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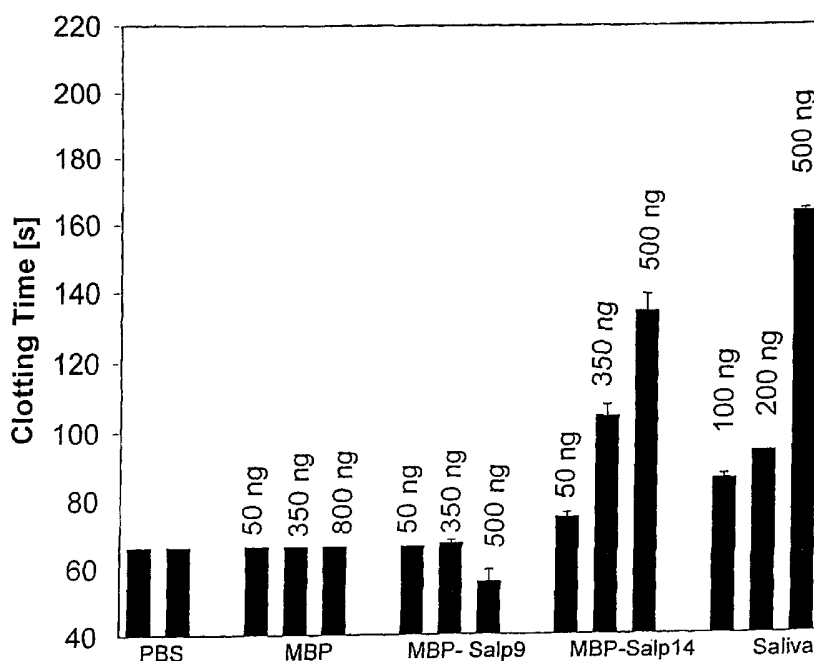
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[Continued on next page]

(54) Title: TICK POLYPEPTIDES AS ANTICOAGULANTS AND VACCINES



(57) Abstract: Polypeptides comprising tick polypeptides, portions and fragments thereof, including fusions, multimeric and chimeric proteins; nucleic acid molecules encoding them and antibodies directed against them. The various compositions are useful as anticoagulants, in compositions and methods for inducing tick immunity and preventing or reducing the transmission of tick-borne pathogens, and in diagnostic kits and methods.



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Tick Polypeptides as Anticoagulants and Vaccines

Background of the Invention

[0001] Ticks are vectors of pathogens that cause medically important diseases in humans and animals. *Ixodes scapularis* can transmit *Borrelia burgdorferi*, the causative agent of Lyme disease (Burgdorfer et al., "The western black-legged tick, *Ixodes pacificus*: a vector of *Borrelia burgdorferi*," Am. J. Trop. Med. Hyg. 34: 925-30 (1985)), *Anaplasma phagocytophila*, the etiologic agent of human granulocytic ehrlichiosis (Telford et al., "Perpetuation of the agent of human granulocytic ehrlichiosis in a deer tick-rodent cycle," Proc. Natl. Acad. Sci. USA 93: 6209-14 (1996)), *Babesia microti* (Piesman et al., "Concurrent *Borrelia burgdorferi* and *Babesia microti* infection in nymphal *Ixodes dammini*", J. Clin. Microbiol. 24: 446-47 (1986)) and flaviviruses within the tick-borne encephalitis virus complex (Burgdorfer, "Tick-borne diseases in the United States: Rocky Mountain spotted fever and Colorado tick fever. A review," Acta Trop. 34: 103-26 (1977)).

[0002] Ticks are pool feeders that tear their way into the dermis of their mammalian hosts in order to obtain a blood meal. In contrast to other shorter feeding hematophagous arthropods, ixodid ticks remain attached to the host for 3-10 days (Nuttall, "Displaced tick-parasite interactions at the host interface," Parasitology 116: S65-72 (1998)). Therefore, to successfully feed to repletion, tick saliva contains factors that circumvent host inflammatory, hemostatic and immune responses.

[0003] Several immunomodulatory activities have been identified in tick saliva. The inhibitory effect of tick salivary extracts on neutrophil function, NK cells and interferon has been reported (Ribeiro et al., "Saliva of the tick *Ixodes dammini* inhibits neutrophil function," Exp. Parasitol. 70: 382-88 (1990); Kopecky et al., "Salivary gland extract from *Ixodes ricinus* ticks inhibits production of interferon-gamma by the upregulation of interleukin-10," Parasite Immunol. 21: 351-56 (1999); and Hajnicka et al.,

"Inhibition of the antiviral action of interferon by tick salivary gland extract," Parasite Immunol. 22: 201-06 (2000)). Tick saliva and salivary gland extracts have been shown to inhibit the activation of the alternative pathway of complement fixation and a protein containing this activity has been purified and characterized (Ribeiro 1987a, "*Ixodes dammini*: salivary anti-complement activity," Exp. Parasitol. 64: 347-53 (1987); Valenzuela et al., "Purification, cloning, and expression of a novel salivary anticomplement protein from the tick, *Ixodes scapularis*," J. Biol. Chem. 275: 18717-23 (2000)). A protein from *I. scapularis* saliva has also been shown to suppress CD4⁺ T cell activation (Anguita et al. 2002). We also identified an *I. scapularis* polypeptide with anticoagulant activity (United States patent application 09/728,914).

[0004] Blood coagulation is involved in both hemostasis (i.e. prevention of blood loss from a damaged vessel) and in pathological conditions such as thrombosis (i.e. the formation of a blood clot in a blood vessel). Coagulation is initiated in injured tissues and propagated by the coagulation cascade, an interlocking network of enzymatic activation, propagation, and control events. These complex reactions ensure that blood coagulation happens quickly and yet remains localized. Blood coagulation results in the formation of a fibrin clot which controls bleeding and facilitates subsequent tissue repair. The fibrin clot is lysed after several days and replaced with a more permanent scaffolding of connective tissue matrix molecules. Abnormalities that delay clot formation or result in premature lysis of clots are associated with increased bleeding.

[0005] Coagulation and fibrinolysis involve many blood plasma proteins (see, for example, tollefsen.wustl.edu/projects/coagulation/coagulation.html). The proteins are structurally and functionally related and fall into several families of proteins. For example, the coagulation proteins Factors II, VII, IX, X, XI, XII, protein C and tissue plasminogen activator (TPA) are members of the serine protease family of proteins which also includes, e.g., trypsin, chymotrypsin, and elastase.

[0006] Blood coagulation can be initiated by exposure of blood to tissue factor, which is displayed on the surface of damaged cells (the "extrinsic system") or by activation of contact factors of plasma (the "intrinsic system"). Both coagulation systems lead to conversion of Factor X to active Factor Xa, which catalyzes the conversion of prothrombin to thrombin (Factor IIa). Two major coagulation tests mentioned above differentiate these pathways. In the prothrombin time (PT) test, tissue factor is added to plasma so that activation proceeds by the extrinsic pathway. In the partial thromoplastin time (PTT) test, blood plasma is activated by the intrinsic pathway.

[0007] Studies on various arthropod-borne pathogens confirm that the saliva of arthropod vectors contains biologically active molecules that enhance the infectivity of the pathogens being transmitted.

Therefore, components of the arthropod saliva serve as novel vaccine for blocking transmission of arthropod-borne pathogens. See, e.g., United States patent application 09/728,914, filed December 1, 2000, the disclosure of which is incorporated by reference herein in its entirety.

