

**METHODS FOR IMPROVING THE DESIGN, BIOAVAILABILITY, AND
EFFICACY OF DIRECTED SEQUENCE POLYMER COMPOSITIONS VIA
SERUM PROTEIN-BASED DETECTION OF DIRECTED SEQUENCE POLYMER
COMPOSITIONS**

RELATED APPLICATIONS

[0001] This application claims the benefit of US Provisional Application No. 61/281,470, filed November 17, 2009, and US Provisional Application No. 61/386,909, filed September 27, 2010.

BACKGROUND

[0002] Complex peptide mixtures are an emerging class of peptide therapeutics, of which Copaxone (glatiramer acetate) is a leading example. Complex peptide mixtures include diverse peptides with one or more shared characteristics (such as amino acid composition and/or sequence similarity) and include altered peptide ligands (APLs), peptide pools, peptide libraries, random sequence polymers (RSP) compositions (e.g., glatiramer acetate, and compositions disclosed in WO 03/029276, WO 05/ 112972, and WO 05/ 085323), and directed sequence polypeptide (DSP) compositions (see, for example, WO 2007/120834, WO 2009/051797, and WO 2009/128948). DSP and RSP compositions are alike in that both comprise a large number of different peptides whose sequences vary randomly within certain defined common parameters. RSP compositions are mixtures of amino acid polymers (typically linked via peptide bonds) comprising two or more randomly ordered amino acid residues in various ratios. In RSP compositions, the sequence similarity springs from the restricted amino acid content of the peptides, because all peptides consist of the same few amino acids, arranged in a random order. In DSP compositions, the sequence similarity springs from a shared base peptide sequence, with certain amino acid positions substituted randomly by a restricted slate of amino acids at defined frequencies.

[0003] There exist in the art methods of detecting simple peptides, but fewer methods are suitable for detecting and measuring complex peptide mixtures. Methods to determine the effective plasma concentration of directed sequence polymers (DSPs), are complicated because DSPs are complex mixtures of peptides, as opposed to individual peptides with a defined amino acid sequence. Improved methods for evaluating the

consistency and composition of DSPs through multiple manufacturing preparations are needed. Determining the *in vivo* status of DSP compositions has immunologic significance because, depending on the route and/or frequency of administration and the serum proteins that bind the DSPs, a mixture can invoke primarily inflammatory (TH1 type) or primarily regulatory (TH2 type) responses, leading to variations in pharmacokinetic and pharmacodynamic effects in the subject. More rigorous design and consistent administration of a DSP composition may increase the therapeutic efficacy, or reduce the potential for adverse inflammatory responses.

[0004] Thus, there is a need for methods of quantitative analysis of DSP compositions, e.g., to facilitate the *in vivo* evaluation of such mixtures and to determine the suitable amount and means of administration for therapeutic purposes.

SUMMARY OF THE INVENTION

[0005] This application provides improved methods of detecting and assessing DSP compositions. The instant invention provides methods for the detection and quantitation of DSP compositions. The instant invention provides a means to determine and enrich a subset of peptides in a DSP composition based on the subset's interactions with certain capture polypeptides. The instant invention further provides methods for administering DSP compositions to a subject in need thereof, wherein the dosage regimen and quantity may be determined or evaluated based on the above-mentioned methods for detection and quantitation.

[0006] The present disclosure also provides a method for detecting a DSP composition comprising the steps: (a) affixing said DSP composition to a solid support; (b) contacting said solid support in (a) with a protein-containing biological fluid; (c) identifying the proteins from (b) specifically bound to the solid support in (a); (d) obtaining substantially pure preparations of bound proteins in (c); (e) affixing said proteins in (c) to a means for quantitatively detecting said DSP composition; and (f) determining binding of said DSP composition to each individual said protein in (e).

[0007] This disclosure also provides a method for improving the design of a DSP composition comprising the steps: (a) affixing said DSP composition to a solid support; (b) contacting said solid support in (a) with a protein-containing biological fluid; (c)

identifying the proteins from (b) specifically bound to the solid support in (a); (d) obtaining substantially pure preparations of bound proteins in (c); (e) affixing said proteins in (c) to a means for quantitatively detecting said DSP composition; (f) determining binding of said DSP composition to each individual said protein in (e); (g) adjusting the design of said DSP composition to either enhance or reduce binding to one or more proteins in (e); (h) repeating step (f); (i) optionally repeating steps (f-h), wherein the adjustments to design of said DSP composition results in any one or more of the group comprising: increased bioavailability, reduction in toxicity, and increase in efficacy.

[0008] Furthermore, this application provides a method for detecting species within a DSP composition comprising the steps: (a) affixing said DSP composition to a solid support; (b) contacting said solid support in (a) with a protein-containing biological fluid; (c) identifying the proteins from (b) specifically bound to the solid support in (a); (d) obtaining substantially pure preparations of bound proteins in (c); (e) affixing said proteins in (c) to a solid support; (f) contacting said solid support in (e) with said DSP composition; and (g) determining binding of individual species of said DSP composition to said solid support in (f).

[0009] In addition, this application provides a method for improving the design of species within a DSP composition comprising the steps: (a) affixing said DSP composition to a solid support; (b) contacting said solid support in (a) with a protein-containing biological fluid; (c) identifying the proteins from (b) specifically bound to the solid support in (a); (d) obtaining substantially pure preparations of bound proteins in (c); (e) affixing said proteins in (c) to a solid support; (f) contacting said solid support in (e) with said DSP composition; (g) determining binding of individual species of said DSP composition to said solid support in (f); (h) adjusting the design of said DSP composition to either enhance or reduce binding to one or more proteins in (f); (i) repeating step (g); and (j) optionally repeating steps (g-i), wherein the adjustments to design of a species of said DSP composition result in any one or more of the group comprising: increased bioavailability, reduction in toxicity, and increase in efficacy.

[0010] Using the methods of the instant application, investigators can not only more reliably detect lower amounts of components of the DSP compositions, but also specifically detect species within DSP compositions that are responsible for or contribute towards a biological activity of interest, for example toxicity or efficacy.

[0011] A finding that underlies the instant invention is the specific binding of a single peptide or a multiplicity of peptides within a YEAK or YFAK peptide composition by certain proteinaceous materials. The proteinaceous materials, herein termed “capture polypeptides”, preferably also bind DSP compositions. Conversely, once the “capture polypeptides” are identified, one or more of the capture polypeptides can be used to quantitatively analyze peptides of a DSP composition, isolate functionally superior subsets of the peptides within the DSP composition, or classify components of the DSP composition based on the binding specificity. To practice the instant invention, a capture polypeptide that binds to the peptides is identified and prepared in a form useful to practice the instant invention, i.e., isolated and purified to a sufficient degree that its binding to the peptides is not compromised by the presence of other components.

[0012] An aspect of the instant invention provides a method to assess or determine variations in the products of distinct manufacturing preparations, different methods of manufacture, or different post-manufacturing processing methods of a DSP composition. A particular method of the invention is to compare the binding of different preparations of a DSP composition to a capture polypeptide to determine the similarities and/or differences between preparations.

[0013] A further aspect of the invention provides a method to quantitatively analyze peptides that are found in a DSP composition or a sample comprising a DSP composition. Some embodiments of the invention are methods to determine a biologically available quantity or concentration *in vivo* (e.g., a plasma concentration) of an administered DSP composition.

[0014] A method of the instant invention is to detect the presence of DSP compositions in a subject's tissue, said subject having previously been in contact with or treated with the DSP composition, wherein the method is carried out one or more times immediately after such contact, or after at least about 10, 20, 30, or 45 minutes, or 1, 2, 4, 6, 12, 24, 36, or 48 hours, or 3, 4, 5, 6, 7, or 10 days, or 2, 3, 4, 6, 8, or 12 weeks after such contact. A particular method of the instant invention is to detect the presence of constituents of a DSP composition in the serum or plasma of a mammal, said mammal having been previously treated with said DSP composition prior to carrying out said method within a time period described above. In certain embodiments, said mammal is a human.

[0015] In certain embodiments, the method comprises determining the presence of, and optionally the quantity of, DSP compositions by binding the DSPs to one or more predetermined capture polypeptides followed by a detection method, such as an immunologic detection method. Thus, one aspect of the invention comprises selecting or identifying a serum protein that preferentially binds a DSP composition. In certain embodiments, a method of identifying one or more serum protein comprises contacting a DSP composition with a biological sample comprising serum, detecting the binding, if any, of peptides in the DSP composition to one or more components of the serum, isolating the bound components, and identifying one or more of the bound components. In some embodiments, the bound components can be isolated by contacting the sample with an affinity column designed to bind the peptides of the DSP composition and subsequently eluting the bound fraction, followed by identifying the bound component(s).

[0016] Any serum binding proteins that bind peptides of DSP compositions can be used in the above methods. Suitable detection methods include Direct Competitive Enzyme-Linked Immunosorbent Assay (ELISA), Western blot, immunoflow cytometric detection, radioimmunoassay (RIA), or any other immunologic detection method that allows quantitative detection of specific antigens.

[0017] One aspect of the instant invention is a method for detecting the presence of a DSP composition in a biological sample, comprising: contacting the biological sample with at least one capture polypeptide; and detecting the presence or absence of binding of the capture polypeptide to the DSP composition, wherein the presence of binding indicates the presence of peptide components of the DSP composition in the biological sample. Further, such method can be extended to measure the amount or concentration of a DSP composition in a sample.

[0018] Another aspect of the instant invention is a method for measuring bioavailability of a DSP composition in a mammal, comprising: administering to a mammal a dose of a DSP composition; removing a biological sample from the subject; and contacting the biological sample with at least one capture polypeptide; thereby determining the bioavailability, or the degree of bioavailability, of the DSP composition in the biological sample.

[0019] Another aspect of the instant invention provides methods of administering DSP compositions to a mammalian subject, such amount determined based on the bioavailable portion of the dosed amount as determined by the method described above or other methods described herein. In certain embodiments, the method further comprises including a control sample, performing a pharmacodynamic test to determine changes of physiological markers, such as hormones, enzymes, serum proteins, cytokines, immunomodulators, or an effector or regulator of any of these functional proteins, between the control sample and test samples by comparing the two results, and determining the dosage effective to induce the desired changes in a pharmacodynamic parameter. In certain embodiments, behavioral changes, subjective changes as reported by a subject such as amelioration of pain or a symptom of a disease, or other evidence of indirect effects are observed. In certain embodiments, said mammalian subject is a rodent, such as a mouse or rat. In other embodiments, said subject is human.

[0020] Certain embodiments of this aspect of the invention provide a method for determining a suitable dose of a DSP composition to administer to a subject in need thereof, comprising: (a) administering to the subject a dose of the DSP composition; (b) removing a biological sample from the subject; (c) contacting the biological sample with at least one capture polypeptide; (d) determining a level of components of the DSP composition in the biological sample; (e) optionally repeating steps (a) through (d) using a different dose; and (g) comparing the levels to a predetermined suitable level of the DSP composition in the biological sample; wherein the suitable dose is a dose that results in the predetermined suitable level of the DSP composition in the biological sample.

[0021] Some embodiments of the invention provide methods to predict a portion of bioavailable fraction of a DSP composition. Such methods comprise contacting a sample comprising a DSP composition with a predetermined capture polypeptide that is found *in situ* at a site where administration and delivery of such DSP composition is contemplated, and determining binding of the DSP composition to the capture polypeptide. Binding by a large fraction of the DSP composition may be indicative of a larger proportion of peptides that are therapeutically and/or physiologically relevant, and tighter binding (per dissociation constant determination) may be indicative of a protective effect that extends the half-life of those peptides *in vivo*.

[0022] A further aspect of the instant invention provides methods to predict a therapeutically effective amount of a DSP composition to be administered to a subject (e.g., a human subject) based on data obtained from experimental subjects. In certain embodiments, the method comprises administering a DSP composition to a non-human experimental mammalian subject, determining the bioavailable portion of the dosed amount (e.g., using a method of quantitative detection described herein), determining functional read-outs, and predicting a therapeutically effective amount of the DSP composition to be delivered to the therapeutic subject based on the data obtained for the experimental mammalian subject and a correlation ratio between the therapeutic and experimental subjects. For the purposes of the instant invention, a "functional read-out" may be a phenotype or function of the subject, a phenotype or function of cellular material derived from the subject, or the composition of one or more fluids derived from the subject. A functional read-out may additionally or alternatively include a measurement of one or more biosynthetic or metabolic components such as hormones, enzymes, serum proteins, cytokines, chemokines, growth factors, immunomodulators, and an effector or regulator of said functional read-outs. In certain embodiments, the detection step may be repeated at various regular or irregular time intervals to determine the time-course of bioavailability, metabolism, and/or clearance after administration. In certain embodiments, a plasma half-life of the DSP composition as a group may be determined in this manner. In a further embodiment, a half-life of a species within the DSP composition may be determined in this manner. In particular embodiments, the experimental subject is a rodent, such as a mouse or rat.

[0023] Yet another aspect of the instant invention provides an efficient and effective method of treating a patient by administering a DSP composition, comprising: preparing a DSP composition by synthesizing peptides (e.g., simultaneously by using pools of amino acid monomers at each cycle of elongation), preparing a pharmaceutically acceptable formulation of said DSP composition, administering said DSP composition to a subject, obtaining a tissue sample from said subject, determining the amounts and/or concentrations of the DSP composition in said tissue sample, determining a functional read-out, correlating the amounts of the DSP composition to the functional read-out, and adjusting the dosage of the DSP composition to the subject to improve the functional readout.

[0024] Another aspect of the invention is a method for treating or preventing an unwanted immune response in a subject, comprising administering to the subject a suitable dose of a DSP composition, wherein such suitable dose is determined by: (i) administering to the subject a dose of the DSP composition; (ii) removing a biological sample from the experimental subject; (iii) contacting the biological sample with at least one capture polypeptide; (iv) determining a level of the capture polypeptide in the biological sample; (v) optionally repeating steps (i) through (iv) using a different dose; and (vi) comparing the level(s) against a predetermined suitable level of the DSP composition in the biological sample; wherein a suitable dose is the dose that results in the predetermined suitable level of the DSP composition in said biological sample.

