Medical Grand Rounds

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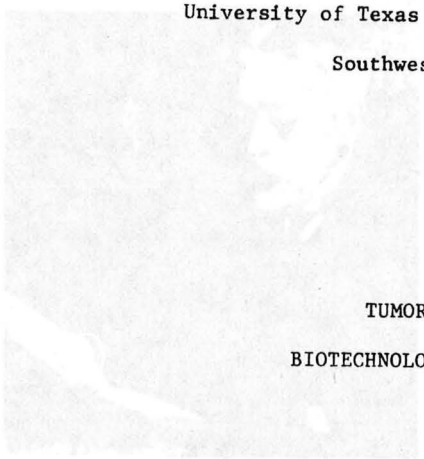

TUMOR NECROSIS FACTOR:

BIOTECHNOLOGY'S ANSWER TO CANCER ?

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INTRODUCTION

In the 1890's Dr. William B. Coley, a surgeon at the New York Cancer Hospital (the predecessor to Memorial Sloan-Kettering Cancer Center), observed that tumors in patients who simultaneously contracted a bacterial infection sometimes shrank or even disappeared. Coley began to treat cancer patients with a mixture of several bacteria - referred to as "Coley's Toxins" or "Coley's Cancer Cocktail." This therapy sometimes succeeded dramatically in causing cancer regression, but more often than not it failed. When it succeeded, it was a remarkable cure, for unlike modern radiotherapy and chemotherapy Coley's Cancer Cocktail did not damage healthy tissues.

WILLIAM B. COLEY, M.D.
1862-1936

Coley's unique contribution to medical science was his persistence in studying the use of mixed bacterial toxins in cancer treatment: he made a 40 year thorough investigation in this one line of cancer research. In addition, Coley devoted himself to promoting a general public interest in the cancer crusade.

For the past 90 years, the mechanism of the tumor regression noted by Coley has remained a mystery. An understanding of the Coley phenomenon has now become possible as a result of the recent identification of the active ingredient in Coley's Cancer Cocktail by Lloyd J. Old and his associates at the Memorial Sloan-Kettering Cancer Center. The active ingredient is called tumor necrosis factor.

Old and associates discovered tumor necrosis factor by studying an experimental counterpart of Coley's clinical observations. The experimental model consists of treating mice with two agents that had been shown previously to cause regression of tumors in animals. The first agent - the priming agent - consists of treating mice with BCG, an attenuated form of tuberculosis which produces a rapid proliferation of macrophages throughout the body. The second agent - the challenging agent - consists of giving mice an intravenous injection of a sublethal dose of endotoxin. This combined treatment leads to the appearance in the serum of tumor necrosis factor, an extremely potent molecule that can selectively destroy malignant cells without killing their normal counterparts.

In the past year, tumor necrosis factor has advanced from a complex biological activity detectable in the serum of BCG-primed and endotoxin-challenged animals to a well defined protein molecule whose site of synthesis in the body has been identified and whose gene has been cloned. Milligram quantities of purified tumor necrosis factor have recently been produced by the techniques of recombinant DNA and are now available for study by basic scientists and clinical investigators. The molecular cloning of tumor necrosis factors opens the way to clinical trials in which the promise of Coley's original observations can now be put to test.

Figure 1

Tumor necrosis factor has turned out to be a versatile and fascinating molecule: it has several potent actions in addition to the original one that led to its discovery. 1) It can kill malarial parasites in vitro and in vivo; 2) it is one of the endogenous mediators of endotoxic shock produced by gram negative bacteria; and 3) it is structurally identical to cachectin, a hormone that mobilizes triglyceride from adipose tissue and produces a state of cachexia in animals.

The gene for TNF has been cloned and its complete amino acid sequence is known. The mature protein consists of a single polypeptide chain of 157 amino acids (molecular weight of ∿ 17,000). The protein contains one intrachain disulfide bond between two cysteine residues. The integrity of this disulfide bond is crucial for the action of TNF.