Brief Summary of the Invention

[0008] The present invention provides a newly identified family of polypeptides from the saliva of *I. scapularis*. This family includes polypeptides that have anticoagulant activity as well as polypeptides that have antigenic activity for detecting and inducing tick immunity. More specifically, the invention provides tick polypeptides, portions of the polypeptides with anticoagulant activity and immunogenic fragments of the polypeptides; nucleic acid sequences encoding the polypeptides, portions and fragments; and antibodies (or antigen-binding portions thereof) specific for the polypeptides, portions and fragments. The invention further provides compositions and methods comprising the polypeptides, nucleic acid sequence and antibodies.

[0009] In one aspect, the invention provides *Ixodes scapularis* polypeptides and polypeptides having at least 75% sequence identity to them. In some embodiments of the invention, the *I. scapularis* polypeptides are full-length polypeptides, while in other embodiments the polypeptides are portions of the full-length polypeptides or immunogenic fragments of the full-length polypeptides. In some embodiments, the polypeptides and portions of the invention have anticoagulant activity. In some embodiments, the polypeptides, portions and immunogenic fragments are antigenic.

[0010] Another aspect of the invention relates to nucleic acid molecules that encode the polypeptides, portions and fragments of the invention. The invention further provides host cells comprising these nucleic acid molecules and methods for producing the polypeptides, portions and fragments of the invention.

[0011] Also within the scope of the invention are compositions comprising the polypeptides, portions and immunogenic fragments. These compositions include pharmaceutical compositions and vaccines.

[0012] In still another aspect, the invention provides therapeutic methods using the polypeptides, portions and fragments of the invention. These include methods for conferring tick immunity or preventing tick-borne disease as well as methods of treating patient where anticoagulant therapy is indicated.

[0013] In yet another aspect, the invention provides diagnostic methods using a polypeptide, portion or fragment or an antibody of antigen-binding portion thereof. These methods are useful for the

detection of tick bite and tick immunity, including tick immunity induced according to a method of the invention or induced using a composition of the invention.

[0014] The invention also provides antibodies specific for polypeptides of the invention, and antigen-binding portions thereof, which optionally may be formulated as a vaccine, as well as therapeutic methods of using the antibodies and compositions. The invention also provides methods of using the antibodies and antigen-binding portions thereof to purify the polypeptides of the invention.

Brief Description of the Drawings

[0015] Figure 1A shows host immunity against ticks in guinea pigs. Fifty *I. scapularis* nymphs were placed on naive (control), tick-immune and animals that were passively immunized with tick-immune sera. There were at least 3 animals in each group. The duration of tick attachment was recorded from experimental and control guinea pigs. An (*) mark on each data point denotes a statistically significant difference at least at the level of $P < 0.05$ (Student's t-test).

[0016] Figure 1B shows host immunity against ticks in rabbits. Fifty *I. scapularis* nymphs were placed on naive (control), tick-immune and animals that were passively immunized with tick-immune sera. There were at least 3 animals in each group. The duration of tick attachment was recorded from experimental and control rabbits. An (*) mark on each data point denotes a statistically significant difference at least at the level of $P < 0.05$ (Student's t-test).

[0017] Figure 1C shows host immunity against ticks in guinea pigs. Fifty *I. scapularis* nymphs were placed on naive (control), tick-immune and animals that were passively immunized with tick-immune sera. There were at least 3 animals in each group and the weight of recovered ticks was recorded from experimental and control guinea pigs. An (*) mark on each data point denotes a statistically significant difference at least at the level of $P < 0.05$ (Student's t-test). The severity of erythema (C and D) was measured on a scale from 0 to 3, where 0 represents a lack of erythema and 1, 2, and 3 represent mild, moderate and severe erythema.

[0018] Figure 1D shows host immunity against ticks in rabbits. Fifty *I. scapularis* nymphs were placed on naive (control), tick-immune and animals that were passively immunized with tick-immune sera. There were at least 3 animals in each group and the weight of recovered ticks was recorded from experimental and control rabbits. An (*) mark on each data point denotes a statistically significant difference at least at the level of $P < 0.05$ (Student's t-test). The severity of erythema was measured on a scale from 0 to 3, where 0 represents a lack of erythema and 1, 2, and 3 represent mild, moderate and severe erythema.

[0019] Figure 2 shows the fractionations of tick saliva. Peak 19 is indicated with an (*) in the bottom panel.

[0020] Figure 3 shows the APTT analysis of HPLC peaks. Fraction 19 contained the major anticoagulant activity, delaying coagulation of human plasma by 45 s over the background (tube 0).

[0021] Figure 4 shows matrix assisted laser desorption spectroscopy (MALDI-MS) of fraction 19.

[0022] Figure 5 shows a comparison of the primary structures of Salp9A (SEQ ID NO: _), Salp14 (SEQ ID NO: _) and amino terminal sequence obtained from polypeptides in HPLC fractions 17 (SEQ ID NO: _) and 19 (SEQ ID NO: _).

[0023] Figure 6 shows a comparison between Salp9A, Salp14 and the factor Xa inhibitors from *O. moubata* (TAP) and *O. savignyi* (fXaI).

[0024] Figure 7 shows temporal and tissue specific expression of Salp9A and Salp14 examined by RT-PCR. Lane 1 shows unengorged salivary glands, lane 2 shows unengorged guts, lane 3 shows engorged salivary glands and lane 4 engorged guts.

[0025] Figure 8 is a graph of the number of ticks attached to control guinea pigs and guinea pigs immunized with polypeptides Salp25C and Salp14B (described in United States patent application 09/728,914) or by tick bite over time (hours).

[0026] Figure 9 is a graph of engorged tick weights and percent survival of attached ticks on control guinea pigs and guinea pigs immunized with Salp25C and Salp14B or by tick bite.

[0027] Figure 10 shows an SDS-PAGE gel of recombinant MBP-Salp14 and MBP-Salp9A.

[0028] Figure 11 shows an APTT assay using recombinant MBP-Salp14 and MBP-Salp9A. Recombinant Salp14 prolonged coagulation of human plasma in a concentration dependent manner. The anticoagulant activity of tick saliva was evident at as low as 100 ng/ μ l total protein.

[0029] Figure 12 shows inhibition of factor Xa by Salp14. Factor Xa mediated cleavage of chromogenic substrate (1 nM enzyme, 300 μ M substrate) was measured in the presence of increasing concentrations of recombinant MBP-Salp14 (10-300 nM) or adult tick saliva (0.1-0.5 μ g).

[0030] Figure 13 shows a western blot analysis of tick saliva. In A and B, MBP fusion tag (lane 1), recombinant Salp9A (lane 2), recombinant Salp14 (lane 3), one microgram of adult tick saliva (lane 4) were electrophoresed on a 12% SDS-PAGE gel and proteins transferred to duplicate nitrocellulose membranes. The membranes were probed with (A) naive guinea pig sera or (B) tick immune guinea pig sera. Part C is a western blot of adult tick saliva (lane 1) and recombinant MBP-Salp14 (lane 2) probed with anti-recombinant Salp14 antisera.

[0031] Figure 14 shows absorption of tick salivary anticoagulant activity with antibodies. The lanes are: (1) human plasma incubated with PBS; (2) tick saliva incubated with PBS; (3) tick saliva incubated with antisera to MBP; (4) naive guinea pig sera; (5) tick immune guinea pig sera; (6) anti-recombinant MBP-Salp14 antisera.

[0032] Figure 15 shows the position of primers used to generate portions of Salp9A and Salp14 polypeptides as described in Example 16.

[0033] Figure 16 shows the post-feeding weight of ticks with reduced expression of the Salp14 family.

[0034] Figure 17 shows the reduced anticoagulant activity in saliva in ticks with reduced expression of the Salp14 family.