[0025] In some of the foregoing aspects and embodiments, the capture polypeptide is labeled. In some embodiments, the capture polypeptides are affixed to solid support. In some embodiments, the complex comprising a capture polypeptide and one or more peptide components of a DSP composition is detected and/or isolated. In particular embodiments, the complex is detected and/or isolated by antibodies specific to the complex but not to the capture polypeptide or to the peptide component of the DSP composition.

[0026] Yet another aspect of the instant invention provides a method to isolate a selected subset of the peptides that make up the DSP composition. In particular instances, the subset may consist of peptides having one or more different amino acid sequences. In other instances, capture polypeptides may be used to classify the components of the DSP composition based on the binding specificity.

[0027] In certain embodiments, a method for isolating peptides from a sample comprising a DSP composition comprises: (a) contacting the sample with at least one capture polypeptide; and (b) separating peptides that bind to the capture polypeptide from the mixture. In certain such embodiments, the capture polypeptides are affixed to a solid support. In some embodiments, the capture polypeptides are epitope-tagged or labeled. In some embodiments, the method further comprises separating bound peptides from the capture polypeptides in order to isolate the peptides. In particular embodiments, the method further comprises determining the characteristics of the isolated peptides, such as amino acid compositions of the pool of isolated peptides and/or amino acid sequences of the isolated peptides.

[0028] In certain embodiments, a method of identifying bioavailable peptides in a DSP composition in a subject comprises: (a) administering the DSP composition to the subject; (b) removing a tissue sample from the subject after conducting step (a); and (c) identifying peptides in the sample that bind to at least one capture peptide.

[0029] In certain embodiments, a method of identifying a subset of peptides that bind to a capture polypeptide comprises preparing a DSP composition according to a protocol, contacting said DSP composition with a predetermined capture polypeptide (e.g., that is desirable as in vivo target or carrier), determining the binding of peptides within the DSP composition, identifying characteristics that differentiate the peptides that bind from peptides that do not, and preparing an improved DSP composition reflecting one or more of the differentiating characteristics.

[0030] Another aspect of the invention is a method of improving the manufacturing process of a composition comprising a DSP composition. In some embodiments, a DSP composition is designed based on the foregoing method of identifying a subset of peptides that bind to a capture polypeptide. In some embodiments, the DSP composition is designed so that the amino acid composition and/or the amino acid sequence approximates that of the subset of peptides that bound to the capture polypeptide. In some embodiments, the DSP composition has enhanced potency compared to a reference DSP composition, wherein the reference DSP composition is or is substantially the same as the original DSP composition that was contacted with the capture polypeptide. In other embodiments, the DSP composition has lower toxicity compared to the reference DSP composition.

[0031] In alternative embodiments, a method comprises preparing a DSP composition according to a protocol, formulating a composition comprising DSPs, determining the bioavailable amount of the DSPs in said composition by detecting the level or degree of functional read-out, comparing such read-out against a standard, and adjusting the protocol or formulation of the composition to obtain a desired bioavailability.

[0032] Yet another aspect of the invention is targeting of therapeutic agents to specific tissues by associating a DSP composition (e.g., a reference DSP composition or an improved DSP composition generated by the methods disclosed herein) or a component of a DSP composition with a therapeutic agent of interest, where said DSP composition or component thereof binds to a capture polypeptide that has tissue-specific targeting

properties. Such associated agents can be administered to a patient to target the agent to a tissue associated with the corresponding capture polypeptide.

[0033] Some embodiments of this aspect of the invention provide a method for delivering a therapeutic agent to a specific tissue in a subject, such method comprising: (a) isolating a peptide tag by contacting a DSP composition with a tissue specific peptide and separating peptides that bind to the tissue specific peptide from the mixture; (b) coupling the peptide tag to a therapeutic agent; and (c) administering the conjugate to a subject. Other embodiments of the invention include a method of preparing such targeted therapeutic agent by step (a) and (b) of the above described method, and a targeted therapeutic prepared thereby.

[0034] A further aspect of the instant invention is a composition useful and used in any of the methods described above. An embodiment of this aspect of the invention is a composition for detecting a DSP composition in a biological sample, comprising at least one capture polypeptide. In certain embodiments, the capture polypeptide is selected from a component of normal human sera, normal non-human primate sera, normal rabbit sera, normal mouse sera, normal rat sera, normal ferret sera, normal pig sera, normal dog sera, normal horse sera, normal sheep sera, normal cow sera, a component of mammalian-derived HDL proteome, a component of mammalian-derived LDL proteome, complement component C3, apolipoprotein A-1 preproprotein, apolipoprotein A-II preproprotein (apolipoprotein D), complement component C4A, trypsin inhibitor, inter-alpha-trypsin inhibitor family heavy chain-related protein (IHRP), alpha-1-B-glycoprotein, alpha-1-antitrypsin, apolipoprotein A-IV, ceruloplasmin, unnamed protein product (BLAST search IDs it as a IgM heavy chain), apolipoprotein E, complement factor B, prealbumin, apolipoprotein C-III, alpha2-HS glycoprotein, apolipoprotein J precursor, Chain C, Immunoglobulin M, immunoglobulin lambda light chain, Coagulation factor II (thrombin), Ig kappa chain V-III (KAU cold agglutinin), apolipoprotein J precursor, Ig A1 Bur, histidine-rich glycoprotein precursor, Alpha-2-HS-glycoprotein, gelsolin isoform a precursor, inhibitor Kunitz type proteinase, unnamed protein product (NCBI Locus/Accession No. CAA28659), and Ig J-chain.

[0035] In particular embodiments the capture polypeptide may be a serum binding protein. In more particular embodiments, the capture polypeptide is selected from alpha-1-antitrypsin, apolipoprotein A-I, alpha-1-B-glycoprotein, apolipoprotein A-IV,

apolipoprotein D, and prealbumin, or from the capture polypeptides enumerated in the paragraph immediately preceding this paragraph, or from serum polypeptides disclosed herein.

[0036] Further, in any of the foregoing embodiments, the binding of peptides in a DSP composition to a capture polypeptide, such as a serum protein, may be carried out in the presence of additional physiologically relevant components. In particular embodiments, the additional component is a lipid, such as cholesterol or triglycerides. In particular embodiments, the additional component is an HDL or LDL complex substantially free of any proteinaceous component other than the capture polypeptide.

BRIEF DESCRIPTION OF THE DRAWINGS

[0037] Figure 1 is a schematic representation of an assay used to determine binding of a DSP composition to support-bound serum binding proteins. After the serum proteins have been identified, they are bound on solid-support. A DSP composition, either alone or contained within serum, is added to the support. A primary antibody against the DSP composition (or against the conjugate between the DSP composition and the serum protein) is added, and binding of the primary antibody to its target(s) is detected by a secondary antibody and detection reagent.

[0038] Figure 2 shows the A450 colorimetric absorbance of HRP conjugated anti-YFAK and anti-YEAK antibodies, after the antibodies have bound to their targets. Targets comprise complex peptides mixtures comprising YEAK or YFAK peptides bound to serum proteins contained in (or spiked into) normal human serum. At higher concentrations of complex peptide mixtures, the detection of conjugates by anti-YEAK or anti-YFAK antibodies is higher than lower concentrations of complex peptide mixtures. 12.5 ng/mL corresponds to a dose of approximately 2 mg in a human patient.

[0039] Figure 3 shows a list of serum proteins which bind to PI-2301 or Copaxone. The origin of serum proteins is either normal mouse serum or normal human serum, as indicated. PI-2301 may be acetylated or non-acetylated. Binding complexes of PI-2301 or Copaxone are recognized by anti-YFAK or anti-YEAK antibodies, and detected with

secondary antibodies and detection reagents. Serum proteins are eluted from the complex and identified. Proteins are assigned a score based on the A450 absorbance of the detection reagent. A score of 70 corresponds to a $p = 0.001$, as compared to background absorbance, and is considered statistically significant.

[0040] Figure 4 shows YEAk in the serum of mice dosed IV with 4mg/kg of YEAk or SC with 21 mg/kg using the A450 colorimetric absorbance of HRP conjugated anti-YEAk antibodies after YEAk has bound to its target comprised of YEAk peptides bound to serum proteins contained in normal human serum. The Figure shows that GA (glatiramer acetate) fragments reach maximum serum concentration of 1800ng/mL at around 15 min post dosing. The estimated bioavailability of Copaxone® administered SC was 12% as compared to Copaxone® administered IV. GA fraction was still detected in serum at 2 hours post-dosing.

[0041] Figure 5 shows an example of the acute release of soluble factors in serum or plasma in response to YEAk administration in mice, in this case CCL22, also known as MDC. As seen in the figure, there exists a linear correlation between the dose of YEAk administered SC to mice, and the observed maximum CCL22 plasma concentration.

[0042] Figure 6 shows peptide patterns observed by LC-MS from serum proteins eluted from YEAk fragments immobilized on a CNBr-Seph column. Peptide sequences were identified using the search engine Mascot. Briefly, YEAk fragments generated by tryptic enzyme digestion were coupled to Cyanogen Bromide Sepharose (CNBr-Seph) 4b, and incubated for two hours at room temperature with either human or mouse sera. Serum proteins bound to the YEAk fragments were eluted using a solution of 0.1M Glycine-HCL, pH 2.8, and digested with trypsin in 50% methanol/50mM ammonium bicarbonate, dried, separated using liquid chromatography (LC), desolvated, ionized, sprayed into a mass spectrometer (MS), visualized, and identified using the Mascot search engine.

[0043] Figure 7 shows an ELISA assay using methods of the instant invention depicted in Figure 1 where YEAk was spiked into male and female normal human sera and pooled male and female normal human sera. The assay demonstrates a linear range detecting YEAk in sera of between 1 and 100 ng/ml. This assay can not be replicated using sera from mice, nor when irrelevant controls such as anti-Keyhole Limpet Hemocyanin (KLH) polyserum was used.

[0044] Figure 8 shows an SE-HPLC profile of Copaxone® (YEAKE) lots P53218, and 119142 with the molecular weights demonstrated to have similar profiles.

[0045] Figure 9 shows the two lots of Copaxone® seen in Figure 8 used in methods of the instant invention depicted in Figure 1.

[0046] Figure 10 shows the two lots of Copaxone® used in Figures 8 and 9 in a bioassay where the monocyte cell line RAW264.7 exposed to YEAKE released CCL22 in a concentration dependent manner.

[0047] Figure 11 shows using MALDI-TOF the strict linear relationship between the actual and theoretical mean molecular weights of YEAKE copolymers of different defined lengths. Theoretical values were calculated by multiplying the copolymer length in amino acids, i.e., 20, 40, 60 and 80, by the average molecular weight of one theoretical amino acid plus one molecule of water. The weight of one theoretical amino acid was calculated by using the respective mass of Y, E, A and K minus one molecule of water lost during amino acid coupling and the amino acid ratio of 1.0, 1.5, 4.5, 3.6

[0048] Figure 12 shows the output ratios as normalized to 100 amino acids of YEAKE copolymers of different lengths manufactured by solid phase synthesis determined by amino acid analysis, as well as the same analysis performed on the two lots of Copaxone® seen in Figures 8, 9, and 10.

[0049] Standard curves were generated, using YEAKE copolymers of 20, 40, 60, and 80 amino acids. For comparison, a standard curve using Copaxone was also generated. Figure 12 illustrates the relationship between size of the YEAKE copolymers and detection by the competitive ELISA-based PK assays. The 20-mer YEAKE copolymer has little inhibitory effect, but the standard curve generated with the 80-mer YEAKE copolymer overlays the curve obtained with Copaxone.

[0050] Figure 13 shows an ELISA assay where the Ig fraction of rabbit polyserum interacts strongly with Copaxone®, and demonstrates an increasing recognition as the length of the solid phase synthesized YEAKE copolymers increases.

[0051] Figure 14 shows an ELISA assay using a previous PK method (as described in PCT publication WO2009/075854 hereby incorporated by reference in its entirety) with

solid phase synthesized YEAK copolymers, demonstrating a relationship between the size of the YEAK copolymers and detection by the methods of the previous assay system.

[0052] Figure 15 shows the monocyte cell line RAW264.7 cultured with the solid phase synthesized copolymers of different sizes seen in Figures 12, 13, and 14 produce an increasing amount of CCL22 as the length of the copolymer increases.

[0053] Figure 16 shows the ability of the two lots of Copaxone® used in Figures 8, 9, 10, and 12, and the solid phase synthesized YEAK copolymers of different lengths used in Figures 12,13,14, and 15 to induce *ex vivo* proliferation of splenocytes from mice immunized weekly for 3 weeks with 2.5mg/kg of Copaxone®. A week after the last SC administration, spleens were collected, cell suspensions made, and the cells were cultured for 4 days with various concentrations of the different copolymers. Splenocyte proliferation was determined by measuring tritiated thymidine incorporation using methods well known in the art.

DETAILED DESCRIPTION OF THE INVENTION

Directed Sequence Polymer (DSP) compositions

[0054] A DSP is a peptide having a sequence derived from a base known peptide sequence, which may be but is not limited to a native epitope associated with an immune response, as a starting point. A DSP has one or more amino acid residues that differ from those of the base peptide sequence, the substitution of which is determined by a defined rule. Because of the semi-random diversity of a DSP composition, a large number of peptide sequences are present in the composition. Diversity of peptide sequences may confer increased efficacy over less diverse compositions, particularly as epitope shifting and spreading occurs. In some embodiments, a DSP composition comprising multiple DSPs is useful in modulation of unwanted immune responses, or eliciting immune responses when the base peptide is weakly or undetectably immunogenic.