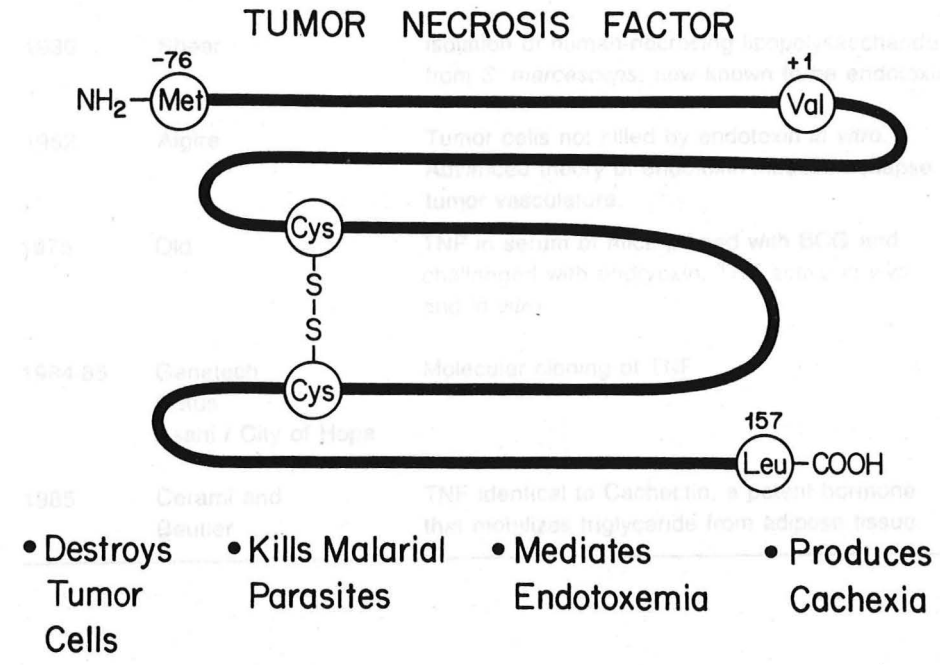


Figure 2

HISTORICAL EVENTS LEADING TO DISCOVERY OF TNF

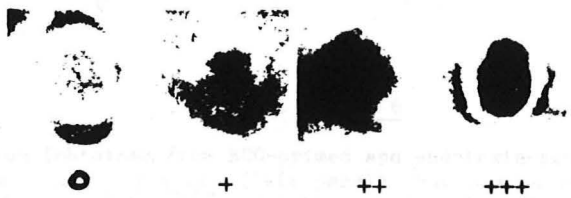
Date	Pioneer	Observation
1891	Coley	Spontaneous regression of certain human tumors during bacterial infection - "Coley's Cancer Cocktail"
1931-36	Gratia and Linz Shwartzmann	Hemorrhagic necrosis of animal tumors after injection of bacterial toxins.
1936	Shear	Isolation of human-necrosing lipopolysaccharide from *S. marcescens*; now known to be endotoxin.
1952	Algire	Tumor cells not killed by endotoxin *in vitro*. Advanced theory of endotoxin-induced collapse of tumor vasculature.
1975	Old	TNF in serum of mice primed with BCG and challenged with endotoxin. TNF active *in vivo* and *in vitro*.
1984-85	Genetech Cetus Asahi / City of Hope	Molecular cloning of TNF
1985	Cerami and Beutler	TNF identical to Cachectin, a potent hormone that mobilizes triglyceride from adipose tissue.

Figure 3

TNF appears in the serum of animals that have been primed with an attenuated form of tuberculosis (BCG) and then sensitized with endotoxin. [Data from ref. 6].

NECROSIS OF TRANSPLANTED SARCOMA IN VIVO

Serum from Mice Treated with:		TNF Assay: Necrotic Response			
		3+	2+	1+	0
BCG	Endotoxin	Number of Mice			
−	−				9
+	−			2	7
−	+				9
+	+	171	109		



Necrotic response of transplanted tumor is graded 0 to 3+

Figure 4

Macrophages are the cells in the body that produce TNF.

What Cells in the Body are Responsible for Producing TNF?

