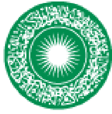

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Diphtheria Toxin Fusion Proteins

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1 Introduction 63
2 Diphtheria Toxin 64
3 Construction of the Diphtheria Toxin Fusion Proteins 66
4 The Interleukin-2 Fusion Proteins 67
4.1 The DAB_{486}IL-2 Fusion Protein 67
4.2 DAB_{486}IL-2 Clinical Studies 67
4.3 Construction of the DAB_{389}IL-2 Fusion Protein 69
4.4 Clinical Studies of DAB_{389}IL-2 in Lymphoma 69
4.5 Application of DAB_{389}IL-2 to Lymphocyte-Mediated Cutaneous Diseases 76
References 77

1 Introduction

Two different approaches have been undertaken to develop targeted biomolecules for therapeutics. The first was the construction of immunotoxins consisting of monoclonal antibodies chemically linked through a disulfide bond to a plant or bacterial toxin or radionuclide. Instability of the chemical conjugation of some of the earlier immunotoxins led to the concept of using protein engineering and recombinant DNA to assemble fusion genes combining the sequences for the enzymatically active and translocation domains of a toxin with those of a specific targeting ligand. From the outset, the prospect of using recombinant DNA methods to assemble the structural genes encoding bacterial toxin growth factor fusion toxins, or fusion proteins, offered significant advantages over chemical conjugation in the assembly of chimeric proteins. Most importantly, the fusion junction, or point at which the substitute receptor binding domain was linked to the toxin fragment, could be precisely determined. Expression of the fusion gene in recombinant *Escherichia coli* would then result in the synthesis of a single homo-

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geneous gene product rather than the mixture of isomeric forms which result from the chemical conjugation process used in the generation of immunotoxins, thereby leading to a theoretically more uniform agent for clinical studies.

A number of years ago, Murphy and colleagues explored the use of diphtheria toxin as a platform for the development of genetically engineered toxins, in which substitution of the native receptor binding domain with specific growth factors would result in a family of biologically active fusion proteins. These "new" toxins would combine the potent cytotoxic active of diphtheria toxin with the cell receptor specificity of the growth factor employed as the substitute receptor binding domain, creating a targeted therapeutic agent for human disease.

2 Diphtheria Toxin

The choice of diphtheria toxin as the toxophore for receptor binding domain substitution was based upon a detailed understanding of the structure-function relationship of the molecule. In their classic study, Uchida, Gill, and Pappenheimer (Uchida et al. 1971) demonstrated that the structural gene for diphtheria toxin was carried by corynebacteriophage β. This study also provided the foundation for subsequent studies on the structure function relationships of diphtheria toxin by demonstrating that the enzymatically active A fragment was positioned on the NH_2-terminal end of the toxin, whereas the receptor binding domain of the toxin was carried on fragment B. Shortly thereafter, Murphy et al. (1974) used β-phage DNA to program S-30 extracts of *E. coli* and demonstrated, in this coupled transcription translation system, that biologically active diphtheria toxin could be synthesized in vitro.

It was known quite early that native diphtheria toxin was a three domain protein consisting of the enzymatically active domain (fragment A), the hydrophobic domain (NH_2-terminal portion of fragment B), and the receptor binding domain (COOH-terminal portion of fragment B). The process by which diphtheria toxin intoxicates sensitive eukaryotic cells involves at least the following steps, as shown in Fig. 1: (1) binding of the toxin to its cell surface receptor, (2) activation of the catalytic domain by a proteolytic cleavage ("nicking") of the toxin in a sensitive exposed 14 amino acid loop that is subtended by Cys-186 and Cys-201, (3) internalization of the bound toxin into endosomes by receptor-mediated endocytosis, and following acidification of the endocytic vesicle, (4) the facilitated delivery of the catalytic domain across the endocytic vesicle membrane and into the cytosol. Once delivered to the cytosol, fragment A rapidly catalyzes the adenosine diphosphate ribosylation of elongation factor 2 which results in the inhibition of protein synthesis and subsequent death of the cell.

The first step in the intoxication process is the specific binding of diphtheria toxin to its cell surface receptor. Middlebrook et al. (1978) were able to correlate the apparent sensitivity of a given cell line to diphtheria toxin with the number of