Detailed Description of the Invention

[0035] This invention relates to tick polypeptides useful as antigens and as anticoagulants, nucleic acid sequences encoding the polypeptides, antibodies directed against those polypeptides, and compositions comprising the polypeptides, nucleic acids or antibodies. This invention further relates to methods for conferring and detecting tick immunity, for preventing or lessening the transmission of tick-borne pathogens, and for treating a condition where anticoagulant therapy is indicated.

[0036] In one aspect, this invention provides twelve *I. scapularis* polypeptides and compositions and methods comprising the polypeptides. More specifically, this invention provides Salp9A (SEQ ID NO: _); Salp14 (SEQ ID NO: _); H1 protein (SEQ ID NO: _); H2 protein (SEQ ID NO: _); H3 protein (SEQ ID NO: _); H7 protein (SEQ ID NO: _); L1 protein (SEQ ID NO: _); L2 protein (SEQ ID NO: _); L3 protein (SEQ ID NO: _); L4 protein (SEQ ID NO: _); L8 protein (SEQ ID NO: _); M1 protein (SEQ ID NO: _); 128-HOMO protein (SEQ ID NO: _); 13-HOMO protein (SEQ ID NO: _); 14-HOMO protein (SEQ ID NO: _); 154-HOMO protein (SEQ ID NO: _); 155-HOMO protein (SEQ ID NO: _); 156-HOMO protein (SEQ ID NO: _); 159-HOMO protein (SEQ ID NO: _); 168-HOMO protein (SEQ ID NO: _); 188-HOMO protein (SEQ ID NO: _); 190-HOMO protein (SEQ ID NO: _); 191-HOMO protein (SEQ ID NO: _); 199-HOMO protein (SEQ ID NO: _); 19-HOMO protein (SEQ ID NO: _); 203-HOMO protein (SEQ ID NO: _); 209-HOMO protein (SEQ ID NO: _); 210-HOMO protein (SEQ ID NO: _); 214-HOMO protein (SEQ ID NO: _); 21-HOMO protein (SEQ ID NO: _); 220-HOMO protein (SEQ ID NO: _); 22-HOMO protein (SEQ ID NO: _); 24-HOMO protein (SEQ ID NO: _); 32-HOMO protein (SEQ ID NO: _); 35-HOMO protein (SEQ ID NO: _); 36-HOMO protein (SEQ ID NO: _); 3-HOMO protein (SEQ ID NO: _); 45-HOMO protein (SEQ ID NO: _); 46-HOMO protein (SEQ ID NO: _); 55-HOMO protein (SEQ ID NO: _); 67-HOMO protein (SEQ ID NO: _); BEKO4891 protein (SEQ ID NO: _); BEKO4892 protein (SEQ ID NO: _);

BEKO4893 protein (SEQ ID NO: _); BEKO4894 protein (SEQ ID NO: _); BEKO4895 protein (SEQ ID NO: _); BEKO6691 protein (SEQ ID NO: _); BEKO6692 protein (SEQ ID NO: _); BEKO6693 protein (SEQ ID NO: _); BEKO6694 protein (SEQ ID NO: _); BEKO48142 protein (SEQ ID NO: _); BEKO48143 protein (SEQ ID NO: _); BEKO48144 protein (SEQ ID NO: _); and BEKO48145 protein (SEQ ID NO: _). The invention also includes polypeptides that have at least 75% sequence identity to one of the aforementioned polypeptides. In other embodiments, these polypeptides have at least 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% sequence identity to one of the aforementioned polypeptides. As used herein, "sequence identity" in the context of amino acid sequences refers to the residues in the two sequences which are the same when aligned for maximum correspondence. There are a number of different algorithms known in the art which can be used to measure amino acid sequence identity. For instance, amino acid sequences can be compared using NCBI BLASTp software.

[0037] In certain embodiments, the above-described polypeptides of the invention have anticoagulant activity. The invention also includes portions of these polypeptides, wherein the portions have anticoagulant activity. In certain embodiments, the anticoagulant activity of the polypeptides and portions is mediated through inhibition of Factor Xa.

[0038] In some embodiments, the above-described polypeptides and portions of the invention are antigenic or immunogenic and accordingly may be used to generate an immune response in an animal. As used herein, "antigenic" or "immunogenic" is used to designate a polypeptide that, when administered to an animal, is capable of eliciting a corresponding antibody.

[0039] Also within the scope of the invention are fragments of the polypeptides. Fragments of the polypeptides have an amino-terminal and/or carboxy-terminal deletion, but are otherwise identical in sequence to one of the longer polypeptides of the invention. A fragment of the invention is typically about 5 to about 80 amino acids long, e.g., a fragment of the invention may be 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 40, 50, 60, 70 or 80 amino acids long. In certain embodiments, the fragments of the invention are antigenic or immunogenic.

[0040] In certain embodiments, the polypeptides, portions and fragments elicit the formation of a tick immune response or tick immunity in animals. As used herein, a "tick immune response" or "tick immunity" is manifested by one or more of the following: (1) reduction in the duration of tick attachment to a host, (2) a reduction in the weight of ticks recovered after detaching from the host compared to those values in ticks that attach to non-immune hosts, (3) failure of the ticks to complete their development and (4) failure to lay the normal number of viable eggs. In related embodiments, this invention provides compositions comprising one or more tick polypeptides, portions or fragments or one

or more antibodies directed against a polypeptide, portion or fragment of this invention. These compositions include vaccines for conferring tick immunity or treating tick-borne disease. The invention further provides methods for conferring tick immunity or treating tick-borne disease using an antigenic polypeptide, portion or fragment of the invention or a composition comprising an antigenic polypeptide, portion or fragment.

[0041] In some embodiments, the polypeptides and portions of the invention have anticoagulant activity. In certain of these embodiments, the anticoagulant activity is mediated through inhibition of Factor Xa. In related embodiments, this invention provides compositions and methods comprising one or more polypeptides or portions having anticoagulant activity. The invention also provides methods for treating a condition where anticoagulant therapy is indicated using one of these polypeptides or portions or a composition comprising one of these polypeptides or portions.

[0042] In some embodiments, the polypeptides and portions of the invention have anticomplement activity. In related embodiments, this invention provides compositions and methods comprising one or more polypeptides or portions having anticomplement activity. The invention also provides methods for treating a condition where anticomplement therapy is indicated using one of these polypeptides or portions or a composition comprising one of these polypeptides or portions.

[0043] As used herein, a substantially pure polypeptide is a polypeptide that is detectable as a single band on an immunoblot probed with polyclonal anti-serum.

[0044] The invention also provides derivatives of the above-described antigenic polypeptides, portions or fragments of the invention in addition to derivatives of the above-described polypeptides or portions having anticoagulant activity. As used herein, a "derivative" of a polypeptide, portion or fragment of the invention is a polypeptide, portion or fragment in which the native form has been modified or altered. Such modifications include, but are not limited to: amino acid modifications, additions or deletions; alterations in the pattern of lipidation, glycosylation or phosphorylation; reactions of free amino, carboxyl, or hydroxyl side groups of the amino acid residues present in the polypeptide with other organic and non-organic molecules; and other modifications, any of which may result in changes in primary, secondary or tertiary structure. Such modifications may enhance or reduce, e.g., immunogenicity, anticoagulant activity, solubility, and half-life *in vivo*.