• MACROPHAGES

Evidence:

1. Priming agents cause macrophage hyperplasia.
2. Certain cultured macrophages secrete TNF after exposure to endotoxin.

Figure 5

Regression of a human melanoma transplanted into TNF-treated mice. [Data from ref. 13].

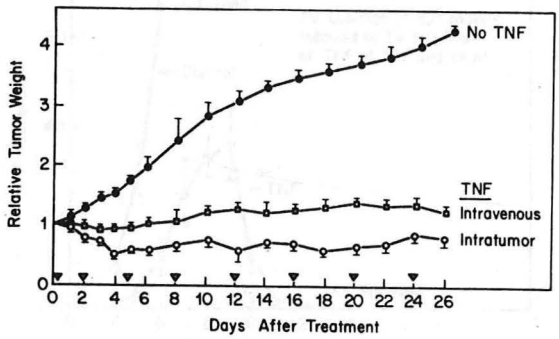


Figure 6

TNF-rich serum (obtained from BCG-primed and endotoxin-sensitized mice) kills cultured tumor cells in vitro (left panel), but has no effect on the growth of normal embryonic fibroblasts (right panel). [Data from ref. 6].

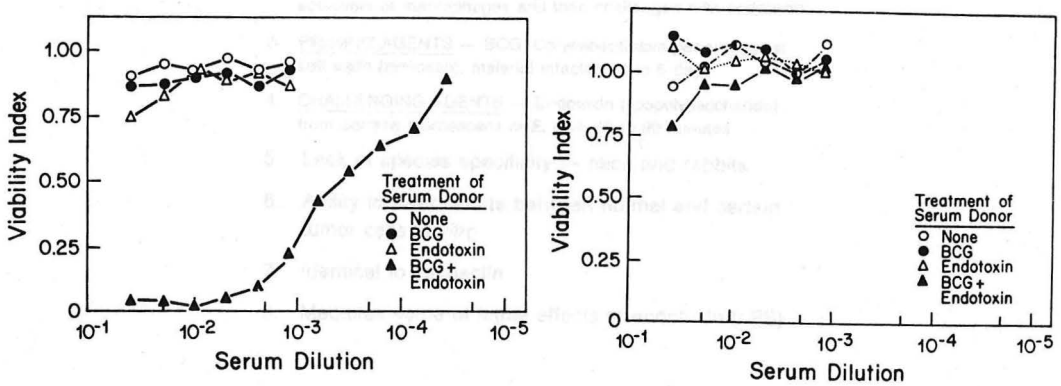


Figure 7

TNF prevents the lethal effect of malaria in a in vivo mouse model. [Data from ref. 21].

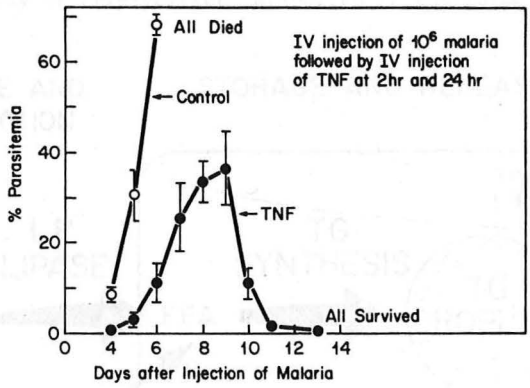
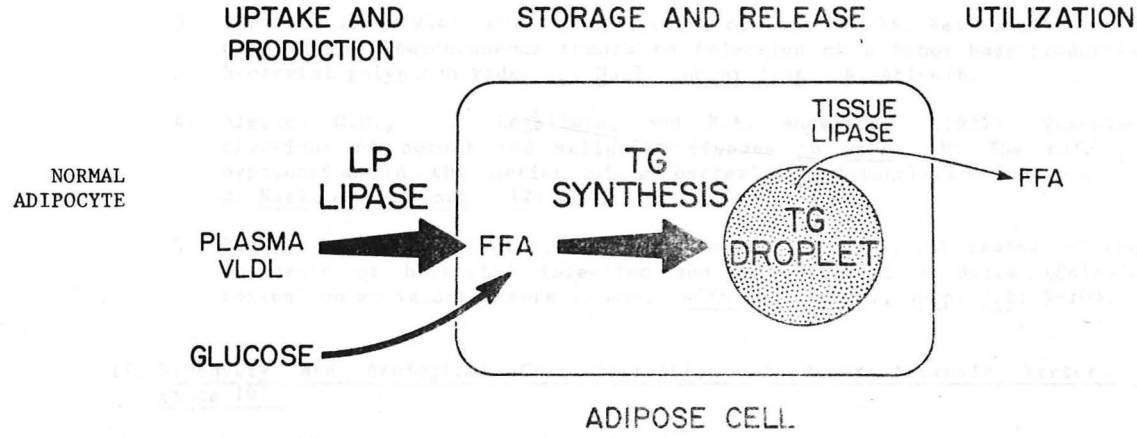


