The therapeutic P140 peptide, a new immunomodulating tool for lupus may have applications in other chronic inflammatory conditions

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Abstract

P140 is a synthetic peptide issued from the U1-70K protein. It was chemically modified and contains a phosphoserine residue at position 140. P140/Lupuzorâ had no adverse safety signals and met its primary efficacy end points in a multicenter, randomized, placebo-controlled phase IIb study for lupus. A phase III-clinical trial is currently on-going for this indication. The mechanism of action of P140 has been recently elucidated in MRL/lpr lupus-prone mice. P140 binds HSPA8/ HSC70 chaperone protein, decreases its expression and reduces autophagic flux in B-lymphocytes of peptide-treated MRL/ lpr mice. P140 interferes with a selective form of autophagy called chaperone-mediated autophagy or CMA. It induces a lower expression class II-MHC molecules and alters the presentation of peptides to autoreactive T cells, leading to a reduction T and B cells activation and a drop of potentially pathogenic autoantibodies. This process does not affect the resistance of mice to an infectious agent. Based on this unique mechanism of action, we anticipated that the peptide could be efficient in other pathological conditions in which reduction of CMA activity would be beneficial. This was evaluated in several murine models of chronic inflammatory diseases.These models notably include a rat model of experimental autoimmune neuritis for chronic inflammatory demyelinating polyradiculoneuropathy, an autoimmune-mediated inflammatory disease of the peripheral nervous system. Our first results show that P140 peptide can curb the course of the disease and protect treated animals. These findings provide arguments to conclude that P140 peptide might efficiently work in indications other than lupus, most particularly in conditions of inflammatory, chronic diseases. Peptides and peptidomimetics can function as immunomodulating agents by either blocking the immune response or stimulating the immune response to generate tolerance. Knowledge of B- or T-cell epitopes along with conformational constraints is important in the design of peptide-based immunomodulating agents. Work on the conformational aspects of peptides, synthesis and modified amino acid side chains have contributed to the development of a new generation of therapeutic agents for autoimmune diseases and cancer. The design of peptides/peptidomimetics for immunomodulation in autoimmune diseases such as multiple sclerosis, rheumatoid arthritis, systemic lupus and HIV infection is reviewed. In cancer therapy, peptide epitopes are used in such a way that the body is trained to recognize and fight the cancer cells locally as well as systemically. To evaluate treatment with the peptide-based agent, Lupuzor, in a double-blind, randomised, placebo-controlled study of patients with systemic lupus erythematosus. T cells of the immune system recognize invasive antigens or foreign materials and neutralize the invaders while sparing the body's own tissues. Thus, T cells should be able to distinguish between 'self' and 'non-self' when neutralizing or destroying cells [1]. One of the earliest models proposed for T-cell activation of naive T cells to effector T cells requires several protein molecules that interact with one another at the junction of T cells and antigen-presenting cells (APCs) and consists of two signals. The first signal is provided by the interaction between a polymorphic receptor expressed on T cells and its ligand on the target cells or the APC as a MHC. The specificity of the immune response is determined by engagement of the T-cell receptors by peptide antigens bound within the groove of MHC proteins expressed on the surface of APCs. These APCs generally include dendritic cells, macrophages and B-lymphocytes. The second signal is provided by adhesion molecules and/or costimulatory ligands on the APC through corresponding counter-receptors on the T cells. Once the T cells are activated, they undergo clonal expansion to produce the immune response. During the immune response generation, the costimulatory signal (signal 2) is delivered by cell adhesion molecules, including CD2-CD58, LFA-1-ICAM-1 (CD11a-CD18-CD54) and CD28-B7 (CD28-CD80). The activation of effector T cells occurs through a multistep process activated by signal 1 and 2 with different adhesion molecules to generate the immune response.The immune system can also cause pathological consequences due to various reasons. One of the first consequences occurs in the normal immune system when a healthy immune response to a transplant leads to transplant rejection. A second case is when tolerance to self is deregulated, leading to autoimmune diseases. Although the immune system is well regulated, autoimmunity occurs when autoreactive immune cells are triggered to activate their responses against self-tissues. This happens due to a lack of immunotolerance or to a breakdown of the mechanism that controls immune tolerance, resulting in failure of the host system to distinguish self from nonself cells. Autoimmune diseases may affect a single organ or multiple organs. Organ-specific diseases include celiac disease, Type 1 diabetes mellitus, multiple sclerosis (MS) and myasthenia gravis. Systemic diseases include rheumatoid arthritis (RA) and systemic