[0045] The invention also contemplates nucleic acids, including DNA, cDNA and RNA, encoding any of the above-described polypeptides, portions or fragments of the invention. In particular, the invention includes nucleic acid molecules comprising a nucleotide sequence encoding Salp9A (SEQ ID NO: _); Salp14 (SEQ ID NO: _); H1 protein (SEQ ID NO: _); H2 protein (SEQ ID NO: _); H3 protein (SEQ ID

NO: _); H7 protein (SEQ ID NO: _); L1 protein (SEQ ID NO: _); L2 protein (SEQ ID NO: _); L3 protein (SEQ ID NO: _); L4 protein (SEQ ID NO: _); L8 protein (SEQ ID NO: _); M1 protein (SEQ ID NO: _); 128-HOMO protein (SEQ ID NO: _); 13-HOMO protein (SEQ ID NO: _); 14-HOMO protein (SEQ ID NO: _); 154-HOMO protein (SEQ ID NO: _); 155-HOMO protein (SEQ ID NO: _); 156-HOMO protein (SEQ ID NO: _); 159-HOMO protein (SEQ ID NO: _); 168-HOMO protein (SEQ ID NO: _); 188-HOMO protein (SEQ ID NO: _); 190-HOMO protein (SEQ ID NO: _); 191-HOMO protein (SEQ ID NO: _); 199-HOMO protein (SEQ ID NO: _); 19-HOMO protein (SEQ ID NO: _); 203-HOMO protein (SEQ ID NO: _); 209-HOMO protein (SEQ ID NO: _); 210-HOMO protein (SEQ ID NO: _); 214-HOMO protein (SEQ ID NO: _); 21-HOMO protein (SEQ ID NO: _); 220-HOMO protein (SEQ ID NO: _); 22-HOMO protein (SEQ ID NO: _); 24-HOMO protein (SEQ ID NO: _); 32-HOMO protein (SEQ ID NO: _); 35-HOMO protein (SEQ ID NO: _); 36-HOMO protein (SEQ ID NO: _); 3-HOMO protein (SEQ ID NO: _); 45-HOMO protein (SEQ ID NO: _); 46-HOMO protein (SEQ ID NO: _); 55-HOMO protein (SEQ ID NO: _); 67-HOMO protein (SEQ ID NO: _); BEKO4891 protein (SEQ ID NO: _); BEKO4892 protein (SEQ ID NO: _); BEKO4893 protein (SEQ ID NO: _); BEKO4894 protein (SEQ ID NO: _); BEKO4895 protein (SEQ ID NO: _); BEKO6691 protein (SEQ ID NO: _); BEKO6692 protein (SEQ ID NO: _); BEKO6693 protein (SEQ ID NO: _); BEKO6694 protein (SEQ ID NO: _); BEKO48142 protein (SEQ ID NO: _); BEKO48143 protein (SEQ ID NO: _); BEKO48144 protein (SEQ ID NO: _); or BEKO48145 protein (SEQ ID NO: _). In some embodiments, these nucleic acid sequences are those shown in Table 5. The invention also includes nucleic acid molecules encoding polypeptides that have at least 75% sequence identity to one of these polypeptides as described above. The invention includes polypeptides both with and without any leader sequence and with or without the initial methods as well as nucleic acid molecule encoding them.

[0046] In yet other embodiments, this invention provides antibodies, or antigen-binding portions thereof, that specifically bind a polypeptide, portion or fragment of this invention. In certain embodiments, these antibodies bind to a single polypeptide and related portions and fragments. In other embodiments, the antibodies bind to multiple members of the tick polypeptide family described herein. In related embodiments, the invention provides compositions comprising those antibodies.

[0047] The antibodies of this invention may be used in a variety of applications, including to detect expression of tick antigens, preferably *I. scapularis* antigens, to screen for expression of novel tick polypeptides, to purify novel tick polypeptides and to confer tick immunity. Antigen-binding portions may be produced by recombinant DNA techniques or by enzymatic or chemical cleavage of intact antibodies. Antigen-binding portions include, e.g., Fab, Fab', F(ab')₂, Fv, dAb, and complementarity

determining region (CDR) fragments, single-chain antibodies (scFv), chimeric antibodies, diabodies and polypeptides that contain at least a portion of an immunoglobulin that is sufficient to confer specific antigen binding to the polypeptide. Nucleic acid molecules encoding heavy and/or light chain or antigen-binding portions as well as human or humanized antibodies that can be made using techniques known in the art.

[0048] In still other embodiments, this invention relates to diagnostic means and methods characterized by a tick polypeptide, portion or fragment thereof; nucleic acid sequence, or an antibody or antigen-binding fragment thereof of the invention.

[0049] Another embodiment of this invention is a method for preventing or reducing the transmission of tick-borne pathogens by administering a polypeptide, antibody or composition of this invention that are effective to induce tick immunity. One such embodiment is a method for preventing or reducing the severity for some period of time of *B. burgdorferi* infection.

[0050] The antigenic polypeptides, portions and fragments disclosed herein are immunologically reactive with antisera generated by immunization with *I. scapularis* polypeptides, extracts and/or by tick bite. Accordingly, they are useful in methods and compositions to detect tick immunity.

[0051] The antigenic polypeptides, portions and fragments disclosed herein are particularly useful in single and multicomponent vaccines against tick bites and infection by tick-borne pathogens. In this regard, multicomponent vaccines are preferred because such vaccines may be formulated to more closely resemble the immunogens presented by tick bite, and because such vaccines are more likely to confer broad-spectrum protection than a vaccine comprising only a single tick polypeptide.

[0052] Multicomponent vaccines according to this invention may also contain polypeptides that characterize other vaccines useful for immunization against diseases such as, for example, Lyme disease, human monocytic ehrlichiosis, babesiosis, diphtheria, polio, hepatitis, and measles. Such multicomponent vaccines are typically incorporated into a single composition.

[0053] Compositions and methods of this invention for conferring tick immunity or treating tick-borne disease typically comprise polypeptides, portions and fragments having enhanced immunogenicity. Such polypeptides may result when the native forms of the polypeptides, portions and fragments thereof are modified or subjected to treatments to enhance their immunogenic character in the intended recipient.

[0054] Numerous techniques are available and well known to those of skill in the art which may be used, without undue experimentation, to substantially increase the immunogenicity of the polypeptides, portions and fragments herein disclosed. For example, a polypeptide, portion or fragment of this

invention may be modified by coupling to dinitrophenol groups or arsanilic acid, or by denaturation with heat and/or SDS. Particularly when the polypeptide, portion or fragment is a small, chemically synthesized polypeptide, it may be desirable to couple it to an immunogenic carrier. The coupling, of course, must not interfere with the ability of either the polypeptide, portion or fragment or the carrier to generate an immune response. For a review of some general considerations in coupling strategies, see *Antibodies, A Laboratory Manual*, Cold Spring Harbor Laboratory, ed. E. Harlow and D. Lane (1988).

[0055] Polypeptides, portions and fragments can be combined with any immunogenic carrier. Such immunogenic carriers are well known in the art. Examples of such carriers are keyhole limpet hemocyanin (KLH); albumins such as bovine serum albumin (BSA) and ovalbumin; PPD (purified protein derivative of tuberculin); red blood cells; tetanus toxoid; cholera toxoid; agarose beads; activated carbon; and bentonite.

[0056] Modification of the amino acid sequence of the polypeptides, portions and fragments disclosed herein to alter the lipidation state is also a method that may be used to increase their immunogenicity or alter their biochemical properties. For example, the polypeptides or fragments thereof may be expressed with or without the signal and other sequences that may direct addition of lipid moieties.

[0057] As will be apparent from the disclosure to follow, the polypeptides, portions or fragments also may be prepared with the objective of increasing stability or rendering the molecules more amenable to purification and preparation. One such technique is to express them as fusion proteins comprising other sequences.