Figure 8

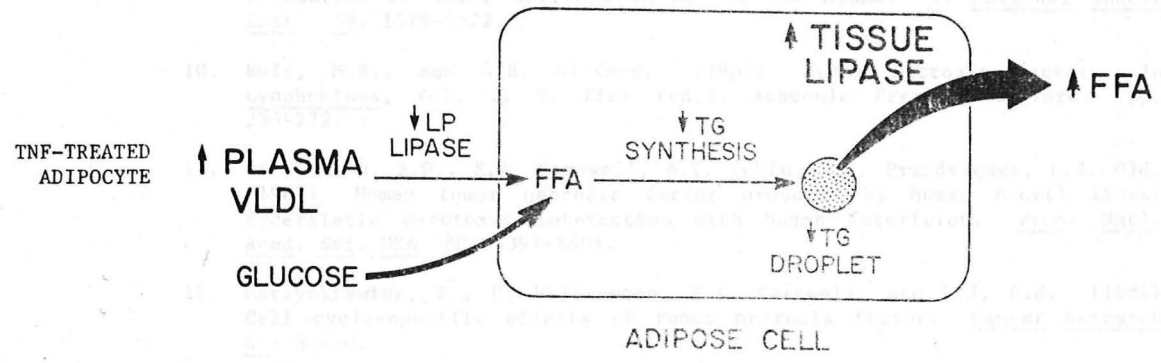
Biological characteristics of TNF

1. Protein molecule with subunit molecular weight of 17,000
2. Appears in serum of animals that have been primed with activators of macrophages and then challenged with endotoxin
3. PRIMING AGENTS — BCG, *Corynebacterium parvum*, yeast cell walls (zymosan), malarial infection. 4 to 6 days
4. CHALLENGING AGENTS — Endotoxin (lipopolysaccharide) from *Serratia marcescens* or *E. Coli*. 60 to 90 minutes
5. Lack of species specificity — mice and rabbits
6. Ability to discriminate between normal and certain tumor cells *in vitro*
7. Identical to cachectin
8. Mediates some of lethal effects of endotoxin (LPS)

Figure 9. TNF is identical to a protein called cachectin, a hormone that mobilizes triglyceride from adipose tissue and produces a state of cachexia in experimental animals. Under normal circumstances, the triglycerides (TG) of adipose tissue are synthesized from free fatty acids (FFA) that originate from two sources: 1) the hydrolysis of triglyceride-rich plasma very low density lipoproteins (VLDL) by the action of lipoprotein (LP) lipase, and 2) the metabolism of glucose within the adipocyte. Net accumulation and storage of triglyceride occurs when triglyceride synthesis exceeds triglyceride hydrolysis. Hydrolysis is mediated by tissue lipase. The pathway of triglyceride metabolism in the normal is shown below.



TNF depletes the triglyceride of adipose tissue by acting at three sites: 1) it decreases the activity of lipoprotein lipase, decreasing FFA availability; 2) it decreases the activity of two key enzymes responsible for converting FFA to triglyceride; and 3) it stimulates the activity of tissue lipase, thus increasing the hydrolysis of triglyceride. These effects of TNF are shown below.



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