[0055] DSPs are designed to include a defined amino acid variation at a defined rate of occurrence of introduction of such amino acid residues at any given position of the sequence to the base peptide sequence. Unlike RSPs such as Cop-1, the resulting peptides, though they may be substituted to varying degrees, maintain their similarity to the natural

sequence of amino acid residues of a defined predetermined peptide sequence of a specified length. Each amino acid position is subjected to change based on a defined set of rules, such substituting amino acid selected from chemically related amino acids, amino acids with steric similarities, phylogenic variations found in xenogeneic analogous proteins of the base peptide, known allelic variants that do not result in dysfunction of the base peptide, or small amino acid residues introduced to disrupt secondary structure of the peptides. In certain embodiments, the amino acid is substituted according to the methods seen in Kosiol *et al.*, *J. Theoretical Biol.*, 2004, 228:97-106). Alternatively, amino acids can be changed in accordance with the exemplary substitutions described in PCT/US2004/032598, pages 10-11.

[0056] DSPs may be prepared by solid phase peptide synthesis, and for each cycle of the synthesis, a mixture of appropriately protected amino acids at a defined ratio, selected for reasons described above, rather than a single amino acid, presented for incorporation into the synthesized polypeptides. Which of the selected amino acids is introduced varies according to the mixture ratio. Thus, a DSP composition, like an RSP composition, is not synthesized as a single peptide, but is always synthesized as part of a composition comprising multiple related DSPs based on a common template sequence, the overall mixture of which is reproducible and consistent with the rules of synthesis that were applied. The result is a mixture of related therapeutically useful proteins, which is described herein as a composition comprising “directed-sequence polymers” or “DSPs”. For a solid phase synthesis procedure, the mixture of amino acids for a given position in the peptide is defined by a *ratio one to another*. Prior to starting the synthesis, such ratio of amino acids in the mixture available for a variant position is determined for each position along the peptide. The resulting directed order peptide mixture comprises a multiplicity of related peptide sequences. Some DSPs which may be used in the invention include those described in international applications WO 2007/120834, WO 2009/051797, WO 2009/128948 and US application publication US 2009/0036653. These references describe methods of synthesizing DSPs, compositions comprising DSPs, therapeutic formulations of DSPs, methods of administering DSP compositions to a subject, diseases that may be treated with DSPs, and additional therapeutically effective agents which may be co-administered to a subject in with the DSPs. The teachings of all these patents, applications and publications are herein incorporated by reference in their entirety, with

particular attention to those portions discussing the structure, preparation, and function of the DSPs.

[0057] DSPs are designed and prepared by selecting a protein, either having no known function, having a known or anticipated research interest, having a known or anticipated diagnostic interest, or having a known or anticipated disease association, and selecting a portion within the protein, which portion may be an epitope within a range of immunogenicity, from no known immunogenicity to being weakly immunogenic to being strongly immunogenic, or where it is known to be relevant to the pathology of a disease. Base peptide sequences for preparing DSP compositions may be selected from various sources. In certain instances, peptide sequences with some significance to a disease state or an adverse reaction may be identified through experimental investigation of a relevant epitope. These sequences may include non-naturally occurring peptide sequences that proved to be useful in treating a disease or a condition, an example found in the international patent application publication WO 2006/031727, US Pat. No. 6,930,168 and the related scientific publication by Stern *et al.*, Proc. Nat. Acad. Sci. USA, 2005, 102:1620-25.

[0058] Further, base peptide sequences that may be epitopes are empirically determined by identifying candidate sequences by positional scanning of synthetic combinatorial peptide libraries (see, for example, D. Wilson *et al.*, above; R. Houghten *et al.*, above; Hernandez *et al.*, Eur. J. Immunol., 2004, 34:2331-41), or by making overlapping peptide sequences of the entire protein of interest, and testing those peptides for immune reactivity using, for example, any read-out assay useful for such purposes, such as the HI assay, a viral challenge model, or one described in Current Protocols in Immunology Edited by John E Coligan, Ada M Kruisbeek, David H Margulies, Ethan M Shevach, Warren Strober NIH, John Wiley & Sons, in an *in vitro* or *in vivo* assay system appropriate for the disease and species the epitope is sought for. Candidate molecules may include peptides that are modified during or post-synthesis by, for example, sugar- and modified sugar addition such as glycosylation and glycogenation, which may be either N or S-linked, fatty acid modification such as myristoylation, or creation of disulfide bonds.

[0059] After identifying a candidate epitope, a probable set of additional related epitopes may be generated using sub-strain variants, cluster variants, drift variants, shift variants of a pathogen, via modeling and prediction algorithms described in readily

available references, for example WO 2000/042559, by aligning and analyzing the mutations, probable antibody accessible epitopes, or predicted binding of these probable epitopes using available prediction methods described in, for example, WO 2005/103679, WO 2002/073193 and WO 99/45954.

[0060] In some embodiments, base peptide sequences for designing DSPs are epitopes related to an autoimmune disease selected from multiple sclerosis, systemic lupus erythematosus, type I diabetes mellitus, myasthenia gravis, rheumatoid arthritis, and pemphigus vulgaris.

[0061] In other embodiments, the base peptide sequence is an epitope relevant to the pathology of a cancer selected from leukemia, breast, skin, bone, prostate, liver, lung, brain, larynx, gallbladder, pancreas, rectum, parathyroid, thyroid, adrenal, neural, head and neck, colon, stomach, bronchi, kidneys, basal cell carcinoma, squamous cell carcinoma, melanoma, metastatic skin carcinoma, osteosarcoma, Ewing's sarcoma, veticulum cell carcinoma, myeloma, giant cell tumor, small-cell lung tumor, islet cell tumor, lymphocytic, granulocytic, hairy-cell, adenoma, hyperplasia, medullary carcinoma, pheochromocytoma, ovarian tumor, cervical dysplasia, in situ carcinoma, neuroblastoma, retinoblastoma, soft-tissue sarcoma, kaposi's sarcoma, and osteogenic sarcoma.

[0062] In other embodiments, the base peptide sequence is an epitope relevant to the pathology of a viral infectious disease selected from AIDS, AIDS Related Complex, Chickenpox (Varicella), Common cold, Cytomegalovirus Infection, Colorado tick fever, Dengue fever, Ebola haemorrhagic fever, Hand, foot and mouth disease, Hepatitis, Herpes simplex, Herpes zoster, HPV, Influenza (Flu), Lassa fever, Measles, Marburg haemorrhagic fever, Infectious mononucleosis, Mumps, Poliomyelitis, Progressive multifocal leukencephalopathy, Rabies, Rubella, SARS, Smallpox (Variola), Viral encephalitis, Viral gastroenteritis, Viral meningitis, Viral pneumonia, West Nile disease, and Yellow fever.

[0063] In other embodiments, the base peptide sequence is an epitope relevant to the pathology of a bacterial infectious disease selected from Anthrax, Bacterial Meningitis, Botulism, Brucellosis, Campylobacteriosis, Cat Scratch Disease, Cholera, Diphtheria, Gonorrhea, Impetigo, Legionellosis, Leprosy (Hansen's Disease), Leptospirosis, Listeriosis, Lyme disease, Melioidosis, MRSA infection, Nocardiosis, Pertussis (Whooping Cough),

Plague, Pneumococcal pneumonia, Psittacosis, Q fever, Rocky Mountain Spotted Fever (RMSF), Salmonellosis, Scarlet Fever, Shigellosis, Syphilis, Tetanus, Trachoma, Tuberculosis, Tularemia, Typhoid Fever, Typhus (including epidemic typhus), and Urinary Tract Infections.

[0064] In other embodiments, such base peptide sequence is an epitope relevant to the pathology of a parasitic infectious disease selected from Amoebiasis, Ascariasis, Babesiosis, Chagas Disease, Clonorchiasis, Cryptosporidiosis, Cysticercosis, Diphyllbothriasis, Dracunculiasis, Echinococcosis, Enterobiasis, Fascioliasis, Fasciolopsiasis, Filariasis, Free-living amoebic infection, Giardiasis, Gnathostomiasis, Hymenolepiasis, Isosporiasis, Kala-azar, Leishmaniasis, Malaria, Metagonimiasis, Myiasis, Onchocerciasis, Pediculosis, Pinworm Infection, Plasmodium, Scabies, Schistosomiasis, Taeniasis, Toxocariasis, Toxoplasmosis, Trichinellosis, Trichinosis, Trichuriasis, Trichomoniasis, and Trypanosomiasis (including African trypanosomiasis).

[0065] In some embodiments, the base peptide sequence is an epitope relevant to the pathology of protein conformational disorders affecting the central and/or peripheral nervous system, selected from: Alzheimer's disease (AD), Dutch hereditary cerebral hemorrhage with amyloidosis (a.k.a. cerebrovascular amyloidosis), congophilic angiopathy; Pick's disease, progressive supranuclear palsy; familial British dementia; Parkinson's disease (PD), Lewy-body related diseases, multiple system atrophy, Hallervorden-Spatz disease; amyotrophic lateral sclerosis (ALS); Huntington's disease (HD); spinocerebellar ataxia; neuronal intranuclear inclusion disease; hereditary dentatorubral-pallidoluysian atrophy; prion-related diseases such as scrapie, bovine spongiform encephalopathy, variant Creutzfeldt-Jakob disease, Gerstmann-Sträussler-Scheinker syndrome, kuru, fatal familial insomnia, and related disorders; hereditary cystatin c amyloid angiopathy; dementia pugilistica; and other disorders characterized by cerebral atrophy and detection of intracellular and/or extracellular fibrillar aggregates as the disorder progresses.

[0066] In a particular embodiment, the protein conformational disorder is Parkinson's disease. In another embodiment, the protein conformational disorder is Alzheimer's disease. In another embodiment the conformational disorder is a prion-related disease. In another embodiment, the conformational disorder is amyotrophic lateral sclerosis. In a particular embodiment, the conformational disorder is Huntington's disease.

[0067] In other embodiments, the base peptide sequence used for the process to manufacture the DSP composition is an epitope relevant to the pathology of protein conformational disorders affecting multiple organs or organs other than the central nervous system, selected from: spinal and bulbar muscular atrophy; hereditary systemic and cerebral amyloidosis, Finnish-type familial amyloidosis; senile systemic amyloidosis (a.k.a. senile cardiac amyloidosis), familial amyloid polyneuropathy; Type-2 diabetes, in particular pancreatic islet amyloidosis; dialysis-related amyloidosis (DRA); inflammation-associated reactive systemic amyloidosis (a.k.a. AA amyloidosis); aortic medial amyloidosis; medullary carcinoma of the thyroid; hereditary renal amyloidosis; light chain associated amyloidosis, light chain deposition disease, light chain cast nephropathy, light chain cardiomyopathy; atrial amyloidosis; injection-localized amyloidosis; cystic fibrosis (CF); sickle cell anemia, and other disorders wherein fibrillogenesis is observed in the affected organs or tissues.

[0068] Examples of natively unfolded proteins and peptides, and those suspected to be natively unfolded, that undergo fibrillogenesis, and therefore are associated with protein conformational disorders and may be used as the source sequences of the base peptides for the preparation of a DSP composition, include: prion protein and its fragments, amyloid beta protein and its fragments, abri protein, tau protein, alpha-synuclein and its central fragment, islet amyloid polypeptide (a.k.a. amylin), exon I of huntingtin, prothymosin alpha, amino-terminal domain of androgen receptor protein, ataxin-1, DRPLA protein (a.k.a. atrophin-1), and calcitonin.

[0069] Examples of globular proteins that undergo fibrillogenesis and therefore associated with protein conformational disorders and may be use as the source sequences of the base peptides for the preparation of a DSP composition, include: cystatin c, transthyretin, beta 2 microglobulin, serum amyloid A protein and its fragments, huntingtin, immunoglobulin light chain variable domains, insulin, lysozyme (in particular human lysozyme), alpha lactalbumin, and monellin, ligand- and DNA-binding domains of androgen receptor protein, lactadherein and more specifically its fragment (a.a. residue 245-294, a.k.a. medin), gelsolin, apolipoprotein A1, fibrinogen and its fragments, and atrial natriuretic factor.

[0070] As specific examples, in Alzheimer's disease, pathology correlates strongly with the presence of a 4 kDa amyloid beta (A β) peptide that is part of A β peptide precursor

(APP), cleaved by enzyme presenilin 1 (PS1). Most A β are 40 amino acids long, and designated A β 40, A β 40, A β 1-40, or, having varied amino terminal, A β x-40. Further, studies have indicated that the fibrillar form of A β 1-40 stimulates the microglia, which cell type is currently thought to play an important role in the pathogenesis of Alzheimer's disease. (Jekabsone, A. *et al.*, J. Neuroinflammation 3:24 (2006)). The peptide sequence of A β 1-40 is shown as SEQ ID NO: 7 in Table I. On the other hand, A β 1-42, which is a minor fraction of plaque-forming A β , is thought to contribute to the initiation of the formation of fibrillar A β . This "long form" of the peptide is described as SEQ ID NO: 8 in Table I. Therefore, the base peptide sequence may be A β peptide, exemplified by SEQ ID NO: 8. The base peptide sequence may also be that of shorter peptide, i.e., A β x-40, A β 1-11, which has been reported in some cases to have clinical significance, A β 14-23, or A β 16-20. Tjernberg, L.O. *et al.*, Biochem. J. 366:343-351 (2002).

Table I: Examples of epitopes

Relevance	Peptide Sequence	Source/ Original Protein	Residue Number	Ref	SEQ ID NO:
Neuro- degeneration	DAEFRHDSGYEVHHQKLVFFA EDVGSNKGAIIGLMVGGVV	Amyloid beta	1-40	54	7
	DAEFRHDSGYEVHHQKLVFFA EDVGSNKGAIIGLMVGGVVIA	Amyloid beta	1-42	55	8
	MGKGEEGYPQEGILEDMPVDP GSEAYEMPSEEGYQDYEEA	Mouse alpha synuclein	100-140	56	9
	DNEAYEMPSEEGYQDYE	Human alpha synuclein	121-137	57	10
	MATLEKLMKAFESLKSF	Huntingtin	1-17	58	11
Dialysis- related amyloidosis	IQRTPKIQVYSRHPAENGKS	Beta-2 microglobulin	21-40	59	12

Reference:

- [0071] Näslund, J. *et al.*, *Proc. Nat. Acad. Sci. USA*, 91: 8378-8382 (1994) 54
- [0072] Gandy, S., *J. Clin. Invest.* 115(5): 1121-1129 (2005) 55
- [0073] Benner, E.J. *et al.*, *PLoS ONE* 3(1): e1376 (2008) 56
- [0074] Campion, D. *et al.* "The NACP/synuclein gene: chromosomal assignment 57

and screening for alterations in Alzheimer disease" *Genomics* 26 (2), 254-257 (1995)

[0075] Lecerf, J.-M. *et al.*, *Proc Natl Acad Sci USA*. 98(8): 4764–4769 (2001) 58

[0076] Kozhukh, GV *et al.*, *JBC*, Vol. 277, No. 2, Issue of January 11, pp. 1310– 59
1315, 2002.