lupus erythematosus (SLE) [4–6]. Another case occurs when the immune system becomes overreactive to harmless antigens, leading to allergy or hypersensitivity. In all of the above pathological cases, immunomodulation is necessary to control the consequences of a deregulated immune system. Immunomodulating agents can be separated into different categories, depending on their actions. For example, agents that suppress or block the immune system as in the case of autoimmune diseases, allergy/asthma [5], inflammation and transplantation; agents that stimulate or activate the immune system as in the case of viral infections and cancer; and agents that remove unwanted cellular subtypes of the immune system via specific antigens as in the case of autoimmune diseases and cancer. These modulating agents can be small organic molecules, antibodies, or peptides and peptidomimetics. In the present review, our focus is on peptides and peptidomimetics that modulate the immune response. We have covered the roles of peptides and peptidomimetics in immunomodulation and their possible therapeutic effects on autoimmune diseases and cancer. Since the list of autoimmune diseases is long (more than 100), we have concentrated our review on a few major autoimmune diseases and have tried to include the research efforts in this area for the past 5–7 years. The use of peptides as drugs began as early as the 1950s with the discovery of hormones and neurotransmitters and treatment with peptide-based drugs for hormonal therapy [7,8]. Peptide-based drug design gained momentum as 3D structures of proteins and their functions on cell surfaces as well as in cells were delineated. Particularly for the immune response, several proteins are involved on cell surfaces that interact with one another (Figure 1), forming an immunological synapse [9,10]. Structural and functional studies of the proteins have suggested that protein–protein interaction (PPI) is required for any cell signaling process. Protein–protein complexes are transient and reversible. In the immune response, these interactions are dynamic in nature and the signal depends on the strength and duration of interaction. The amino acids that are present on the surface of proteins provide them with high specificity and affinity yet dynamic binding character. Since PPI surfaces are made up of epitopes of amino acids, peptides are a relevant choice to modulate such interactions. Peptides act as a mimicking surface of one of the proteins, interfere with PPI and modulate the signaling. This is particularly important in immune response, since these molecules will not completely shut down the signaling process but, rather, modulate the signaling. Traditionally, PPIs are modulated by antibodies and there are several antibody drugs for immunomodulation in the market [11]. However, antibodies have limitations in terms of stability, delivery and, more importantly, immunogenicity. Even the humanized versions of antibodies elicit an immune response. In some cases, long-term administration of antibodies results in chronic problems [12,13]. Peptide-based drugs have advantages in terms of specificity and, generally, are nonimmunogenic and can be synthesized in large amounts. Most of the peptides will not have tertiary and quaternary structures, making them more stable compared with antibodies. Peptides combine the favorable properties of small-molecule drugs and protein therapeutics [14–16]. However, peptides have limitations in terms of in vivo enzymatic stability, short half-life, fast renal clearance and formulation challenges [17]. To overcome short half-life and low bioavailability, several strategies have been investigated that can be adopted in the design of peptide-based drugs [18]. In vivo stability of peptides can be enhanced by peptide backbone modification; this can be accomplished by introduction of unnatural amino acids or D-amino acids, peptide-bond modification, N- and C-termini modifications and constraining the backbone by introducing cyclization, resulting in molecules that are stable against enzymatic degradation [19–21]. Bioavailability and renal clearance problems can be overcome by PEGylation of the peptides. Modification of the backbone or side chain of peptides produces peptidomimetics. Peptidomimetics are compounds whose pharmacophore mimics a natural peptide or protein in 3D space with the ability to interact with the biological target and produce the same biological effect [8]. The idea behind this design is that proteins exert their biological effects through small regions on their surface called epitopes. A short sequence of peptides or functional groups that are close together can be reproduced in smaller, conformationally similar fragments that can bind to the receptor and provide steric hindrance between the receptor and the native protein ligand. Peptidomimetics have advantages over peptides in terms of stability and bioavailability associated with a natural peptide. Therefore, peptidomimetics have great potential in drug discovery. Peptidomimetics can have main- or side-chain modifications of the parent peptide designed for biological function (Figure 2A–2D) [22–25]. Some examples of peptidomimetics structures that are therapeutically useful and that are already in the market for cardiovascular disorder are shown in Figure 2E [26]. In terms of design considerations, peptidomimetics can be designed from protein epitopes with global or local conformational restrictions. Global conformational restrictions impose a particular shape or secondary structure on the peptide and also provide stability against enzymatic degradation. Examples of global conformational constraints include cyclization of the peptide using nonpeptide moieties, lactam bridges or inclusion of penicillamine (dimethyl cysteine) to form disulfide bonds. Local conformational restrictions can be applied using backbone modifications at particular amino acid residues or between two amino acid residues in the peptide. Backbone amides can be replaced by amide bond-like surrogates and isosteric substituents (Figure 2B) [27]. These backbone-modified mimetics can have regular amino acids. Side chains of amino acids in the peptides can be replaced with analogs of amino acids that have functional properties similar to those of amino acid side chains but

