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Receptor-Directed Aptamers for Modulation of Immune Responses against Microbial Infection

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Aptamers are nucleic acid molecules that have been selected from random pools based on their ability to bind other molecules. Because a huge diversity of molecular shapes can exist within the universe of all possible nucleotide sequences, aptamers may be obtained for various targets, including small organic molecules, proteins, nucleic acids, and even cells, tissues and entire organisms. This emerging class of therapeutics has many potential uses in various areas including biomedical field. Aptamers may prove useful in the treatment of a wide variety of human maladies, including infectious diseases as well as cancer and cardiovascular disease.

Several properties of aptamers make them an attractive target for new drug development. Although they have the molecular recognition properties similar to antibodies, they can be chemically synthesized, obviating the need for cumbersome production procedures in cell lines and animals. Aptamers elicit little or no immunogenicity in therapeutic applications. Another advantage over antibodies can be their stability in high temperature. Moreover, during chemical synthesis, modifications can be easily introduced into the aptamer to enhance the stability, affinity and specificity. Also, *in vitro* selection process can be manipulated to obtain aptamers that bind a specific region of the target with specific binding properties in different binding conditions. In this respect, aptamers can distinguish between closely related but non-identical members of a protein family, or between different functional or conformational states of the same protein. Affinity and specificity of aptamers for a given protein make it possible to isolate a ligand to virtually any target, and adjusting their bioavailability expands their clinical utility [1-2].

In many cases, aptamers could inhibit the function of their targets, presumably by blocking binding of the cognate ligand [2]. An aptamer targeting VEGF, pegaptanib (Macugen; Eyetech Pharmaceuticals/ Pfizer), was the first aptamer therapeutic approved for use in humans, paving the way for future aptamer applications [3]. In a first demonstration of using aptamers to modulate immune responses *in vivo*, the

generation of aptamers that bind and inhibit the function of murine CTLA-4 was reported [4]. Very recently, aptamers that function as agonists, such as aptamers that induce downstream signaling upon binding to cell surface receptors, have been described [5]. In their study, an aptamer that binds 4-1BB on the surface of activated T cells was generated and conditions whereby it functions as an agonist was defined. Specifically, they showed that bivalent and multivalent configurations of the aptamers costimulated T cell activation *in vitro* and promoted tumor rejection *in vivo*. Thus, agonistic aptamers could represent a superior alternative to Abs for the therapeutic manipulation of the immune system.

Currently, there is a growing interest in the biology of toll-like receptors (TLRs). These pattern-recognition receptors (PRRs), expressed on various immune and non-immune cell types, recognize pathogen-associated molecular patterns and play a crucial role in the innate immune response to microbial infection. Their activation by various ligands triggers a signalling cascade leading to cytokine production and initiation of an adaptive immune response. It is also suggested that activation of TLRs contributes to the development of certain cardiovascular diseases such as atherosclerosis, congestive heart failure and cardiac dysfunction in sepsis [6-7]

Despite these important aspects of TLRs, there are few therapeutic agents directed against these receptors for the treatment of the relevant diseases. Here, I tried to develop RNA-based aptamers that can specifically bind to and modulate the function of TLR2. To achieve this goal, a cDNA encoding hTLR2 was cloned from U937 cells using RT-PCR procedure. Then the soluble form of human TLR2 (sTLR2) lacking the intracellular and transmembrane domains was generated. This truncated sTLR2 protein (Met1-589) was then purified and subjected to SELEX (Systematic Evolution of Ligands by Exponential enrichment) procedure to obtain an RNA aptamer that can selectively bind to the ectodomains of TLR2. In addition to this TLR2 aptamer, another aptamer for TLR6 is being developed, since it is known that TLR6 interacts with TLR2 and helps to recognize the ligands such as proteoglycan and bacterial lipoprotein. Similarly, the cDNA encoding hTLR6 was cloned from U937 cells using RT-PCR procedure. The putative ectodomain of hTLR6 protein (sTLR6), which consists of the amino acid residues 1-586, was expressed and purified using the pET vector that has six his-tag sequences. This truncated hTLR6 protein was then subjected to SELEX procedure to obtain an RNA aptamer that can selectively bind to the ectodomain of hTLR6. Isolation of the best performing aptamers are currently underway and subsequent characterization studies will be presented. Since TLRs recognize specific molecular patterns of microbial components and their stimulation relates to both innate and adaptive immunity, both TLR2 and TLR6 aptamers might provide novel options in the treatment of many infectious diseases.

References

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