[0077] DSPs can also be used to treat Parkinson's Disease (PD). PD is a degenerative neurological disorder currently without a cure affecting 1-2% of the individuals over 50 years of age. The neuropathological hallmarks are characterized by progressive loss of neuromelanin containing dopaminergic neurons in the substantia nigra pars compacta (SNpc) with the presence of eosinophilic, intracytoplasmic, proteinaceous inclusions termed Lewy Bodies (LB). α -Synuclein is the most abundant protein in Lewy Bodies, and appears to be an important mediator, perhaps even a causal factor, of toxicity in PD. Thus, reduction of toxic α -Synuclein is thought to be beneficial to PD patients. The sequence of one such mouse α -Synuclein peptide, derived from the C-terminal region of the full length protein, is shown as SEQ ID NO: 9 in Table I. (Benner, E.J. *et al.*, *PLoS ONE* 3(1): e1376 (2008)). Further, elimination or sequestration of nitrated α -Synuclein and fragments thereof, appear to have favorable effects on the patients suffering from PD. Therapeutically effective antibodies are said to be directed at the nitrated α -Synuclein but not native. Therefore, the base peptide sequence may be, for example, SEQ ID NO: 9. In other embodiments, the base peptide sequence may be a fragment comprising amino acids 121-137 of human α -Synuclein (DNEAYEMPSEEGYQDYE) (SEQ ID NO: 10). In yet other embodiments, the α -Synuclein fragment (121-137) sequence is substituted at positions 121 and 122 in different species, tri-nitrated at each Y (tyrosine) position, and/or phosphorylated at S115.

[0078] DSPs may also be derived from base peptide sequence relevant to prion-diseases. SEQ ID NO: 13 (AAH22532) is human prion protein sequence. A relevant peptide is selected from partial sequences of SEQ ID NO: 13. Various species' prion sequences are disclosed by Harmeyer, S. *et al.*, *J Gen Virol*. 79(Pt 4):937-45 (1998), the entirety of which is incorporated herein by reference. The amino acid variations by species can be used to design the substituting amino acids.

[0079] A base peptide sequence may also be derived from superoxide dismutase I (SOD1). SOD1 mutation is known to have a causal relationship with the pathology of some forms of familial ALS. It has been reported that the antisera raised against a mutant form of SOD1, human G93A SOD1 recombinant protein, had protective effect on a mouse model of ALS carrying G37R mutant SOD1 (line 29), which overexpress human SOD1 protein by 4-fold higher than endogenous mouse SOD1. Urushitani, M. *et al.*, Proc. Nat. Acad. Sci. USA, 104(7): 2495–2500 (2007). An example of SOD1 protein sequence is SEQ ID NO: 14 (CAG46542). Therefore, a base peptide sequence may be a partial sequence of SEQ ID NO: 14.

[0080] Misfolded protein also plays a role in Huntington's disease, a genetic disorder caused by the pathological expansion of a polyglutamine (polyQ) tract in the huntingtin (htt) protein (SEQ ID NO: 15, human huntingtin), resulting in neurodegeneration and premature death of the afflicted individual. A single-chain antibody that binds to an epitope formed by the N-terminal 17 amino acids of htt (Lecerf, J.-M. *et al.*, Proc Natl Acad Sci USA. 98(8): 4764–4769 (2001) SEQ ID NO:11) has been shown to reduce symptoms in a Drosophila model of Huntington's disease. (Wolfgang, W.J. *et al.*, Proc Natl Acad Sci USA. 102(32): 11563–11568 (2005)) Therefore, a base peptide sequence may be SEQ ID NO: 11.

[0081] DSP compositions may also be used to treat Dialysis-related Amyloidosis (DRA). DRA may be caused by different forms of blood filtration, such as haemodialysis, hemofiltration, or Continuous Ambulatory Peritoneal Dialysis (CAPD). DRA has an incidence of greater than 95% of patients on dialysis for more than 15 years with beta-2-microglobulin (B2M, SEQ ID NO:13) amyloidosis being prevalent and predictably increasing over time. Conformational isomers of B2M have been observed in a clinical setting (Uji *et al.*, Nephron Clin Pract 2009;111:c173–c181). B2M is part of the human leukocyte antigen (HLA) class I molecule, and has a prominent beta-pleated structure characteristic of amyloid fibrils. B2M is known to circulate as an unbound monomer distributed in the extracellular space. B2M undergoes fibrillogenesis to form amyloid deposits in a variety of tissues. This deposition causes renal failure, which causes an increase in synthesis and release of B2M, exacerbating the condition. Thus, in an embodiment of the invention, a protein the base sequence of which is used for preparation of a DSP composition is beta 2 microglobulin (SEQ ID NO: 16) and fragments thereof. An

exemplary fragment of B2M may be that spanning amino acid residues 21-40, SEQ ID NO: 12 in Table I, useful as a base peptide for DRA.

[0082] In other embodiments, the base peptide sequence is a partial sequence of a protein selected from: osteopontin, an HLA protein, myelin oligodendrite glycoprotein, myelin basic protein (MBP), proteolipid protein, and myelin associated glycoproteins, S100Beta, heat shock protein alpha, beta crystallin, myelin-associated oligodendrocytic basic protein (MOBP), 2',3' cyclic nucleotide 3'-phosphodiesterase, hsp60, hsp70, Ro60, La, SmD, and 70-kDa U1RNP, glutamic acid decarboxylase (GAD65), insulinoma-antigen 2 (IA-2), insulin, acetylcholine receptor (AChR) α -subunit and muscle-specific receptor tyrosine kinase (MuSK), type II collagen, desmoglein 1 (Dsg1), desmoglein 3 (Dsg3), G-protein coupled receptors (GPCR), inflammatory related proteins, allergic related proteins, interleukins and their receptors, chemokines and their receptors, chaperones and their receptors. In other embodiments, the base peptide sequence is derived from CD20, vascular endothelial growth factor (VEGF), CD52, epidermal growth factor receptor (EGFR+), CD33, HER2; non-oncology related proteins, e.g., TNF alpha, CD25 or immunoglobulin E, for immunosuppression, CD11a, alpha4-beta1 integrin; infectious disease related beta chemokine receptor CCR5, RSVgpP.

[0083] Alternatively, a base peptide sequence may be created from a discontinuous epitope, that is, selecting the amino acids that make up the epitope, combining the amino acids into a linear peptide to performing directed permutations to create the DSP composition.

[0084] Yet other embodiments of the instant invention comprise selecting two or more proteins of interest, from which two or more epitopes are selected with at least one epitope deriving from each protein of interest, and combining the epitopes into a linear sequence to performing directed permutations to create the DSP composition.

[0085] In still further embodiments, a base sequence to prepare DSPs is taken from the group of proteins comprising: a protein known only as containing a domain having a primary, secondary tertiary or quaternary structural attribute, such as beta pleated sheet or alpha helices, a protein known only as containing a domain having a certain activity, such as serotonin binding, a protein known only as having a known origin, a protein known only as belonging to a specific cellular compartment such as the nucleus or cytoplasm, a protein

known only as having a cellular function, such as a cellular process producing a specific protein of interest, a protein known only as having an antioxidant activity or a metabolic activity, or a biosynthesis activity, or a catabolic activity, or a kinase activity, or a transferase activity, or a lyase activity, or a ligase activity, or a signal transduction activity or a binding activity, or a motility activity, or a membrane fusion activity, or a cellular communication activity, or a biological process regulation activity, response to stimulus activity, a cellular death related activity, a T cell activation related activity, a B cell activation related activity, an APC activation related activity, an inflammatory immune response related activity, an allergic response related activity, an infectious disease response related activity, a transporter activity, a channel activity, a secretion activity, a pathogenic activity, and a cytoskeleton organization activity.

[0086] DSP compositions can be classified according to their preferential binding targets and their physiological functions, which derive directly from the amino acid composition and their ratios. Any available method can be used to ascertain whether a DSP composition binds to a candidate or known target proteins. For example, the polypeptide can be labeled with a reporter molecule (such as a radionuclide or biotin), mixed with a crude or pure preparation of a target protein and binding is detected if the reporter molecule adheres to the target protein after removal of the unbound polypeptide.

[0087] In particular embodiments, DSP compositions useful for the present invention bind to one or more DQ isotypes with an average K_d of 1 μ M or less, and more preferably an average K_d less than 100 nM, 10 nM or even less than 1 nM. Another way to identify preferred DSPs is based on the measure of a DSP composition to displace another in competitive binding assays, using assays akin to those described in Sidney et al., 2002, J. Immunol. 169:5098, which is expressed as an IC_{50} value. In some embodiments, DSPs of the present invention have IC_{50} 's less than 1 μ M, more preferably less than 500 nM, and even more less than 100 nM.

[0088] In the methods herein, DSPs can be substituted with peptide pools, peptide libraries, or pools of altered peptide ligands (APLs). Like DSP compositions, APL compositions comprise a mixture of related polypeptides. APLs are defined as a series of peptides each of which has a small number of amino acid changes from a starting sequence of interest, such as that of a native immunogenic peptide ligand. Variant peptides with such altered amino acid sequences may be pooled to prepare a composition having the

advantages of a heterogeneous peptide mixture. Fairchild *et al.*, *Curr. Topics Peptide & Protein Res.* 2004, 6:237-44. Each APL would have a defined sequence, but the composition may be a mixture of APLs with more than one sequence. In some embodiments, pools of peptides or APLs or peptide libraries which may be used in the instant invention include those described in U.S. Pat. No. 7,118,874.

Pharmacokinetic methods

[0089] In some embodiments, the absorption and distribution of DSP compositions may be determined. The rate at which a DSP composition effects a change and the persistence of the effect, as well as chemical alterations to the composition of the DSP composition may also be determined.

[0090] Different DSP compositions will persist for different lengths of time in the serum and other biological fluids than other mixtures. In some instances, the administered peptides are sequestered by or bound to some *in vivo* component *in situ*, the result of which is longer half-life in that environment, with or without enhancement in bioavailability. In certain embodiment, the environment is blood plasma or lymph. In an alternative embodiment, the environment is spinal or cerebral fluid. In yet other embodiments, the environment is any tissue or organ locale to which peptides from DSP compositions are delivered.

Identification of physiological polypeptides and proteins that bind amino acid polymers from DSP compositions

[0091] One aspect of the present invention is identification of a capture polypeptide that binds a DSP composition. The term "capture polypeptide" is used herein to mean any polypeptide, protein, protein fragment, proteolipid, or other molecule containing proteinaceous material, found in normal tissues and organs. It may be a single polypeptide or a protein comprising multiple polypeptides and/or subunits, or a complex comprising a protein associated (covalently or non-covalently) with other materials such as lipids, which may further have defined structures that are desirable or necessary for the capture polypeptide to bind a DSP composition. Often a capture polypeptide is not transient, i.e., there is a base, stable amount that is found at all times, regardless of whether there is an induced or enhanced presence transiently. Preferably, a capture polypeptide is a protein.

More preferably, a capture polypeptide is a protein found in a biological fluid, such as a serum protein.

[0092] Some embodiments of this aspect of the invention are methods of identifying a capture polypeptide that binds to peptides that compose a DSP composition, wherein the methods comprise: contacting a sample containing an amount of the DSP composition with a normal tissue sample; and detecting binding of the peptides of the DSP composition to any component of the normal tissue sample. In certain embodiments, the peptides of DSP composition are immobilized either on a resin (through covalent bond by reacting the peptides with activated resin) or on a solid substrate such as polystyrene. For example, a tissue sample may be contacted with the immobilized peptides and incubated, washed to remove non-specific binding, and the materials bound to the peptides that were in the tissue sample identified. The bound materials may be identified by any suitable method, such as by subjecting the materials to a panel of specific antibodies; microsequencing of materials if such materials are suspected to be polypeptides or nucleotides; tryptic digestion followed by liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) subjecting such materials to specific dyes if such materials are suspected to be polysaccharides; or any analytical method with sufficiently high sensitivity.

[0093] As a non-limiting example of the above described identification, a DSP composition may be used in a direct ELISA assay to identify serum proteins that bind to the DSP composition using a protocol like that in Example 1. Table II below lists serum proteins experimentally shown to bind to RSPs, YEAk and/or YFAk peptides, in normal human serum. It has been observed that YEAk and YFAk peptides have different binding specificities; conversely, serum proteins can be said to bind YEAk and YFAk peptides with different specificities. Tables III and IV list serum proteins which associate with HDL and LDL, respectively. Any serum proteins may bind to the DSPs described herein by varying affinities and selectivities.

[0094] Once a capture polypeptide that binds DSPs is identified, the specificity of the binding against similar peptides or against completely random peptides may be determined. The identified and characterized capture polypeptide (either the same molecules actually identified or like molecules obtained from a different source) then in turn may be used to quantitatively analyze the DSP compositions that it was found to bind.