with conformational restrictions of χ angles for side-chain rotation .The side chain-modified peptidomimetics can expose the proper functional groups to bind with the targeted receptors with high affinity compared with normal side chains of amino acids. Another tactic to design the peptidomimetics is a minimalistic approach where the secondary structure of the peptide epitope is mimicked using α-helical, β-turn or β-strand constraints to introduce organic functional groups .The entire peptide backbone can be modified to mimic turn or helical structures using organic functional groups without any peptide bonds. The design of helical or turn mimetics provided by Hamilton et al. and Hirschmann et al. provides such peptidomimetics. However, synthesis of such mimetics requires extensive expertise in synthesis to achieve the desired product for biological investigation. In recent years, peptides and peptidomimetics have gained significant importance in various clinical areas such as immunology, endocrinology, urology and oncology. Most of the diseases in the body occur as a result of either overexpression or underexpression of certain proteins or PPIs. Since the epitope of a PPI is a peptide, strategies to design peptidomimetics to modulate this interaction are utilized in many pathological conditions. In this review, we will be focusing on the use of peptides and peptidomimetics as immunomodulators in the pathology of several autoimmune disorders, cancer and HIV. Furthermore, we will give a brief overview of cyclotides [31], which are used as templates to translate the pharmacophore designed in the peptide design strategy to multicyclic structures of naturally occurring, enzymatically stable peptides or miniproteins. After a long period where the potential of therapeutic peptides was let into oblivion and even dismissed, there is a revival of interest in peptides as potential drug candidates. Novel strategies for limiting metabolism and improve their bioavailability, and alternative routes of administration have emerged. This resulted in a large number of peptide-based drugs that are now being marketed in different indications. Regarding autoimmunity, successful data have been reported in numerous mouse models of autoimmune inflammation, yet relatively few clinical trials based on synthetic peptides are currently underway. This review reports on peptides that show much promises in appropriate mouse models of autoimmunity and describes in more detail clinical trials based on peptides for treating autoimmune patients. A particular emphasis is given to the 21-mer peptide P140/Lupuzor that has completed successfully phase I, phase IIa and phase IIb clinical trials for systemic lupus erythematosus.

Biography:

Sylviane Muller has received her Doctoral degrees in Molecular Biology (1978) and Science (1984) from the University of Strasbourg, France. She has worked as a Post-doctoral Fellow at the Max-Planck Institute for Immunobiology in Freiburg, Germany. She is currently a Distinguished class Research Director at the CNRS and Professor at the Institute of Advanced Studies of the Strasbourg University (Chair Therapeutic Immunology). She is a Deputy Director of the Molecular and Cellular Biology Institute, Director of the CNRS Unit Immunopathology and Therapeutic Chemistry and Head of the Drug Discovery Center for Cancer and Inflammation. Medal is awarded 'Laboratory of Excellence'. Her research interests focus on molecular and cellular events involved in autoimmunity, especially in the lupus disease. She has discovered the P140/Lupuzor peptide that is currently evaluated in a phase III clinical trial for lupus. She is the co-author of over 345 publications, Co-Inventor of ~30 patents and Co-Founder of NeoMPS (1986) and ImmuPharma (2002) companies. She has received the CNRS Silver Medal (2009) and the CNRS Innovation Award (2015). Her research interests include molecular and cellular events involved in autoimmunity, especially in the lupus disease.

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