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Basic and Clinical Studies of Soluble Tumor Necrosis Factor/Lymphotoxin Receptors

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The mechanisms through which TNF and LT mediate their activities on cells and tissues are initiated by binding to specific cell membrane receptors (TNF-R). There are two different TNF/LT membrane receptors: one of 55 kD [1, 2] and the other one of 75 kD [3]. These two receptors have 40% amino acid homology in their extracellular domains [4] but differ in their intracellular domains and both receptors are expressed on many types of cells although one of the receptors may predominate depending on the cell types.

Two groups originally identified soluble forms of these receptors of 30 and 40 kD in urine of patients with chronic inflammatory diseases [5–7]. These materials bound to and inactive TNF and LT activity, they were subsequently termed blocking factors (BF). We were the first to identify and characterize soluble forms of these receptors from the serum of human cancer patients and demonstrate they can block the activity of human TNF and LT both in vitro and in vivo [8, 9]. Moreover, we have shown these BF can affect the growth, development, and functional activity of human T cells in vitro. These studies have led to the formulation of a new concept, namely that the extracellular domain of cytokine membrane receptors can be shed by cells and these soluble receptors may be important in the regulation of cytokine action. However, the cell and tissue source of these soluble TNF receptors and the cellular processes involved in the release of these molecules in cancer patients is not yet known. The present article focuses on basic and clinical studies of these receptors in women with gynecologic cancers.

Cell Source and Regulation of the Release of Soluble TNF/LT Receptors in Women with Gynecologic Cancer

Recently, we identified TNF/LT BF activity in the cell-free ascites derived from 15 women with ovarian cancer [10]. We extended these

studies and found by ELISA techniques soluble forms of both receptors in the ascites and employing primary culture techniques that both ascites cells and solid tumor tissues were releasing high levels of BF activity and these receptors. However, release stopped after 24–48 h in culture. Thus BF activity in these samples appears to be due to soluble receptors and local tumor tissues and ascites cells were probably the source of BF in the ascites of these patients. We next studied release of soluble receptors by several different continuous human ovarian cancer cell lines in vitro. PA-1 cells were co-cultured for 24–48 h with various levels of recombinant human cytokines (IL-1β, IL-4, IL-6, IFN-γ) and different levels of the stimulators (lipopolysaccharide (LPS), phorbol myristate acetate (PMA) and formyl-methionyl-leucyl-phenylalanine (FMLP)). We found PA-1 cells spontaneously release high levels of the 55-kD receptor and low levels of the 75-kD receptor. The release of both 55 and 75 kD TNF-R was stimulated when PA-1 cells were treated with IL-1β, IL-6 and IFN-γ; however, IFN-γ was the most potent stimulator and most strongly up-regulate release of the 75-kD receptor. These data suggest that ovarian cancer cells may be one of the sources of soluble TNF-R in the ascites fluid in these patients and cytokines released by effector cells may be involved in controlling or regulating their release [11].

These results support the concept that receptor release is a selective process, and cells may be able to specifically release one or both of these receptors in response to different inducing stimuli.

The Mechanism of Release of Soluble TNF Receptors by Human Monocytic THP-1 Cells in vitro

Monocytes and macrophage are major effector cells in the peritoneal cavity so we have begun to examine the mechanism of receptor release by these cells in vitro. We first examined receptor release by PMA-stimulated human monocytic THP-1 cells [9]. We found that these cells release soluble receptors spontaneously. However, release is greatly enhanced by co-culture with PMA. The optimal effect of PMA is achieved by 24-hour exposure to 10^{-8} *M*. Different inhibitors were tested for their ability to affect PMA-activated release in culture maintained for 2 h in vitro. Actinomycin D (1 μg/ml) showed no effects while cycloheximide (20 μg/ml) showed significant inhibitory effect on release of both receptors. Colchicine (1 m*M*) and cytochalasin B (0.1 m*M*) inhibited the stimulated release of both receptors by about 90 and 70% respectively, while methylamine (1 m*M*) had no effect. The serine protease inhibitor PMSF (4 m*M*) has significant inhibitory effects on receptor release. Finally, we found receptor

shedding from paraformaldehyde-fixed THP-1 cells was induced by incubation in medium from PMA-stimulated THP-1 cells. This shedding was also blocked by PMSF. Collectively, these data indicate that the release of soluble receptors involves two different mechanisms: (a) spontaneous release through endosomal/lysosomal pathway by resting THP-1 cells and (b) PMA activation may be promoting release through secreting a serine protease which cleaves receptors off the surface of the cell membrane.

The Clinical Significance of Soluble TNF Receptors in Patients with Gynecologic Malignancies

We were very curious if serum levels of soluble TNF-R might provide an objective measure of response to treatment or assessment of disease status in women with gynecologic cancers. We have measured soluble TNF-R levels in the sera of women with gynecologic malignancy and compared it to CA-125 measurements in these same patients with respect to disease status and response to therapy. CA-125 was chosen for comparison since it is the most frequently used and most reliable marker of tumor status and response to therapy in ovarian cancer patients. All of these studies were conducted retrospectively from a bank of serum samples collected over a 5-year period.

We found that serum levels of both soluble TNF-R were elevated in 79 patients with various gynecologic malignancies (55 kD of 3.07 ± 3.79 ng/ml ($p < 0.02$) and 75 kD of 2.93 ± 1.27 ng/ml ($p < 0.001$)) as compared to 16 normal controls (55 kD of 0.65 ± 0.22 ng/ml and 75 kD of 1.62 ± 0.37 ng/ml). Serum levels of 55 and 75 kD TNF/LT receptors were a more sensitive indicator of active cancer and had greater predictive value for detecting tumor in patients with ovarian cancer than CA-125. Additional studies were conducted on serial serum samples collected from women who were undergoing therapy over a 1- to 3-year period. These studies were very revealing and we found that soluble TNF-R were also more sensitive than CA-125 in detecting persistent or recurrent tumor and measuring response to therapy.

The soluble TNF receptor may have an important role in regulating the interaction of host effector cells with tumor cells in the microenvironment of solid tumors. While the role is not yet clear it appears that serum levels of these receptors may also be useful probes for detection and patient response to therapies in gynecologic cancer.

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