Serum Proteins

[0095] In some embodiments, binding of DSP compositions to serum proteins constitutes an important aspect of their biological activity. The binding of DSP compositions to serum proteins may facilitate their tissue distribution and capture by antigen-presenting cells such as monocytes and macrophages. As stated above, binding of peptides to serum proteins may protect them from degradation and/or turnover. In an analogous experiment involving RSPs, PI-2301 (plovamer, a YFAK random sequence polymer) and Cop-1 (glatiramer acetate, a YFAK RSP) can be detected in serum of various species, including man, several hours after subcutaneous administration, whereas a control RSP disappeared from serum after a short time (US App. Pub. 2009-027496). Copolymer 1 (Cop-1) is also referred to as glatiramer acetate. Cop-1 has been approved in several countries for the treatment of multiple sclerosis (MS) under the trade name, COPAXONETM (trademark of Teva Pharmaceuticals Ltd., Petah Tikva, Israel). Molecular weight ranges and processes for making a preferred form of Cop-1 are described in U.S. Pat. No. 5,800,808.

[0096] Accordingly, serum proteins may be used to capture and/or identify one or more peptides from a DSP composition. As a whole, DSP compositions contain a large number, even billions, of individual peptides, of which one or more sub-fractions may be responsible for the serum protein binding properties, while other sub-fractions are not. This is especially true for mixtures made by solution phase peptide synthesis, where different lots of DSP compositions may contain variations in the percentage of peptides capable of binding serum proteins. For example, it may be important to monitor DSP compositions in serum to demonstrate bioequivalence among different lots in order demonstrate that the serum protein-binding fractions are equivalent quantitatively and qualitatively across different lots of DSP compositions.

[0097] Serum proteins may be used *in vitro* to select and/or characterize binding partners from a DSP composition. Serum proteins may also be used *in vivo* to select, measure, and/or otherwise characterize peptides which bind the serum proteins, thus providing a means for distinguishing specific peptides or subsets of peptides on the basis of their binding to serum proteins and/or their persistence *in vivo*. Specific characteristics of peptides that bind to serum proteins may comprise specific amino acid sequences, ratios of amino acids in the mixture, structures, unique motifs, configuration of charged residues.

Table II: Examples of serum proteins experimentally shown to bind to YEAk and YFAk peptides in human serum:

Protein	NCBI locus/Accession No.
alpha-1-antitrypsin (SEQ ID NO: 1)	AAA51546 (CAJ15161)
alpha-1-B-glycoprotein (SEQ ID NO: 3)	OMHU1B
alpha2-HS glycoprotein	BAA22651
Alpha-2-HS-glycoprotein	P02765
apolipoprotein A-I preproprotein (SEQ ID NO: 4)	AAA51747
Apolipoprotein A-I (SEQ ID NO: 2)	Q9Z2L4 (AAS68227)
apolipoprotein A-II preproprotein (apolipoprotein D)	NP_001634 (AAB32200)
apolipoprotein A-IV	AAA51744
apolipoprotein C-III	AAB59372
apolipoprotein D (SEQ ID NO: 5)	AAB35919
apolipoprotein E	AAB59518
apolipoprotein J precursor	AAA51765
ceruloplasmin	AAA51975
Chain C, Immunoglobulin M	2RCJ_C
Coagulation factor II (thrombin)	3F68_H
complement component 3	NP_058690
complement component C3	AAA85332
complement component C4A	AAA51855
complement factor B	AAA16820
gelsolin isoform a precursor	NP_000168
histidine-rich glycoprotein precursor	NP_000403
Ig A1 Bur	763134A
Ig J-chain	AAA58902
Ig kappa chain V-III (KAU cold agglutinin)	A23746
immunoglobulin lambda light chain	CAA40939
inhibitor, Kunitz type proteinase	0511271A
inter-alpha-trypsin inhibitor family heavy chain-related protein (IHRP)	BAA07602
Inter-alpha-trypsin inhibitor heavy chain H1	Q61702
Inter-alpha-trypsin inhibitor heavy chain H2	Q61703
lumican	AAB35361
Prealbumin (SEQ ID NO: 6)	BAA00059
trypsin inhibitor	CAA30160
unnamed protein product (putative IgM heavy chain)	CAA34971
unnamed protein product (putative vitronectin)	CAA28659
vitronectin	AAA40558

Table III: serum proteins associated with HDL

Proteins	Accession No.
Apo A-I	P02647
Apo A-II	P02652
Apo A-IV	P06727
Apo C-II	P02655
Apo C-III	P02656
Apo D	P05090
Apo E	P02649
Apo J	P10909
Apo L1	O14791
Apo M	gi 13645390
LPL	gi 3293305
CETP	P11597
C-RP	P02741
Ceroplasmin	gi 13645230
Complement component 3	gi 13649325,
Haptoglobin	gi 1212947,P00738
SAA	P35542
SAP	P02743
Transthyretin	P02766
Transferrin	gi 4557871,P02787
PON	P27169
Complement component 1 inhibitor	P05155
Macrophage stimulating factor 1	gi 10337615
Lymphocyte antigen	gi 553540
Meningioma expressed antigen 5	gi 11024698
HLA-A protein	gi 13620230
NOTCH1	gi 11275980
Sialic acid binding Ig-like lectin 5	gi 13633818
C-type lectin super family member1	gi 5031637
H factor 1 (complement)	gi 4504375
Complement component 3	gi 13649325,
Insulinoma-associated protein I A-6	gi 14211925
Latent transforming growth factor beta	gi 3327808
LTBP-2	gi 1272664
Growth arrest-specific gene-6	gi 4557617
Receptors ryanodine receptor 2	gi 13638463
POU 5 domain protein	gi 12382246
Plasma kallikrein B1	gi 11436257
TFPI	P10646/P48307
Unnamed protein product	gi 10435007
Unknown protein	gi 12653035
Unknown protein	gi 12802992
KIAA1095	gi 5689527
KIAA1730protein	gi 12698005
KIAA0675 gene product	gi 13643803

Proteins	Accession No.
CIP-interacting zing finger protein	gi 12643326
dj675G8.1(novel zinc finger protein)	gi 11137825
dj733D15.1	gi 3702137
TAT-interactive protein, 72-kDa	gi 1427566
dj758N20.1 (protein kinase)	gi 11493357
Protein tyrosine phosphatase	gi 13645209
Hypothetical protein dj1057B20.2	gi 11034845
Desmocollin	gi 13435361
Coagulation factor VIII-associated protein	gi 13652210
IgG	gi 10334541,P99007
HSA	gi 178345, P02728
α -1 β -glycoprotein	P04217

Table IV: serum proteins associated with LDL

Proteins	
apoE (five isoforms)	
apoL-I (seven isoforms)	
apoC-IV (three isoforms)	
apoA-IV	
apoA-I	
apoM	
apoC-III	
b-actin	
fibrinogen-g (two isoforms)	
albumin (three isoforms)	
Prenylcysteine lyase (two isoforms)	

Binding between serum proteins and DSP compositions

[0098] Without wishing to be bound by theory, mechanistically, binding of peptides within DSP compositions, to the serum proteins such as lipoproteins found associated with HDL and LDL might facilitate their capture by monocytes through receptors such as SR-BI or ABCA1. This binding may induce activation of monocytes and their differentiation into anti-inflammatory cells.

[0099] A serum protein may bind to a DSP composition as a part of a cholesterol complex such as an HDL or LDL complex, and/or in conjunction with other proteins and polypeptides (any of which individually may also function as a capture polypeptide) that are found in association with the serum protein under physiological conditions. Thus, the

methods of the invention contemplate having additional components found in the serum when binding DSP composition to a serum protein.

Detection of a DSP composition in a biological sample determination of bioavailability

[00100] One aspect of the instant invention is a method for detecting the presence of a DSP composition in a biological sample, comprising: contacting the biological sample with at least one capture polypeptide; and detecting the presence or absence of binding of the capture polypeptide to the DSP composition, wherein the presence of binding indicates the presence of peptide components of the DSP composition in the biological sample. Further, such method can be extended to measure the amount or concentration of a DSP composition in a sample.

[00101] In some embodiments, the presence of a DSP composition may be detected in a biological sample by contacting the biological sample with at least one capture polypeptide (e.g., comprising a peptide selected from alpha-1-antitrypsin, apolipoprotein A-I, alpha-1-B-glycoprotein, apolipoprotein A-IV, apolipoprotein D, and prealbumin); and detecting the presence or absence of binding of the capture polypeptide to the DSP composition. In this assay, the presence of binding indicates the presence of DSPs in the biological sample. Further, the invention provides methods for determining an amount of a DSP composition in a biological sample, by contacting the biological sample with at least one capture polypeptide (e.g., comprising a peptide selected from alpha-1-antitrypsin, apolipoprotein A-I, alpha-1-B-glycoprotein, apolipoprotein A-IV, apolipoprotein D, and prealbumin); and quantifying a level of binding of the capture polypeptide to the DSP composition.

[00102] Other embodiments of the invention provide methods of determining the bioavailability of a DSP composition in a subject, comprising administering to a subject a dose of a composition comprising the DSP composition; removing a biological sample from the subject; and contacting the biological sample with at least one capture polypeptide (e.g., comprising a peptide selected from alpha-1-antitrypsin, apolipoprotein A-I, alpha-1-B-glycoprotein, apolipoprotein A-IV, apolipoprotein D, and prealbumin). It is contemplated that the peptides of DSP compositions are extensively bound to a capture polypeptide *in vivo*. Nevertheless, for further characterization, antibodies specific against

the complexes comprising peptides of a DSP composition and a capture peptide, but not each of those singly, may be used for detection of the bioavailable DSP composition.

Improvement of dosage and methods of administration

[00103] Another aspect of the instant invention provides methods of administering DSP compositions to a mammalian subject, in an amount determined based on the bioavailable portion of the dosed amount as determined by the method described above or other methods described herein. In certain embodiments, the method further comprises including a control sample, performing a pharmacodynamic test to determine changes of physiological markers, such as hormones, enzymes, serum proteins, cytokines, immunomodulators, or an effector or regulator of any of these functional proteins, between the control sample and test samples by comparing the two results, and determining the dosage effective to induce the desired changes in a pharmacodynamic parameter. In certain embodiments, behavioral changes, subjective changes as reported by a subject such as amelioration of pain or a symptom of a disease, or other evidence of indirect effects are observed. In certain embodiments, said mammalian subject is a rodent, such as a mouse or rat. In other embodiments, said subject is human.

[00104] More generally, a method for treating or preventing an unwanted immune response in a subject may comprise providing a DSP composition; administering the DSP composition to a test subject; removing a biological sample from the test subject; contacting the biological sample with at least one capture polypeptide (e.g., comprising a peptide sequence selected from alpha-1-antitrypsin, apolipoprotein A-I, alpha-1-B-glycoprotein, apolipoprotein A-IV, apolipoprotein D, and prealbumin); separating DSPs that bind to the capture polypeptide from the mixture; determining characteristics of the separated DSPs; preparing a set of DSPs with the characteristics of the separated DSPs, and administering the prepared set of DSPs to a subject.

[00105] In these methods, DSP compositions may be administered to a subject more than once. DSP compositions may be administered to the subject at intervals of, for example, 1, 2, 3, 4, 6, 12, 18, 24, 36, 48, or 72 hours.

[00106] Thus, some embodiments of the invention are methods of administering a suitable dose of a DSP composition to a subject in need thereof, wherein the suitable dose is determined by administering to the subject a first dose of the DSP composition;

removing a biological sample from the subject; contacting the biological sample with at least one capture polypeptide (e.g., comprising a peptide selected from alpha-1-antitrypsin, apolipoprotein A-I, alpha-1-B-glycoprotein, apolipoprotein A-IV, apolipoprotein D, and prealbumin); determining a level of the capture polypeptide in the biological sample; optionally repeating the previous steps using a second different dose; and comparing the levels to a predetermined suitable level of the DSP composition in the biological sample. Under these conditions, a suitable dose is the dose that results in the predetermined suitable level of the DSP composition in the biological sample. A suitable level of a DSP composition in a biological sample is a level at which a desirable functional read-out, or surrogate marker change, is obtained. A functional read-out can be the phenotype or function of the subject, the phenotype or function of cellular material derived from the subject, or the composition of fluids derived from the subject. In a particular embodiment, the detection step is repeated after certain time intervals to determine the time-course of bioavailability after administration. In certain embodiment, a half-life of the DSP composition as a group is determined from such time course. Examples for functional readouts of immune response enhancement or sequestering are: increase or detection of TNF α , IL-6, CXCL1, CXCL2, and IL-12p70 as indicators of undesired immune stimulation, and increase or detection of IL-1ra, CXCL13, and CCL22 as indicators of desirable positive changes. Changes in these markers are easily determined by skills and materials known and readily available in the art.

[00107] Certain embodiments of the invention facilitate the comparison of effective doses across species. Comparison of effective doses in human and experimental animals such as mice or rats is made difficult not only by the body size difference and the difference in general metabolism, but also because it has been observed that bioavailability of a drug differs between animal species. It is an aspect of the present invention that the bioavailability of DSP compositions is correlated partly by the binding of the component peptides to serum proteins, which may allow for longer half-life and certain tissue distribution. Thus, some embodiments of the invention are methods of determining a suitable dosage of a DSP composition in a subject, such methods comprising determining a first suitable dosage of the DSP composition in an experimental animal model, wherein the first suitable dosage is such dosage that gives a favorable read-out and that corresponds to a level of DSP composition bound to a serum protein in vivo, and determining a second suitable dosage of the DSP composition in the subject by dosing the subject so that the

level of DSP composition bound to the serum protein in vivo in the subject is similar or identical to the level achieved by administering the first suitable dosage to the experimental animal.

[00108] In particular embodiments, administration of a DSP composition may be enhanced using the methods of present invention. One method comprises administering to the subject a suitable dose of a DSP composition, wherein such suitable dose is determined by administering to the subject a dose of the DSP composition; removing a biological sample from the experimental subject; contacting the biological sample with at least one capture polypeptide (e.g., selected from alpha-1-antitrypsin, apolipoprotein A-I, alpha-1-B-glycoprotein, apolipoprotein A-IV, apolipoprotein D, and prealbumin); determining a level of the capture polypeptide in the biological sample; optionally repeating all previous steps, and comparing the level(s) against a predetermined suitable level of the DSP composition in the biological sample. A suitable dosage is determined as described above, based on favorable readouts.