[0058] A derivative of a polypeptide, portion or fragment of the invention may be prepared by a variety of methods, including by *in vitro* manipulation of the DNA encoding the native polypeptide, portion or fragment and subsequent expression of the modified DNA, by chemical synthesis of a derivatized DNA sequences, or by chemical or biological manipulation of an expressed amino acid sequence.

[0059] For example, a derivative may be produced by substitution of one or more amino acids with an amino acid derivative or non-native amino acid. Those of skill in the art will understand that conservative substitution is preferred, e.g., 3-methylhistidine may be substituted for histidine, 4-hydroxyproline may be substituted for proline, 5-hydroxylysine may be substituted for lysine, and the like.

[0060] Furthermore, one of skill in the art will recognize that individual substitutions, deletions or additions which alter a single amino acid or a small percentage of amino acids (typically less than about 5%, more typically about 1% or less) in an encoded sequence are "conservatively modified variations"

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where the alterations result in the substitution of an amino acid with a chemically similar amino acid. Polypeptides comprising conservatively modified amino acids are another embodiment of the invention. Conservative substitution tables providing functionally similar amino acids are well known in the art. See, e.g., Creighton (1984) *Proteins* W.H. Freeman and Company. Conservative substitutions typically include the substitution of one amino acid for another with similar characteristics such as substitutions within the following groups: valine, glycine; glycine, alanine; valine, isoleucine; aspartic acid, glutamic acid; asparagine, glutamine; serine, threonine; lysine, arginine; and phenylalanine, tyrosine. The non-polar (hydrophobic) amino acids include alanine, leucine, isoleucine, valine, proline, phenylalanine, tryptophan and methionine. The polar neutral amino acids include glycine, serine, threonine, cysteine, tyrosine, asparagine and glutamine. The positively charged (basic) amino acids include arginine, lysine and histidine. The negatively charged (acidic) amino acids include aspartic acid and glutamic acid. Other conservative substitutions are described, e.g., by Dayhoff in the *Atlas of Protein Sequence and Structure* (1988).

[0061] Causing amino acid substitutions which are not conservative may also result in desired derivatives, e.g., by causing changes in charge, conformation and other biological properties. Such substitutions would include for example, substitution of a hydrophilic residue for a hydrophobic residue, substitution of a cysteine or proline for another residue, substitution of a residue having a small side chain for a residue having a bulky side chain or substitution of a residue having a net positive charge for a residue having a net negative charge.

[0062] When the result of a given substitution cannot be predicted with certainty, the derivatives may be readily assayed according to the methods disclosed herein to determine the presence or absence of the desired characteristics. For example, the immunogenicity, immunodominance and/or protectiveness of a derivative of this invention can be readily determined using methods disclosed, e.g., in Examples 3, 4, 6, and 7. Similarly, e.g., Examples 2, 10, and 15 disclose methods for determining whether a derivative of this invention has anticoagulant activity.

[0063] In some embodiments of this invention, the polypeptides, portions or fragments disclosed herein are prepared as portion of a larger fusion or chimeric protein. For example, a polypeptide of this invention may be fused at its N-terminus or C-terminus to a different immunogenic polypeptide, which may be an *I. scapularis* or a non-*I. scapularis* polypeptide, or to combinations thereof, to produce fusion proteins.

[0064] In certain embodiments of this invention, fusion proteins are constructed that comprise one or more of the polypeptides, portions or fragments of the invention fused to one or more other

polypeptide(s) from *I. scapularis*, which may be from the same or different isolate of *I. scapularis*. Such fusion proteins are particularly effective in the induction of tick immunity against a wide spectrum of isolates.

[0065] In another embodiment of this invention, the polypeptides, portions or fragments are fused to moieties, such as immunoglobulin domains, that may increase the stability and prolong the *in vivo* plasma half-life of the polypeptide. Such fusions may be prepared without undue experimentation according to methods well known to those of skill in the art, for example, in accordance with the teachings of United States patent 4,946,778, or United States patent 5,116,964. The exact site of the fusion is not critical as long as the polypeptide, portion or fragment retains the desired biological activity. Such determinations may be made according to the teachings herein or by other methods known to those of skill in the art.

[0066] The fusion proteins comprising a polypeptide, portion or fragment of the invention are conveniently produced at the DNA level, e.g., by constructing a nucleic acid molecule encoding the fusion protein, transforming host cells with the molecule, inducing the cells to express the fusion protein, and recovering the fusion protein from the cell culture. Alternatively, the fusion proteins may be produced after gene expression according to known methods.

[0067] The polypeptides, portions and fragments of the invention also may be portion of larger multimeric molecules that may be produced recombinantly or may be synthesized chemically. Such multimers also may include the polypeptides, portions and fragments fused or coupled to moieties other than amino acids, including lipids and carbohydrates.

[0068] Preferably, the multimeric proteins will consist of multiple T- or B-cell epitopes or combinations thereof repeated within the same molecule, either randomly, or with spacers (amino acid or otherwise) between them.

[0069] In a preferred embodiment of this invention, one or more antigenic polypeptides, portions and fragments of the invention are incorporated into a vaccine. As described in Example 3 and 4, the duration time of attachment and feeding of ticks exposed to animals that are immunized with a tick antigen of the invention is reduced. Without being confined to any particular mechanism of action for such a vaccine, it is believed that antibodies generated in an immunized host against tick polypeptides can form complexes that alone or in association with immune cells or serum factors, disturb or block the feeding of ticks. Alternatively or additionally, such antibodies generated in the immunized host cause an irritation at or near the tick attachment site, such irritation resulting in a reduction in the duration of tick attachment or feeding.

[0070] In some embodiments of this invention, a polypeptide, portion or fragment of this invention is incorporated into a single-component vaccine. In other embodiments, a polypeptide, portion or fragment of this invention is incorporated into a multicomponent vaccine comprising other protective tick polypeptides. In addition, a multicomponent vaccine may also contain protective polypeptides useful for immunization against other diseases such as, for example, Lyme disease, human granulocytic ehrlichiosis, babesiosis, diphtheria, polio, hepatitis, and measles. Such a vaccine, by virtue of its ability to elicit antibodies to a variety of protective polypeptides, will be more effective to protect against tick-borne diseases.

[0071] The multicomponent vaccine may contain a polypeptide, portion or fragment of this invention as portion of a multimeric molecule in which the various components are covalently associated. Alternatively, it may contain multiple individual components. For example, a multicomponent vaccine may be prepared comprising two or more of the polypeptides, portions or fragments of this invention, wherein each is independently purified and then combined prior to or during formulation.

[0072] Alternatively, a multicomponent vaccine may be prepared from heterodimers or tetramers wherein the polypeptide, portion or fragment of this invention has been fused to immunoglobulin chains or portions thereof. Such a vaccine could comprise, for example, an H1 polypeptide fused to an immunoglobulin heavy chain and an H2 polypeptide, fused to an immunoglobulin light chain, and could be produced by transforming a host cell with DNA encoding the heavy-chain fusion and DNA encoding the light-chain fusion. One of skill in the art will understand that the host cell selected should be capable of assembling the two chains appropriately. Alternatively, the heavy- and light-chain fusions could be produced from separate cell lines and allowed to associate after purification.

[0073] The desirability of including a particular component and the relative proportions of each component may be determined by using the assay systems disclosed herein, or by using other systems known to those in the art. Most preferably, the multicomponent vaccine will comprise numerous T-cell and B-cell epitopes of the polypeptides, portions or fragments of this invention.

[0074] This invention also contemplates that a polypeptide, portion or fragment of this invention, either alone or combined, may be administered to an animal via a liposome delivery system in order to enhance their stability and/or immunogenicity. Delivery of a polypeptide, portion or fragment of this invention via liposomes may be particularly advantageous because the liposome may be internalized by phagocytic cells in the treated animal. Such cells, upon ingesting the liposome, would digest the liposomal membrane and subsequently present the polypeptide to the immune system in conjunction with other molecules required to elicit a strong immune response.

