; Larsen et al., 1989a; Matsushima and Oppenheim, 1989). MCAF has been identified as a chemotactic and activating factor for monocytes(Matsushima et al., 1989). MCAF belongs to another family

of inducible proteins closely related to the IL-8 family, that includes the PDGF-inducible genes JE, LD 78, murine macrophage inflammatory protein, RANTES, and TCA-3(Matsushima and Oppenheim, 1989).

Collagenase(TSG-21) was also reported earlier to be TNF-inducible in synovial cells and fibrobalsts(Brenner et al., 1989; Dayer et al., 1985). Although, to our knouledge, the induction of stromelysin by TNF has not been reported, transcription of human stomelysin mRNA was recently shown to be inducible by IL-1(Quinones et al., 1989). Like collagenase, stromelysin is a metalloproteinase which can degrade collagen, fibronectin, laminin and proteoglycans, Both collagenase and stromelysin are thought to be important in the increased extracellular matrix degradation occurring in rheumatoid arthritis. The inhibitory effect of cycloheximide on the induction of collagenase and stromelysin mRNAs by TNF seen in our experiments suggests that the inducing effect is indirect. Induction of the stromelysin gene by TPA was also inhibited by cycloheximide(Frisch et al., 1987), as was the induction by EGF of the rat homolog of the stromelysin gene, termed transin (Matrisian et al.,1986).

It is very likely that the ability of TNF to induce collagenase and stromelysin is related to the important role of TNF in tissue distruction, remodeling and wound repair during inflammation. It has been suggested that both TNF and IL-1 are secreted by activated macrophages in the rheumatoid pannus (Meyer, 1990). They may induce the production of collagenase, stromelysin and other neutral proteases in synovial fibrolasts and in chondrocytes located in the adjactent articular cartilage. These enzymes degrade proteoglycans and collagen, resulting in cartilage destruction.

Finally, metallothionein-II has been shown to be inducible by various types of stress, including heavy metal challenge, injection of lipopolysaccharide(LPS) as well as cytokines including interferons and IL-1(Karin, 1985). In addition to its ability to bind heavy metal ions, metallothionein-II may also act as a scavenger of free radicals released by activated macrophages and neutrophils at the site of acute infection

(Thornalley et al., 1985). Metallothionein-II induction by TNF may serve a protective role in the prevention of tissue injury during inflammation.

It is intriguing that all five of the known TSGs represent genes whose products have been implicated in inflammatory responses. This finding is not entirely surprising, however, given the fact that the library that was screened was derived from fibroblasts that were exposed to the proinflammatory cytokine TNF, a condition that mimics a chronic inflammatory situation to some extent. TNF and IL-1 are produced by moncytes/macrophages in response to various stimuli including bacteria, virus, or immune complexes(Old, 1985; Le and Vileck, 1978b). since fibroblasts are ubiquitous and are aften in close contact with macrophages and other cells of the immune system, fibroblast-derived cytokines and other meditators may play important roles in normal and pathologic processes. As depicted in Figure, fibroblasts are one of the major target of TNF and IL-1 actions. Amongthe biological consequences of TNF actions on fibroblasts are the production of chemoattractants such as IL-8 and MCAF that serve to recruit and activate neutrophils and monocytes at the site of inflammation, production of collagenase and stormelysin responsible for tissue damage, and presumably production of other meditators implicated in cartilage resorption, granuloma formation and other chronic inflammatory functions. We believe that elucidation of the biological functions of proteins encoded by the newly identified TSGs should provide new insight into the involvement of TNF in inflammation.