[00109] Peptides may be labeled by any suitable means, such as affixing fluorescent moieties, radioactive labels, forming chemical conjugates, biotinylation, adding epitope tags, or any other moiety that facilitates detection. Serum proteins acting as detector polypeptides as described above may be affixed to a solid support. After serum proteins have bound to one or more peptides from the DSP composition, the bound complex comprising the capture polypeptide bound to the DSP composition may be isolated.

[00110] Methods for isolating bound complexes may include immunoprecipitation, ELISA, immunodetection, or detection of the label the capture polypeptides. Detecting binding of the capture polypeptide to the DSP composition may be performed with antibodies to the capture polypeptide, antibodies to the DSP composition, or antibodies that have been generated to recognize the bound complex.

[00111] DSP compositions may be administered subcutaneously, intramuscularly, intravenously, intranasally, or through any orifice or mucous membrane.

[00112] In some embodiments, a composition for detecting a DSP composition in a biological sample may comprise at least one capture polypeptide comprising a peptide

selected from alpha-1-antitrypsin, apolipoprotein A-I, alpha-1-B-glycoprotein, apolipoprotein A-IV, apolipoprotein D, and prealbumin.

Selection of specific peptides from within a DSP composition

[00113] An aspect of the present invention is its use in identifying and/or isolating peptides or a subset of peptides from a DSP composition. Although one advantageous feature of the DSP compositions compared to a single-species or oligo-specific peptide samples is its heterogeneity, it is conceivable that a subset of the peptides that compose the mixture is more effective than another subset, or that a subset is in fact undesirable. Thus, the present invention provides methods for identifying and/or isolating peptides from a sample comprising a DSP composition based on the peptides' affinity to certain capture polypeptides. In particular instances, the subset may comprise peptides having one or more different amino acid sequences. In other instances, capture polypeptides may be used to classify the components of the DSP composition based on the binding specificity.

[00114] In some embodiments, a method of identifying a subset of peptides that bind to a capture polypeptide comprises preparing a DSP composition according to a protocol, contacting said DSP composition with a predetermined capture polypeptide (e.g., that is desirable as *in vivo* target or carrier), determining the binding of peptides within the DSP composition, identifying characteristics that differentiate the peptides that bind from peptides that do not, and preparing an improved DSP composition reflecting one or more of the differentiating characteristics.

[00115] In certain embodiments, a sample containing a DSP composition is contacted with a capture polypeptide, and the peptides that compose the DSP composition that bind to the capture polypeptide are isolated and identified. In certain embodiments, a DSP composition is contacted with at least one serum protein which acts as a capture polypeptide. In more particular embodiments, such serum protein is selected from alpha-1-antitrypsin, apolipoprotein A-I, alpha-1-B-glycoprotein, apolipoprotein A-IV, apolipoprotein D, and prealbumin capture polypeptide.

[00116] The capture polypeptide may be immobilized on a solid support, and/or may be labeled by methods known in the art. Immobilization and labeling may be used in further steps of separating bound peptides from the capture polypeptides, and/or determining characteristics of isolated peptides. Such characteristics may include the

amino acid sequence of a bound peptide, relative ratios of amino acids in bound peptides, configuration or disposition of charged residues in the sequence, the structure of the peptide, charge, or any other suitable characteristic.

[00117] The binding between DSP compositions and serum proteins may also be used for identifying bioavailable peptides in a DSP composition, such as a biological sample collected from a subject. Here, the DSP composition may be administered to the subject at a first time; and then, at a second time after administration, a tissue sample may be removed from the patient. In the tissue sample, peptides in the sample that bind to at least one capture polypeptide, e.g., comprising a peptide selected from alpha-1-antitrypsin, apolipoprotein A-I, alpha-1-B-glycoprotein, apolipoprotein A-IV, apolipoprotein D, and prealbumin, may be identified.

Improved preparation of DSP compositions

[00118] Another aspect of the invention is a method of improving the manufacturing process of a composition comprising a DSP composition. In some embodiments, a DSP composition is designed based on the foregoing method of identifying a subset of peptides that bind to a capture polypeptide. In some embodiments, the DSP composition is designed so that the amino acid composition and/or the amino acid sequence approximates that of the subset of peptides that bound to the capture polypeptide.

[00119] In certain embodiments, a method for producing a DSP composition having reduced toxicity may comprises contacting the DSP composition with at least one capture polypeptide (e.g., comprising a peptide selected from alpha-1-antitrypsin, apolipoprotein A-I, alpha-1-B-glycoprotein, apolipoprotein A-IV, apolipoprotein D, and prealbumin); separating peptides that bind to the capture polypeptide from the mixture; determining characteristics of the separated peptides; and preparing a set of peptides with the characteristics of the separated peptides.

[00120] Similarly, a method for producing a DSP composition having enhanced potency may comprise contacting the DSP composition with at least one capture polypeptide comprising a peptide (e.g., selected from alpha-1-antitrypsin, apolipoprotein A-I, alpha-1-B-glycoprotein, apolipoprotein A-IV, apolipoprotein D, and prealbumin); and separating peptides that bind to the capture polypeptide from the mixture; determining

characteristics of the separated peptides; and preparing a set of peptides with the characteristics of the separated peptides.

[00121] In some embodiments, a desirable subset of a DSP composition may be obtained by using immobilized capture polypeptides in a preparatory scale. A DSP composition is prepared as previously contemplated and described, and contacted with immobilized capture polypeptides relevant to a desired improvement. Unbound peptides are removed by washing the sample, and bound portion of the DSP composition is eluted using appropriate dissociation condition, such as varied pH, salt concentration, or addition of organic solvents. The pooled bound portion is treated appropriately to concentrate and to remove therapeutically undesirable components, e.g. organic solvent, by evaporation or by further purification through appropriate chromatographic or crystallization or other purification methods. The subset of the DSP composition thus prepared is used as therapeutic agents.

[00122] Further, this aspect of the invention may be combined with the above-described improvements in dosage and administration. When better-tailored DSP compositions are prepared, it is anticipated that the dosage and mode of administration may be adjusted accordingly. Therefore, in alternative embodiments, a method comprises preparing a DSP composition according to a protocol, formulating a composition comprising the DSP composition, determining the bioavailable amount of the DSP composition in said composition by detecting the level or degree of functional read-out, comparing such read-out against a standard, and adjusting the protocol or formulation of the composition to obtain a desired bioavailability.

Tissue-specific targeting of therapeutic agents

[00123] Another potential use of the relationship between DSP compositions and serum proteins is tissue-specific targeting of therapeutic agents. In one embodiment, a method for preparing a therapeutic agent to a target tissue in a subject may comprise providing a DSP composition; and coupling a therapeutic agent to the DSP composition to form a conjugate.

[00124] Thus, some embodiments of the invention are methods for delivering a therapeutic agent to a specific tissue in a subject by isolating a peptide tag by contacting a DSP composition with a tissue-specific peptide (e.g., comprising a peptide selected from alpha-1-antitrypsin, apolipoprotein A-I, alpha-1-B-glycoprotein, apolipoprotein A-IV, apolipoprotein D, and prealbumin); and separating peptides that bind to the tissue-specific peptide from the mixture; coupling the peptide tag to a therapeutic agent; and (c) administering the conjugate to a subject.

[00125] Other embodiments of the invention include a method of preparing a conjugate comprising a therapeutic agent coupled to a peptide tag, and the resulting conjugates themselves. Such a peptide may be isolated from the DSP composition on the basis of binding affinity to alpha-1-antitrypsin, apolipoprotein A-I, alpha-1-B-glycoprotein, apolipoprotein A-IV, apolipoprotein D, and prealbumin.

[00126] A therapeutic agent may be a small organic molecule or a biological macromolecule, and the specific tissue may be brain, lung, or liver tissue. The peptide tag may be coupled to the therapeutic agent by a covalent bond, inclusion complexes, ionic bonds, or hydrogen bonds. Examples of therapeutic agents useful for the practice of this invention are anti-tumor agents including antimetabolites, cytokine and growth factor inhibitors, kinase inhibitors, antiangiogenesis agents, anti-inflammatory agents, disease specific antibodies, vaccines, and antibiotics.

[00127] Standard immunological, biochemical, and molecular biology methods may be used herein and are known in the art. Examples of standard protocols can be found in, for example, Current Protocols series published by John Wiley and Sons, and all updates available to date, including Current Protocols in Molecular Biology, in Immunology, in Cell Biology, in Protein Chemistry, in Pharmacology, and others. All references and patents and patent applications cited herein are incorporated by reference in their entirety.

EXAMPLES

Example 1

Detection of PI-2301 and Cop-1 in normal human serum

[00128] PI-2301 (a YFAK random sequence polymer) or Cop-1 (a YEAK random sequence polymer) were made up at a concentration of 500 ng/mL and were diluted in 5% normal human serum in PBS to concentrations of 100 ng/mL, 50 ng/mL, 25 ng/mL, or 12.5 ng/mL, and added to normal human serum. Binding of PI-2301 or Cop-1 to serum proteins contained in the normal human serum was detected by addition of rabbit anti-YFAK or rabbit anti-YEAK antibodies.

[00129] An uncoated ELISA plate was blocked with PBS/0.1% Tween 20 for 2 hours at room temperature. PI-2301 or Cop-1 samples were serially diluted in PBS/5% normal human serum and added to the blocked and washed wells of the ELISA plate. The PI-2301 or Cop-1 in normal human serum was bound to the plate and unbound PI-2301 or Cop-1 was removed by washing the plate with PBS/0.05% Tween 20. Protein-A-purified anti-rabbit anti-PI-2301 or anti-rabbit anti-Cop-1, diluted to a suitable concentration based on the titer, was added for 1 hr at RT. After another wash step to remove the unbound rabbit anti-2301 or rabbit anti-Cop-1 antibodies, a secondary antibody, a goat anti-rabbit IgG–HRP (horse radish peroxidase conjugated antibody to rabbit IgG) was added to the well. After washing away any unbound secondary antibody, substrate for HRP was added to the wells and incubated for 15 minutes, which yielded a blue color that turns yellow when stop solution is added, the intensity of which color correlates with the amount of total PI-2301 or Cop-1 in the well. The optical density was measured at 450 nm with a ELISA plate reader and a titer curve was generated for each set of the serum samples spiked with PI-2301 and Cop-1, respectively. The limit of serum PI-2301 or serum Cop-1 detection is defined as the concentration which results in an A450 nm absorption which is 3 times above background. ELISA plate wells used to determine background are treated as described above except PI-2301 or Cop-1 was omitted.

[00130] Results are plotted in Fig. 2. On the x-axis, the concentration of complex peptide mixture is indicated. On the y-axis, the A450 colorimetric absorbance of HRP conjugated secondary antibodies is shown. At higher concentrations of complex peptide mixtures, the detection of conjugates by anti-PI-2301 or anti-Cop-1 antibodies is higher than lower concentrations of complex peptide mixtures. 12.5 ng/mL corresponds to a dose of approximately 2 mg in a human patient.

Example 2

Capture of complexes on a column

[00131] Immobilized PI-2301 or Cop-1 was prepared by reacting the peptides with CNBr-activated Sepharose®, a pre-activated large pore chromatography medium used for immobilizing ligands (proteins, peptides, nucleic acids) containing primary amines using the cyanogens bromide method. Briefly, after weighing out the desired amount, the freeze-dried CNBr-Sepharose® was washed 10 X 15 minutes with cold 1mM HCl (use approximately 200 mL 1 mM HCl/gram dried Sepharose) then 2 X with coupling buffer. The ligand was dissolved in coupling buffer to the desired concentration, combined with the CNBr-Sepharose® in a 1:2 ratio (use 1 volume of ligand to 2 volumes of washed CNBr-Sepharose® gel) then incubated overnight at 4°C on a rocking platform. Any remaining active sites on the gel were blocked and then washed to remove any excess ligand. To purify the ligand-specific protein, the coupled gel was washed 2 X in phosphate-buffered saline (PBS), the desired reagent (serum, cell supernatant) was added in a 1:2 ratio (1 volume of reagent to 2 volumes of washed CNBr-Sepharose® gel) then incubated overnight at 4°C on a rocking platform. The gel/reagent slurry was packed into a disposable column, washed to remove unbound reagent, then the ligand-specific protein was eluted with a low pH buffer. After pH neutralization, the absorbance at 280 nm of the eluted fractions was read to identify fractions containing the ligands. The column was washed and stored at 4°C for repeated use.

Example 3

Identification of proteins bound to PI-2301 or Cop-1

[00132] Samples containing PI-2301 binding proteins or Cop-1 binding proteins were obtained by the method of Example 1 or Example 2. These samples were then enzymatically digested and analyzed by liquid chromatography tandem mass spectrometry (LC-MS/MS) for the purpose of identify the proteins which bind PI-2301 or Cop-1. Briefly, an aliquot of each sample was digested with the sequence specific protease, trypsin. After digestion, the protein peptide mixture was analyzed by LC-MS/MS. Peptides were separate based on their retention to a release phase column and then sprayed into a mass spectrometer. During the spraying process the peptide picked up a +2 or +3 charge and the

mass spectrometer monitors the mass overcharge ratio. If a peptide has a significant mass overcharge ratio it is then fragmented by collision with gases and the fragment patterns are recorded. These fragment patterns can then be compared to the theoretical fragment patterns of all known proteins. This molding of experimental fragment patterns to theoretical fragment patterns resulted in the identification of several lipoproteins from the HDL and LDL complexes. These lipoproteins were found both in the PI-2301 sample and the Cop-1 sample. The Cop-1 sample also had some unique proteins including complement proteins such as C3 and C4A.