[0075] The liposome system may be any variety of unilamellar vesicles, multilamellar vesicles, or stable plurilamellar vesicles, and may be prepared and administered according to methods well known to those of skill in the art, for example in accordance with the teachings of United States patents 4,762,915, 5,000,958, 5,169,637 or 5,185,154. In addition, it may be desirable to produce or express a polypeptide, portion or fragment of this invention, as well as other selected polypeptides, as lipoproteins, in order to enhance their binding to liposomes.

[0076] Any of the polypeptides, portions or fragments of this invention may be used in the form of a pharmaceutically acceptable salt. Suitable acids and bases which are capable of forming salts with the polypeptides, portions and fragments of the present invention are well known to those of skill in the art, and include inorganic and organic acids and bases.

[0077] According to this invention, we describe a method which comprises the steps of treating an animal with a therapeutically effective amount of a polypeptide, portion or fragment of this invention or a fusion protein or a multimeric protein comprising a polypeptide, portion or fragment of this invention, in a manner sufficient to confer tick immunity or prevent or lessen the severity, for some period of time, of infection by a tick-borne pathogen. The polypeptides that are preferred for use in such methods are those that contain protective epitopes. Such protective epitopes may be B-cell epitopes, T-cell epitopes, or combinations thereof.

[0078] According to another embodiment of this invention, we describe a method which comprises the steps of treating an animal with a multicomponent vaccine comprising a therapeutically effective amount of a polypeptide, portion or fragment of this invention, or a fusion protein or multimeric protein comprising such a polypeptide, portion or fragment, in a manner sufficient to confer tick immunity or prevent or lessen the severity, for some period of time, of infection by a tick-borne pathogen. Again, the polypeptides, portions and fragments of this invention, as well as fusion proteins and multimeric proteins generally used in such methods are those that contain protective epitopes, which may be B-cell epitopes, T-cell epitopes, or combinations thereof.

[0079] Typically, the polypeptides, portions or fragments as well as fusion proteins and multimeric proteins for use in compositions and methods for eliciting tick immunity or treating tick-borne disease are those containing both strong T-cell and B-cell epitopes. Without being bound by theory, we believe that this is the best way to stimulate high-titer antibodies that are effective to confer tick immunity. Such preferred polypeptides will be internalized by B cells expressing surface immunoglobulin that recognizes the B-cell epitope(s). The B cells will then process the antigen and present it to T cells. The T cells will recognize the T-cell epitope(s) and respond by proliferating and producing lymphokines

which in turn cause B cells to differentiate into antibody-producing plasma cells. Thus, in this system, a closed autocatalytic circuit exists which will result in the amplification of both B- and T-cell responses, leading ultimately to production of a strong immune response which includes high-titer antibodies against the polypeptide, portion or fragment of this invention.

[0080] One of skill in the art will also understand that it may be advantageous to administer a polypeptide, portion or fragment of this invention in a form that will favor the production of T-helper cells type 1 (TH1), which help activate macrophages, and/or T-helper cells type 2 (TH2), which help B cells to generate antibody responses. Aside from administering epitopes which are strong T-cell or B-cell epitopes, the induction of TH1 or TH2 cells may also be favored by the mode of administration of the polypeptide. For example, a polypeptide, portion or fragment of this invention may be administered in certain doses or with particular adjuvants and immunomodulators, for example with interferon-gamma or interleukin-12 (TH1 response) or interleukin-4 or interleukin-10 (TH2 response).

[0081] B-cell epitopes can be readily identified using routine techniques. For example, overlapping polypeptides, portions or fragments of this invention are constructed. Amino acid sequences of the polypeptides, portions or fragments of this invention that contain B-cell epitopes may be identified in a variety of ways for example by their ability to (1) remove protective antibodies from polyclonal antiserum directed against the polypeptide or (2) elicit an immune response which is effective to confer tick immunity.

[0082] Alternatively, a polypeptide, portion or fragment of this invention may be used to produce monoclonal antibodies that are screened for their ability to confer tick immunity when used to immunize naive animals. Once a given monoclonal antibody is found to confer protection, the particular epitope that is recognized by that antibody may then be identified.

[0083] As recognition of T-cell epitopes is MHC-restricted, the polypeptides that contain T-cell epitopes may be identified *in vitro* by testing them for their ability to stimulate proliferation and/or cytokine production by T-cell clones generated from humans of various HLA types, from the lymph nodes, spleens, or peripheral blood lymphocytes of C3H or other laboratory mice, or from domestic animals. Compositions comprising multiple T-cell epitopes recognized by individuals with different Class II antigens are useful for prevention and treatment of human granulocytic ehrlichiosis in a broad spectrum of patients.

[0084] In certain embodiments of the present invention, a polypeptide, portion or fragment of this invention containing a B-cell epitope is fused to one or more other immunogenic *I. scapularis* polypeptides containing strong T-cell epitopes. The fusion protein that carries both strong T-cell and B-

cell epitopes is able to participate in elicitation of a high-titer antibody response effective to confer tick immunity.

[0085] Strong T-cell epitopes may also be provided by non-*I. scapularis* molecules. For example, strong T-cell epitopes have been observed in hepatitis B virus core antigen (HBcAg). Furthermore, it has been shown that linkage of one of these segments to segments of the surface antigen of Hepatitis B virus, which are poorly recognized by T cells, results in a major amplification of the anti-HBV surface antigen response. See, e.g., D.R. Milich et al., "Antibody Production to the Nucleocapsid and Envelope of the Hepatitis B Virus Primed by a Single Synthetic T Cell Site", Nature 329: 547-49 (1987).

[0086] Therefore, in yet another embodiment, B-cell epitopes of the polypeptides, portions or fragments of this invention are fused to segments of HBcAg or to other antigens which contain strong T-cell epitopes, to produce a fusion protein that can elicit a high-titer antibody response against the antigenic polypeptide, portion or fragment of this invention. In addition, it may be particularly advantageous to link a polypeptide, portion or fragment of this invention to a strong immunogen that is also widely recognized, for example tetanus toxoid.

[0087] It will be readily appreciated by one of ordinary skill in the art that the polypeptides, portions and fragments of this invention, as well as fusion proteins and multimeric proteins containing them, may be prepared by recombinant means, chemical means, or combinations thereof. For example, the polypeptides, portions and fragments of this invention may be generated by recombinant means using a DNA sequence encoding the corresponding polypeptide, portion or fragment. DNA encoding serotypic variants of the polypeptides may likewise be cloned, e.g., using PCR and oligonucleotide primers derived from the sequence herein disclosed. In this regard, it may be particularly desirable to isolate additional nucleic acid molecules encoding *I. scapularis* polypeptides from the family disclosed herein including polypeptides that differ antigenically.

[0088] Oligonucleotide primers and other nucleic acid probes derived from the nucleic acid molecules encoding the polypeptides of this invention also may be used to isolate and clone other related proteins from *I. scapularis* and related ticks which may contain regions of DNA sequence homologous to the DNA sequences of this invention.

[0089] If the tick polypeptides, portions and fragments of this invention are produced recombinantly, they may be expressed in unicellular hosts. As is well known to one of skill in the art, in order to obtain high expression levels of foreign DNA sequences in a host, the sequences are generally operatively linked to transcriptional and translational expression control sequences that are functional in the chosen

host. Preferably, the expression control sequences, and the DNA sequence of interest, will be contained in an expression vector that further comprises a selectable marker.

