Peptides as Requirement for Immunotherapy of the Guinea-pig Line-10 Tumor with Endotoxins

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Summary. The transplantable line-10 hepatocellular carcinoma of guinea-pigs has been used as a model for the study of immunotherapy of malignant tumors. Cure rates of up to 100% have been obtained with ReGI-CM from O antigen-deficient (Re) mutant strains of Enterobacteriaceae, provided they were combined with mycobacterial trehalose dimycolate (cord factor, P3). Where-as highly endotoxic LPS extracts from all wild-type strains so far tested have failed to cause tumor regression, acid hydrolys of such LPS samples led to residual fractions (RESI) that cross-reacted serologically with ReGI-CM samples (Chang and Nowotny, 1975) and provided cure rates up to 100%. RESI from Serratia marcescens was essentially nonpyrogenic and 100 times less lethal for chick embryos than potent endotoxins. Antigenic material associated with endotoxic extracts appears to be cryptic or sterically hindered from being effective in wild-type LPS but is exposed in ReGI and RESI samples.

Reduction of the aminoacid content of ReGI-CM by microparticulate silica gel chromatography or by treatment with Triton X-100 significantly lowered the ability to bring about tumor regression without affecting endotoxicity. Antitumor activity could be restored by the addition of synthetic N-acetyl-muramyl-L-2-iso-glutamine (MDP) or a nontoxic lipid side fraction recovered during the isolation of ReGI-CM, which contained a small amount of peptidic substances. It is concluded that the addition of peptidic material, which may act as an adjuvant, to endotoxins is required to make them useful for immunotherapy of the weakly immunogenic line-10 tumor.

Chemical procedures known to 'detoxify' endotoxins while retaining adjuvanticity, such as succinylation and phthalylolation, resulted in complete loss of endotoxicity and tumor-regressive potency of ReGI-CM. Transesterification with sodium methoxide led to a water-soluble phase, which cured 50% of tumor-bearing animals even though lethality and pyrogenicity were reduced by 100 times and 50 times, respectively. Thus there was no direct correlation between endotoxic potency and tumor-regressive activity. In addition, our findings indicate that a low level of toxicity may be required to obtain optimal levels of tumoricidal action.

Introduction

We have described previously the high efficacy of unre fined aqueous phases of phenol-water extracts from polysaccharide-deficient Re mutant (rough) strains of Salmonella typhimurium or S. minnesota (ReGI-PW) when combined with mycobacterial trehalose dimycolate (P3) in regressing line-10 tumors in syngeneic strain-2 guinea-pigs (Ribi et al., 1975a). These bacterial components were admixed with small amounts of oil, dispersed in a relatively large amount of saline, and inoculated directly into 1-week-old tumors. In 90% of animals treated in this way, tumors regressed and metastases in the drain-
ing lymph node were eliminated; all the animals cured rejected a second tumor cell transplant. Inasmuch as similarly prepared LPS extracts from wild-type (smooth) strains of *Salmonella* were significantly less active in tumor regression although their endotoxic properties were comparable (as measured by lethality for chick embryos and pyrogenicity in rabbits), it appeared that endotoxicity itself was not the only requirement for tumor-regressive potency (Ribi et al., 1975a, 1978).

To determine the structural attributes necessary for tumor-regressive potency, we chose as starting materials for fractionation endotoxic glycolipids extracted with chloroform-methanol (ReGI-CM) (Chen et al., 1973) from the same Re mutant strains. In this paper we show these extracts to be highly effective in causing regression of line-10 tumors. Because of their solubility in organic solvents, they were expected to be fractionable by preparative microparticulate gel pressure elution chromatography (Ribi et al., 1974).

From a practical point of view, our interest centered upon the importance of the toxicity of the extracts. Therefore, we also explored chemical modification techniques reported to selectively reduce the toxicity of bacterial extracts and possibly provide clinically useful cancer immunotherapeutic agents.

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**Fig. 1.** Flow chart summarizing procedures used to obtain glycolipid and other fractions from Re mutants of gram-negative bacteria.