[00133] Figure 3 summarizes the serum proteins in normal mouse serum or normal human serum which were identified by binding to PI-2301 or Cop-1. PI-2301 may be acetylated or non-acetylated. The sample proteins were obtained in a method similar to that of Example 1, wherein the PI-2301 or Cop-1 were mixed and bound to components in serum. Binding complexes of PI-2301 or Cop-1 were recognized by anti-YFAK or anti-YEAK antibodies, and detected with secondary antibodies and detection reagents. Serum proteins were eluted from the complex and identified. Proteins are assigned a score based on the A450 absorbance of the detection reagent. A score of 70 corresponds to a significance value of $p < 0.001$, as compared to background absorbance, and is considered statistically significant.

[00134] While capture peptides identified by the method above are expected to bind to DSPs, the above assay may also be performed with DSPs in order to empirically identify the strongest DSP-binding serum proteins.

Example 4

Comparison of peptides composition across various lengths & lots of DSP compositions

[00135] Following synthesis of different DSP compositions, for example by solid phase synthesis or by solution phase synthesis, the individual lots or batches made by the same manufacturing process, and individual batches of mixtures manufactured by different processes may be tested and compared for variation using bioassays. Depending on the indication of the DSP composition, appropriate bioassays include the release of CCL22 by

the monocyte cell line RAW264.7, *ex vivo* proliferation assays, and measuring the binding of serum proteins to peptides in the DSP composition. Using these bioassays, one may determine subsets of peptides or even individual peptides that are present in any given process or lot. Processes and lots of DSP compositions will be compared to determine whether the same subsets of peptides and/or types of peptides are consistently represented across the different processes and lots.

[00136] A plurality of identifying resins are prepared by immobilizing a selection of serum proteins on solid support. In some embodiments, the capture protein is a protein of Figure 3. Each solid support will contain at least one serum protein, and if more than one serum protein is bound to the solid support, then the ratio of the individual serum proteins bound to a given solid support will be consistent across each identifying resin. An aliquot from each lot of the DSP composition will be applied to its own solid support, under conditions that allow a subset of DSPs to bind to the serum proteins. After washing away unbound peptides, the bound peptides will be eluted. The DSP peptides isolated in this manner will be further characterized for (1) presence of distinct DSP peptides, (2) ratios of peptides to one another, (3) proportion of peptides that bind to the serum binding protein, relative to the total DSP composition, (4) presence of binding motifs and peptide sequences, (5) amino acid composition and ratios of amino acids, and/or other characteristics of peptides. The characteristics of isolated DSP peptides from each lot will be compared with each other.

We claim:

1. A method for detecting a DSP composition, comprising:
 - a. providing a substantially pure preparation of one or more capture polypeptides;
 - b. affixing the one or more capture polypeptides to a means for quantitatively detecting the DSP composition; and
 - c. determining binding of the DSP composition to the one or more said capture polypeptide.

2. A method for improving the design of a DSP composition, comprising:
 - a. providing a substantially pure preparation of one or more capture polypeptides;
 - b. affixing the one or more capture polypeptides to a means for quantitatively detecting the DSP composition;
 - c. determining binding of the DSP composition to the one or more said capture polypeptides;
 - d. adjusting the design of the DSP composition to either enhance or reduce binding to one or more capture polypeptides;
 - e. repeating step (c);
 - f. optionally repeating steps (c-e),wherein adjusting the design of said DSP composition results in any one or more of: increased bioavailability, reduction in toxicity, and increase in efficacy.

3. A method for detecting species within a DSP composition, comprising:
 - a. providing a substantially pure preparation of one or more capture polypeptides;
 - b. affixing the one or more capture polypeptides to a solid support;
 - c. contacting the solid support with the DSP composition; and
 - d. determining binding of individual species of the DSP composition to the solid support.

4. A method for improving the design of species within a DSP composition, comprising:
 - a. providing a substantially pure preparation of one or more capture polypeptides;
 - b. affixing the one or more capture polypeptides to a solid support;
 - c. contacting the solid support with the DSP composition;
 - d. determining binding of individual species of the DSP composition to the solid support;
 - e. adjusting the design of the DSP composition to either enhance or reduce binding to one or more capture polypeptides;
 - f. repeating step (d);
 - g. optionally repeating steps (d-f),wherein adjusting the design of species of said DSP composition results in any one or more of: increased bioavailability, reduction in toxicity, and increase in efficacy.
5. The method of any of claims 1-4, wherein the one or more capture polypeptides of (a) are identified by:
 - i. affixing the DSP composition to a solid support;
 - ii. contacting said solid support in (i) with a protein-containing biological fluid;
 - iii. identifying the proteins from (ii) specifically bound to the solid support in (i);wherein a protein identified in (ii) is a capture polypeptide.
6. The method of any of claim 1-5, wherein the capture polypeptide is selected from complement component C3, apolipoprotein A-1 preproprotein, apolipoprotein A-II preproprotein (apolipoprotein D), complement component C4A, trypsin inhibitor, inter-alpha-trypsin inhibitor family heavy chain-related protein (IHRP), alpha-1-B-glycoprotein, alpha-1-antitrypsin, apolipoprotein A-IV, ceruloplasmin, unnamed protein product (NCBI Locus/Accession No. CAA34971), apolipoprotein E, complement factor B, prealbumin, apolipoprotein C-III, alpha2-HS glycoprotein, apolipoprotein J precursor, Chain C, Immunoglobulin M, immunoglobulin lambda light chain, Coagulation factor II (thrombin), Ig kappa chain V-III (KAU cold agglutinin), apolipoprotein J precursor, Ig A1 Bur, histidine-rich glycoprotein

precursor, Alpha-2-HS-glycoprotein, gelsolin isoform a precursor, inhibitor Kunitz type proteinase, unnamed protein product (NCBI Locus/Accession No. CAA28659), and Ig J-chain.

7. A method for determining the presence of a DSP composition comprising the steps:
 - a. affixing one or more proteins selected from complement component C3, apolipoprotein A-1 preproprotein, apolipoprotein A-II preproprotein (apolipoprotein D), complement component C4A, trypsin inhibitor, inter-alpha-trypsin inhibitor family heavy chain-related protein (IHRP), alpha-1-B-glycoprotein, alpha-1-antitrypsin, apolipoprotein A-IV, ceruloplasmin, unnamed protein product (NCBI Locus/Accession No. CAA34971), apolipoprotein E, complement factor B, prealbumin, apolipoprotein C-III, alpha2-HS glycoprotein, apolipoprotein J precursor, Chain C, Immunoglobulin M, immunoglobulin lambda light chain, Coagulation factor II (thrombin), Ig kappa chain V-III (KAU cold agglutinin), apolipoprotein J precursor, Ig A1 Bur, histidine-rich glycoprotein precursor, Alpha-2-HS-glycoprotein, gelsolin isoform a precursor, inhibitor Kunitz type proteinase, unnamed protein product (NCBI Locus/Accession No. CAA28659), and Ig J-chain to a means for quantitatively detecting said DSP composition in a sample; and
 - b. determining the level of said DSP composition in said sample.
8. The method of any of claims 1-4 wherein a capture polypeptide is selected from complement component C3, apolipoprotein A-1 preproprotein, apolipoprotein A-II preproprotein (apolipoprotein D), complement component C4A, trypsin inhibitor, inter-alpha-trypsin inhibitor family heavy chain-related protein (IHRP), alpha-1-B-glycoprotein, alpha-1-antitrypsin, apolipoprotein A-IV, ceruloplasmin, unnamed protein product (NCBI Locus/Accession No. CAA34971), apolipoprotein E, complement factor B, prealbumin, apolipoprotein C-III, alpha2-HS glycoprotein, apolipoprotein J precursor, Chain C, Immunoglobulin M, immunoglobulin lambda light chain, Coagulation factor II (thrombin), Ig kappa chain V-III (KAU cold agglutinin), apolipoprotein J precursor, Ig A1 Bur, histidine-rich glycoprotein precursor, Alpha-2-HS-glycoprotein, gelsolin isoform a precursor, inhibitor Kunitz

type proteinase, unnamed protein product (NCBI Locus/Accession No. CAA28659), and Ig J-chain.

9. A method for detecting presence of a DSP composition in a biological sample, comprising:
 - (a) contacting the biological sample with at least one capture polypeptide contained in normal human sera, normal non-human primate sera, normal rabbit sera, normal mouse sera, normal rat sera, normal ferret sera, normal pig sera, normal dog sera, normal horse sera, normal sheep sera, normal cow sera; and
 - (b) detecting the presence or absence of binding of the capture polypeptide to the DSP composition,wherein the presence of binding indicates the presence of DSP composition in the biological sample.
10. The method of claim 9, wherein the capture polypeptide is selected from a polypeptide comprising at least one component of the HDL proteome, LDL proteome, or at least one serum protein.
11. A method for detecting the presence of a DSP composition comprising YFAK or YEAK peptides in a biological sample, comprising:
 - (a) contacting the biological sample with at least one capture polypeptide comprising a peptide selected from: complement component C3, apolipoprotein A-1 preproprotein, apolipoprotein A-II preproprotein (apolipoprotein D), complement component C4A, trypsin inhibitor, inter-alpha-trypsin inhibitor family heavy chain-related protein (IHRP), alpha-1-B-glycoprotein, alpha-1-antitrypsin, apolipoprotein A-IV, ceruloplasmin, unnamed protein product (NCBI Locus/Accession No. CAA34971), apolipoprotein E, complement factor B, prealbumin, apolipoprotein C-III, alpha2-HS glycoprotein, apolipoprotein J precursor, Chain C, Immunoglobulin M, immunoglobulin lambda light chain, Coagulation factor II (thrombin), Ig kappa chain V-III (KAU cold agglutinin), apolipoprotein J precursor, Ig A1 Bur, histidine-rich glycoprotein precursor, Alpha-2-HS-

glycoprotein, gelsolin isoform a precursor, inhibitor Kunitz type proteinase, unnamed protein product (NCBI Locus/Accession No. CAA28659), and Ig J-chain; and

- (b) detecting the presence or absence of binding of the capture polypeptide to the DSP composition,

wherein the presence of binding indicates the presence of YFAK or YEAK peptides in the biological sample.

- 12. A method for measuring an amount of a DSP composition comprising YFAK or YEAK peptides in a biological sample, comprising:

- (a) contacting the biological sample with at least one capture polypeptide comprising a peptide selected from complement component C3, apolipoprotein A-1 preproprotein, apolipoprotein A-II preproprotein (apolipoprotein D), complement component C4A, trypsin inhibitor, inter-alpha-trypsin inhibitor family heavy chain-related protein (IHRP), alpha-1-B-glycoprotein, alpha-1-antitrypsin, apolipoprotein A-IV, ceruloplasmin, unnamed protein product (NCBI Locus/Accession No. CAA34971), apolipoprotein E, complement factor B, prealbumin, apolipoprotein C-III, alpha2-HS glycoprotein, apolipoprotein J precursor, Chain C, Immunoglobulin M, immunoglobulin lambda light chain, Coagulation factor II (thrombin), Ig kappa chain V-III (KAU cold agglutinin), apolipoprotein J precursor, Ig A1 Bur, histidine-rich glycoprotein precursor, Alpha-2-HS-glycoprotein, gelsolin isoform a precursor, inhibitor Kunitz type proteinase, unnamed protein product (NCBI Locus/Accession No. CAA28659), and Ig J-chain;
- (b) quantifying a level of binding of the capture polypeptide to the DSP composition;

wherein the level of binding indicates the amount of the DSP composition in the biological sample.

- 13. A method for measuring bioavailability of a DSP composition in a mammal, comprising:

- (a) administering to a mammal a dose of a composition comprising the DSP composition;
 - (b) removing a biological sample from the subject; and
 - (c) contacting the biological sample with at least one capture polypeptide comprising a peptide selected from complement component C3, apolipoprotein A-1 preproprotein, apolipoprotein A-II preproprotein (apolipoprotein D), complement component C4A, trypsin inhibitor, inter-alpha-trypsin inhibitor family heavy chain-related protein (IHRP), alpha-1-B-glycoprotein, alpha-1-antitrypsin, apolipoprotein A-IV, ceruloplasmin, unnamed protein product (NCBI Locus/Accession No. CAA34971), apolipoprotein E, complement factor B, prealbumin, apolipoprotein C-III, alpha2-HS glycoprotein, apolipoprotein J precursor, Chain C, Immunoglobulin M, immunoglobulin lambda light chain, Coagulation factor II (thrombin), Ig kappa chain V-III (KAU cold agglutinin), apolipoprotein J precursor, Ig A1 Bur, histidine-rich glycoprotein precursor, Alpha-2-HS-glycoprotein, gelsolin isoform a precursor, inhibitor Kunitz type proteinase, unnamed protein product (NCBI Locus/Accession No. CAA28659), and Ig J-chain;
- thereby determining the bioavailability of the DSP composition in the biological sample.

14. A method for determining a suitable dose of a DSP composition to administer to a subject in need thereof, comprising:
 - (a) administering to the subject a dose of the DSP composition;
 - (b) removing a biological sample from the subject;
 - (c) contacting the biological sample with at least one capture polypeptide comprising a peptide selected from complement component C3, apolipoprotein A-1 preproprotein, apolipoprotein A-II preproprotein (apolipoprotein D), complement component C4A, trypsin inhibitor, inter-alpha-trypsin inhibitor family heavy chain-related protein (IHRP), alpha-1-B-glycoprotein, alpha-1-antitrypsin, apolipoprotein A-IV, ceruloplasmin, unnamed protein product (NCBI Locus/Accession No. CAA34971), apolipoprotein E, complement factor B, prealbumin, apolipoprotein C-III,

alpha2-HS glycoprotein, apolipoprotein J precursor, Chain C, Immunoglobulin M, immunoglobulin lambda light chain, Coagulation factor II (thrombin), Ig kappa chain V-III (KAU cold agglutinin), apolipoprotein J precursor, Ig A1 Bur, histidine-rich glycoprotein precursor, Alpha-2-HS-glycoprotein, gelsolin isoform a precursor, inhibitor Kunitz type proteinase, unnamed protein product (NCBI Locus/Accession No. CAA28659), and Ig J-chain;

- (d) determining a level of the capture polypeptide in the biological sample;
- (e) optionally repeating steps (a) through (d) using a different dose; and
- (g) comparing the levels to a predetermined suitable level of the DSP composition in the biological sample;

wherein the suitable dose is the dose that results in the predetermined suitable level of the DSP composition in the biological sample.