[0090] The nucleotide sequences encoding the polypeptides, portions and fragments of this invention may or may not encode a signal sequence. If the expression host is eukaryotic, it generally is preferred that a signal sequence be encoded so that the mature polypeptide is secreted from the eukaryotic host.

[0091] An amino-terminal methionine may or may not be present on the expressed polypeptides, portions and fragments of this invention. If the terminal methionine is not cleaved by the expression host, it may, if desired, be chemically removed by standard techniques.

[0092] A wide variety of expression host/vector combinations may be employed in expressing a DNA sequence encoding an amino acid sequence comprising a polypeptide, portion or fragment of this invention, including fusion proteins, multimeric proteins, and chimeric proteins comprising them. Useful expression vectors for eukaryotic hosts, include, for example, vectors comprising expression control sequences from SV40, bovine papilloma virus, adenovirus, adeno-associated virus, cytomegalovirus and retroviruses including lentiviruses. Useful expression vectors for bacterial hosts include bacterial plasmids, such as those from *E. coli*, including pBluescript®, pGEX-2T, pUC vectors, colE1, pCR1, pBR322, pMB9 and their derivatives, pET-15, broad-host-range plasmids, such as RP4, phage DNAs, e.g., the numerous derivatives of phage lambda, e.g. λGT10 and λGT11, and other phages. Useful expression vectors for yeast cells include the 2μ plasmid and derivatives thereof. Useful vectors for insect cells include pVL941.

[0093] In addition, any of a wide variety of expression control sequences— sequences that control the expression of a DNA sequence when operatively linked to it — may be used in these vectors to express the DNA sequences of this invention. Such useful expression control sequences include the expression control sequences associated with structural genes of the foregoing expression vectors. Examples of useful expression control sequences include, for example, the early and late promoters of SV40 or adenovirus, the *lac* system, the *trp* system, the TAC or TRC system, the T3 and T7 promoters, the major operator and promoter regions of phage lambda, the control regions of fd coat protein, the promoter for 3-phosphoglycerate kinase or other glycolytic enzymes, the promoters of acid phosphatase, e.g., Pho5, the promoters of the yeast α-mating system and other constitutive and inducible promoter sequences known to control the expression of genes of prokaryotic or eukaryotic cells or their viruses, and various combinations thereof.

[0094] In one embodiment, a DNA sequence encoding a polypeptide, portion or fragment of this invention is cloned in the expression vector lambda ZAP® II (Stratagene, La Jolla, CA), in which expression from the *lac* promoter may be induced by IPTG.

[0095] In another embodiment, DNA encoding a polypeptide, portion or fragment of this invention is inserted in-frame into an expression vector that allows high-level expression of the polypeptide or fragment as a glutathione S-transferase fusion protein. Such a fusion protein thus contains amino acids encoded by the vector sequences as well as amino acids of the polypeptide, portion or fragment of the invention.

[0096] In yet another embodiment, DNA encoding a polypeptide, portion or fragment of this invention is inserted in-frame into an expression vector that allows high-level expression of the polypeptide or fragment as thioredoxin fusion protein. Such a fusion protein thus contains amino acids encoded by the vector sequences as well as amino acids of the polypeptide, portion or fragment of the invention.

[0097] The term "host cell" refers to one or more cells into which a recombinant DNA molecule is introduced. Host cells of the invention include, but need not be limited to, bacterial, yeast, animal, insect and plant cells. Host cells can be unicellular, or can be grown in tissue culture as liquid cultures, monolayers or the like. Host cells may also be derived directly or indirectly from tissues or they may be present in an organism.

[0098] A wide variety of unicellular host cells are useful in expressing the DNA sequences of this invention. These hosts may include well known eukaryotic and prokaryotic hosts, such as strains of *E. coli*, *Pseudomonas*, *Bacillus*, *Streptomyces*, fungal cells including yeast, plant cells, insect cells such as *Spodoptera frugiperda* (SF9) or *Drosophila* cells, animal cells such as CHO and mouse cells, African green monkey cells such as COS 1, COS 7, BSC 1, BSC 40, and BMT 10, and human cells.

[0099] A host cell is "transformed" by a nucleic acid when the nucleic acid is translocated into the cell from the extracellular environment. Any method of transferring a nucleic acid into the cell may be used; the term, unless otherwise indicated herein, does not imply any particular method of delivering a nucleic acid into a cell, nor that any particular cell type is the subject of transfer.

[0100] An "expression control sequence" is a nucleic acid sequence that regulates gene expression (i.e., transcription, RNA formation and/or translation). Expression control sequences may vary depending, for example, on the chosen host cell or organism (e.g., between prokaryotic and eukaryotic hosts), the type of transcription unit (e.g., which RNA polymerase must recognize the sequences), the cell type in which the gene is normally expressed (and, in turn, the biological factors normally present in that cell type).

[0101] A "promoter" is one such expression control sequence, and, as used herein, refers to an array of nucleic acid sequences which control, regulate and/or direct transcription of downstream (3') nucleic acid sequences. As used herein, a promoter includes necessary nucleic acid sequences near the start site of transcription, such as, in the case of a polymerase II type promoter, a TATA element.

[0102] A "constitutive" promoter is a promoter which is active under most environmental and developmental conditions. An "inducible" promoter is a promoter which is inactive under at least one environmental or developmental condition and which can be switched "on" by altering that condition. A "tissue-specific" promoter is active in certain tissue types of an organism, but not in other tissue types from the same organism. Similarly, a developmentally regulated promoter is active during some but not all developmental stages of a host organism.

[0103] Expression control sequences also include distal enhancer or repressor elements which can be located as much as several thousand base pairs from the start site of transcription. They also include sequences required for RNA formation (e.g., capping, splicing, 3' end formation and polyadenylation, where appropriate); translation (e.g., ribosome binding site); and post-translational modifications (e.g., glycosylation, phosphorylation, methylation, prenylation, and the like).

[0104] The terms "operatively linked" and "operably linked" refer to functional linkage between a nucleic acid expression control sequence (such as a promoter, or array of transcription factor binding sites) and a second nucleic acid sequence, wherein the expression control sequence directs transcription of the nucleic acid corresponding to the second sequence.

[0105] The term "polypeptide" refers to any polymer consisting essentially of amino acids regardless of its size. Although "protein" is often used in reference to relatively large polypeptides, and "peptide" is often used in reference to small polypeptides, usage of these terms in the art overlaps and varies. The term "polypeptide" as used herein thus refers interchangeably to peptides, polypeptides and proteins, including portions and fragments as used elsewhere herein, unless otherwise noted.

[0106] It should of course be understood that not all vectors and expression control sequences will function equally well to express a nucleic acid sequence encoding an amino acid sequence comprising a polypeptide sequence of this invention. Neither will all hosts function equally well with the same expression system. However, one of skill in the art may make a selection among these vectors, expression control sequences and hosts without undue experimentation and without departing from the scope of this invention. For example, in selecting a vector, the host must be considered because the vector must be replicated in it. The vector's copy number, the ability to control that copy number, the

ability to control integration, if any, and the expression of any other proteins encoded by the vector, such as antibiotic or other selectable markers, should also be considered.

[0107] In selecting an expression control sequence, a variety of factors should also be considered. These include, for example, the relative strength of the promoter sequence, its controllability, and its compatibility with a nucleic acid sequence of this invention, particularly with regard to potential secondary structures. Unicellular hosts should be selected by consideration of their compatibility with the chosen vector, the toxicity of the product coded for by a nucleic acid sequence of this invention, their secretion characteristics, their ability to fold the polypeptide correctly, their fermentation or culture requirements, and the ease of purification from them of the products coded for by the DNA sequences of this invention.