15. A method for treating or preventing an unwanted immune response in a subject, comprising:

- (a) administering to the subject a suitable dose of a DSP composition, wherein such suitable dose is determined by:
 - (i) administering to the subject a dose of the DSP composition;
 - (ii) removing a biological sample from the experimental subject;
 - (iii) contacting the biological sample with at least one capture polypeptide selected from complement component C3, apolipoprotein A-1 preproprotein, apolipoprotein A-II preproprotein (apolipoprotein D), complement component C4A, trypsin inhibitor, inter-alpha-trypsin inhibitor family heavy chain-related protein (IHRP), alpha-1-B-glycoprotein, alpha-1-antitrypsin, apolipoprotein A-IV, ceruloplasmin, unnamed protein product (NCBI Locus/Accession No. CAA34971), apolipoprotein E, complement factor B, prealbumin, apolipoprotein C-III, alpha2-HS glycoprotein, apolipoprotein J precursor, Chain C, Immunoglobulin M, immunoglobulin lambda light chain, Coagulation factor II (thrombin), Ig kappa chain V-III (KAU cold agglutinin), apolipoprotein J precursor, Ig A1 Bur, histidine-rich glycoprotein precursor, Alpha-2-HS-glycoprotein, gelsolin isoform a

precursor, inhibitor Kunitz type proteinase, unnamed protein product (NCBI Locus/Accession No. CAA28659), and Ig J-chain;

- (iv) determining a level of the capture polypeptide in the biological sample;
- (v) optionally repeating steps (i) through (iv) using a different dose; and
- (vi) comparing the level(s) against a predetermined suitable level of the DSP composition in the biological sample;

wherein a suitable dose is the dose that results in the predetermined suitable level of the DSP composition in said biological sample.

- 16. The method of any of claims 11-15, wherein the capture polypeptide is labeled.
- 17. The method of any of claims 11-15, wherein the capture polypeptide is affixed to a solid support.
- 18. The method of any of claims 11-15, further comprising isolating a complex comprising the capture polypeptide bound to the DSP composition.
- 19. The method of any of claims 11-15, further comprising detecting binding of the capture polypeptide to the DSP composition with antibodies to the capture polypeptide.
- 20. The method of any of claims 11-15, wherein the composition is administered subcutaneously.
- 21. A composition for detecting a DSP composition in a biological sample, comprising at least one capture polypeptide comprising a peptide selected from complement component C3, apolipoprotein A-1 preproprotein, apolipoprotein A-II preproprotein (apolipoprotein D), complement component C4A, trypsin inhibitor, inter-alpha-trypsin inhibitor family heavy chain-related protein (IHRP), alpha-1-B-glycoprotein, alpha-1-antitrypsin, apolipoprotein A-IV, ceruloplasmin, unnamed protein product (NCBI Locus/Accession No. CAA34971), apolipoprotein E, complement factor B, prealbumin, apolipoprotein C-III, alpha2-HS glycoprotein,

apolipoprotein J precursor, Chain C, Immunoglobulin M, immunoglobulin lambda light chain, Coagulation factor II (thrombin), Ig kappa chain V-III (KAU cold agglutinin), apolipoprotein J precursor, Ig A1 Bur, histidine-rich glycoprotein precursor, Alpha-2-HS-glycoprotein, gelsolin isoform a precursor, inhibitor Kunitz type proteinase, unnamed protein product (NCBI Locus/Accession No. CAA28659), and Ig J-chain.

22. A method for isolating peptides from a sample comprising a DSP composition, comprising:
 - (a) contacting the sample with at least one capture polypeptide comprising a peptide selected from complement component C3, apolipoprotein A-1 preproprotein, apolipoprotein A-II preproprotein (apolipoprotein D), complement component C4A, trypsin inhibitor, inter-alpha-trypsin inhibitor family heavy chain-related protein (IHRP), alpha-1-B-glycoprotein, alpha-1-antitrypsin, apolipoprotein A-IV, ceruloplasmin, unnamed protein product (NCBI Locus/Accession No. CAA34971), apolipoprotein E, complement factor B, prealbumin, apolipoprotein C-III, alpha2-HS glycoprotein, apolipoprotein J precursor, Chain C, Immunoglobulin M, immunoglobulin lambda light chain, Coagulation factor II (thrombin), Ig kappa chain V-III (KAU cold agglutinin), apolipoprotein J precursor, Ig A1 Bur, histidine-rich glycoprotein precursor, Alpha-2-HS-glycoprotein, gelsolin isoform a precursor, inhibitor Kunitz type proteinase, unnamed protein product (NCBI Locus/Accession No. CAA28659), and Ig J-chain; and
 - (b) separating peptides that bind to the capture polypeptide from the mixture.
23. The method of claim 22, wherein the capture polypeptide is immobilized on solid support.
24. The method of claim 23, wherein the capture polypeptide is epitope-tagged.
25. The method of claim 22, further comprising separating bound peptides from the capture polypeptides.

26. The method of claim 22, further comprising determining characteristics of isolated peptides.
27. The method of claim 26, wherein determining characteristics comprises determining an amino acid sequence of a bound peptide or determining relative ratios of amino acids in bound peptides.
28. A method for identifying bioavailable peptides in a DSP composition in a subject, comprising:
- (a) administering the DSP composition to the subject at a first time; and
 - (b) at a second time after administration, removing a tissue sample from the patient; and
 - (c) identifying peptides in the sample that bind to at least one capture polypeptide comprising a peptide selected from complement component C3, apolipoprotein A-1 preproprotein, apolipoprotein A-II preproprotein (apolipoprotein D), complement component C4A, trypsin inhibitor, inter-alpha-trypsin inhibitor family heavy chain-related protein (IHRP), alpha-1-B-glycoprotein, alpha-1-antitrypsin, apolipoprotein A-IV, ceruloplasmin, unnamed protein product (NCBI Locus/Accession No. CAA34971), apolipoprotein E, complement factor B, prealbumin, apolipoprotein C-III, alpha2-HS glycoprotein, apolipoprotein J precursor, Chain C, Immunoglobulin M, immunoglobulin lambda light chain, Coagulation factor II (thrombin), Ig kappa chain V-III (KAU cold agglutinin), apolipoprotein J precursor, Ig A1 Bur, histidine-rich glycoprotein precursor, Alpha-2-HS-glycoprotein, gelsolin isoform a precursor, inhibitor Kunitz type proteinase, unnamed protein product (NCBI Locus/Accession No. CAA28659), and Ig J-chain.
29. A method for producing a DSP composition having reduced toxicity, comprising:
- (a) contacting the DSP composition with at least one capture polypeptide comprising a peptide selected from complement component C3, apolipoprotein A-1 preproprotein, apolipoprotein A-II preproprotein (apolipoprotein D), complement component C4A, trypsin inhibitor, inter-

alpha-trypsin inhibitor family heavy chain-related protein (IHRP), alpha-1-B-glycoprotein, alpha-1-antitrypsin, apolipoprotein A-IV, ceruloplasmin, unnamed protein product (NCBI Locus/Accession No. CAA34971), apolipoprotein E, complement factor B, prealbumin, apolipoprotein C-III, alpha2-HS glycoprotein, apolipoprotein J precursor, Chain C, Immunoglobulin M, immunoglobulin lambda light chain, Coagulation factor II (thrombin), Ig kappa chain V-III (KAU cold agglutinin), apolipoprotein J precursor, Ig A1 Bur, histidine-rich glycoprotein precursor, Alpha-2-HS-glycoprotein, gelsolin isoform a precursor, inhibitor Kunitz type proteinase, unnamed protein product (NCBI Locus/Accession No. CAA28659), and Ig J-chain; and

- (b) separating peptides that bind to the capture polypeptide from the mixture;
- (c) determining characteristics of the separated peptides; and
- (d) preparing a set of peptides with the characteristics of the separated peptides.

30. A method for producing a DSP composition having enhanced potency, comprising:

- (a) contacting the DSP composition with at least one capture polypeptide comprising a peptide selected from complement component C3, apolipoprotein A-1 preproprotein, apolipoprotein A-II preproprotein (apolipoprotein D), complement component C4A, trypsin inhibitor, inter-alpha-trypsin inhibitor family heavy chain-related protein (IHRP), alpha-1-B-glycoprotein, alpha-1-antitrypsin, apolipoprotein A-IV, ceruloplasmin, unnamed protein product (NCBI Locus/Accession No. CAA34971), apolipoprotein E, complement factor B, prealbumin, apolipoprotein C-III, alpha2-HS glycoprotein, apolipoprotein J precursor, Chain C, Immunoglobulin M, immunoglobulin lambda light chain, Coagulation factor II (thrombin), Ig kappa chain V-III (KAU cold agglutinin), apolipoprotein J precursor, Ig A1 Bur, histidine-rich glycoprotein precursor, Alpha-2-HS-glycoprotein, gelsolin isoform a precursor, inhibitor Kunitz type proteinase, unnamed protein product (NCBI Locus/Accession No. CAA28659), and Ig J-chain; and
- (b) separating peptides that bind to the capture polypeptide from the mixture;
- (c) determining characteristics of the separated peptides; and

- (d) preparing a set of peptides with the characteristics of the separated peptides.
31. A method for treating or preventing an unwanted immune response in a subject, comprising:
- (a) providing a DSP composition;
 - (b) administering the DSP composition to a test subject;
 - (c) removing a biological sample from the test subject;
 - (d) contacting the biological sample with at least one capture polypeptide comprising a peptide sequence selected from complement component C3, apolipoprotein A-1 preproprotein, apolipoprotein A-II preproprotein (apolipoprotein D), complement component C4A, trypsin inhibitor, inter-alpha-trypsin inhibitor family heavy chain-related protein (IHRP), alpha-1-B-glycoprotein, alpha-1-antitrypsin, apolipoprotein A-IV, ceruloplasmin, unnamed protein product (NCBI Locus/Accession No. CAA34971), apolipoprotein E, complement factor B, prealbumin, apolipoprotein C-III, alpha2-HS glycoprotein, apolipoprotein J precursor, Chain C, Immunoglobulin M, immunoglobulin lambda light chain, Coagulation factor II (thrombin), Ig kappa chain V-III (KAU cold agglutinin), apolipoprotein J precursor, Ig A1 Bur, histidine-rich glycoprotein precursor, Alpha-2-HS-glycoprotein, gelsolin isoform a precursor, inhibitor Kunitz type proteinase, unnamed protein product (NCBI Locus/Accession No. CAA28659), and Ig J-chain;
 - (e) separating peptides that bind to the capture polypeptide from the mixture;
 - (f) determining characteristics of the separated peptides;
 - (g) preparing a set of peptides with the characteristics of the separated peptides, and
 - (h) administering the new set of peptides to a subject.
32. The method of claim 28 or 31, wherein the peptides are administered to the subject more than once.
33. The method of claim 32, wherein the peptides are administered to the subject at intervals of 1, 2, 3, 4, 6, 12, 18, 24, 36, 48, or 72 hours.

34. A method for comparing different preparations of DSP composition, comprising:
- (a) contacting a first DSP composition with at least one capture polypeptide contained in normal human sera, normal non-human primate sera, normal rabbit sera, normal mouse sera, normal rat sera, normal ferret sera, normal pig sera, normal dog sera, normal horse sera, normal sheep sera, normal cow sera; and
 - (b) contacting a second DSP composition with at least one capture polypeptide comprising a peptide selected from: normal human sera, normal non-human primate sera, normal rabbit sera, normal mouse sera, normal rat sera, normal ferret sera, normal pig sera, normal dog sera, normal horse sera, normal sheep sera, normal cow sera; and
 - (c) repeating step (b) as necessary; and
 - (d) separating peptides that bind to the capture polypeptide from the mixtures from steps (a-c);
 - (e) determining characteristics of the separated peptides from step (d); and
 - (f) comparing said separated set of peptides with the characteristics of the separated peptides from step (d).
35. A method for preparing a therapeutic agent to a target tissue in a subject, comprising:
- (a) providing a DSP composition; and
 - (b) coupling a therapeutic agent to the DSP composition to form a conjugate.
36. A method for delivering a therapeutic agent to a specific tissue in a subject, comprising:
- (a) isolating a peptide tag by:
 - (i) contacting a DSP composition with a tissue-specific peptide comprising a peptide selected from complement component C3, apolipoprotein A-1 preproprotein, apolipoprotein A-II preproprotein (apolipoprotein D), complement component C4A, trypsin inhibitor, inter-alpha-trypsin inhibitor family heavy chain-related protein (IHRP), alpha-1-B-glycoprotein, alpha-1-antitrypsin, apolipoprotein A-IV, ceruloplasmin, unnamed protein product (NCBI

Locus/Accession No. CAA34971), apolipoprotein E, complement factor B, prealbumin, apolipoprotein C-III, alpha2-HS glycoprotein, apolipoprotein J precursor, Chain C, Immunoglobulin M, immunoglobulin lambda light chain, Coagulation factor II (thrombin), Ig kappa chain V-III (KAU cold agglutinin), apolipoprotein J precursor, Ig A1 Bur, histidine-rich glycoprotein precursor, Alpha-2-HS-glycoprotein, gelsolin isoform a precursor, inhibitor Kunitz type proteinase, unnamed protein product (NCBI Locus/Accession No. CAA28659), and Ig J-chain; and

- (ii) separating peptides that bind to the tissue-specific peptide from the mixture;
 - (b) coupling the peptide tag to a therapeutic agent; and
 - (c) administering the conjugate to a subject.
37. The method of claim 35 or 36, wherein the therapeutic agent is a small organic molecule or a biological macromolecule.
38. The method of claims 35 or 36, wherein the tissue is brain, lung, or liver tissue.
39. The method of claims 35 or 36, wherein the peptide tag is coupled to the therapeutic agent by a covalent bond, inclusion complexes, ionic bonds, or hydrogen bonds.