[0108] Within these parameters, one of skill in the art may select various vector/expression control sequence/host combinations that will express the DNA sequences of this invention on fermentation or in other large-scale cultures.

[0109] The polypeptides encoded by the nucleic acid molecules of this invention may be isolated from the fermentation or cell culture and purified using any of a variety of conventional methods including: liquid chromatography such as normal or reversed-phase, using HPLC, FPLC and the like; affinity chromatography (such as with inorganic ligands or monoclonal antibodies); size-exclusion chromatography; immobilized metal chelate chromatography; gel electrophoresis; and the like. One of skill in the art may select the most appropriate isolation and purification techniques without departing from the scope of this invention. If the polypeptide is membrane-bound or suspected of being a lipoprotein, it may be isolated using methods known in the art for such proteins, e.g., using any of a variety of suitable detergents.

[0110] In addition, the polypeptides and fragments of the invention may be generated by any of several chemical techniques. For example, they may be prepared using the solid-phase synthetic technique originally described by R. B. Merrifield, "Solid Phase Peptide Synthesis. I. The Synthesis of a Tetrapeptide," J. Am. Chem. Soc. 83: 2149-54 (1963), or they may be prepared by synthesis in solution. A summary of peptide synthesis techniques may be found in E. Gross & H. J. Meinhofer, 4. The Peptides: Analysis, Synthesis, Biology; Modern Techniques of Peptide and Amino Acid Analysis, John Wiley & Sons, (1981) and M. Bodanszky, Principles of Peptide Synthesis, Springer-Verlag (1984).

[0111] Typically, these synthetic methods comprise the sequential addition of one or more amino acid residues to a growing peptide chain. Often peptide coupling agents are used to facilitate this reaction. For a recitation of peptide coupling agents suitable for the uses described herein see M. Bodansky,

supra. Normally, either the amino or carboxyl group of the first amino acid residue is protected by a suitable, selectively removable protecting group. A different protecting group is utilized for amino acids containing a reactive side group, e.g., lysine. A variety of protecting groups known in the field of peptide synthesis and recognized by conventional abbreviations therein, may be found in T. Greene, *Protective Groups In Organic Synthesis*, Academic Press (1981).

[0112] According to another embodiment of this invention, antibodies that specifically bind a polypeptide, portion or fragment of the invention are generated. Such antibodies are immunoglobulin molecules or portions thereof that are immunologically reactive with a polypeptide, portion or fragment of the present invention. It should be understood that the antibodies of this invention include antibodies immunologically reactive with fusion proteins and multimeric proteins comprising such a polypeptide, portion or fragment.

[0113] Antibodies directed against a polypeptide, portion or fragment of the present invention may be generated by a variety of means including immunizing a mammalian host with *I. scapularis* extract, by tick infestation, or by immunization of a mammalian host with an antigenic polypeptide, portion or fragment of the present invention. Such antibodies may be polyclonal or monoclonal. Methods to produce polyclonal and monoclonal antibodies are well known to those of skill in the art. For a review of such methods, see *Antibodies, A Laboratory Manual*, *supra*, and D.E. Yelton, et al., "Monoclonal Antibodies: A Powerful New Tool in Biology and Medicine," *Ann. Rev. of Biochem.* 50: 657-80 (1981). Determination of immunoreactivity with a polypeptide, portion or fragment of the present invention may be made by any of several methods well known in the art, including by immunoblot assay and ELISA.

[0114] An antibody of this invention may also be a hybrid molecule formed from immunoglobulin sequences from different species (e.g., mouse and human) or from portions of immunoglobulin light- and heavy-chain sequences from the same species. It may be a molecule that has multiple binding specificities, such as a bifunctional antibody prepared by any one of a number of techniques known to those of skill in the art including: the production of hybrid hybridomas; disulfide exchange; chemical cross-linking; addition of peptide linkers between two monoclonal antibodies; the introduction of two sets of immunoglobulin heavy and light chains into a particular cell line; and so forth.

[0115] The antibodies of this invention may also be human monoclonal antibodies produced by any of the several methods known in the art. For example, human monoclonal antibodies may be produced by immortalized human cells, by SCID-hu mice, by the expression of cloned human immunoglobulin genes, by phage-display, or by any other method known in the art. A human antibody or an antigen-

binding portion thereof of the invention also can be produced in non-human animals capable of producing human antibodies. See e.g., International Patent publication WO 98/24893.

[0116] In addition, it may be advantageous to couple an antibody of this invention to a toxin such as diphtheria, pseudomonas exotoxin, ricin A chain, gelonin, etc., or antibiotics such as penicillins, tetracyclines and chloramphenicol.

[0117] In sum, one of skill in the art, provided with the teachings of this invention, has available a variety of methods which may be used to alter the biological properties of the antibodies of this invention including methods which would increase or decrease the stability or half-life, immunogenicity, toxicity, affinity or yield of a given antibody molecule, or to alter it in any other way that may render it more suitable for a particular application.

[0118] One of skill in the art will understand that antibodies directed against a polypeptide, portion or fragment of the present invention may have utility in prophylactic compositions and methods directed against tick bite and infection with a tick-borne pathogen. For example, the level of pathogens in infected ticks may be decreased by allowing them to feed on the blood of animals immunized with a polypeptide of this invention.

[0119] The antibodies of this invention also have a variety of other uses. For example, they are useful as reagents to screen for expression of a polypeptide, portion or fragment of the present invention, either in libraries constructed from *I. scapularis* nucleic acid molecules or from other samples in which the proteins may be present. Moreover, by virtue of their specific binding affinities, the antibodies of this invention are also useful to purify or remove polypeptides from a given sample, to block or bind to specific epitopes on the polypeptides and to direct various molecules, such as toxins, to ticks.

[0120] To screen the polypeptides, portions or fragments and antibodies of this invention for their ability to confer protection against tick bite or their ability to lessen the severity of infection with tick-borne pathogens, guinea pigs are preferred as an animal model. Of course, while any animal that can acquire tick immunity may be useful, guinea pigs are not only a classical model for tick immunity but also display skin reactivity that mimics hypersensitivity reactions in humans. Thus, by administering a particular polypeptide, portion or fragment of the present invention or corresponding antibody to guinea pigs, one of skill in the art may determine without undue experimentation whether that polypeptide, portion or fragment or the corresponding antibody would be useful in the methods and compositions claimed herein.

[0121] The administration of a polypeptide, portion or fragment or corresponding antibody of this invention to the animal may be accomplished by any of the methods disclosed herein or by a variety of other standard procedures. For a detailed discussion of such techniques, see *Antibodies, A Laboratory Manual*, supra. If a polypeptide is used, it will generally be administered with a pharmaceutically acceptable adjuvant, such as complete or incomplete Freund's adjuvant, RIBI (muramyl dipeptides) or ISCOM (immunostimulating complexes). Such adjuvants may protect the polypeptide from rapid dispersal by sequestering it in a local deposit, or they may contain substances that stimulate the host to secrete factors that are chemotactic for macrophages and other components of the immune system. Preferably, if a polypeptide is being administered, the immunization schedule will involve two or more administrations of the polypeptide, spread out over several weeks.

[0122] Once the polypeptide, portion or fragment or corresponding antibody of this invention have been determined to be effective in the screening process, they may then be used in a therapeutically effective amount in pharmaceutical compositions and methods to confer tick immunity and to prevent or reduce the transmission of tick-borne pathogens.

[0123] In another aspect, the present invention includes methods of collecting mammalian plasma such that clotting of said plasma is inhibited, comprising adding to a blood collection tube an amount of a polypeptide of the present invention sufficient to inhibit the formation of a clot when mammalian blood is drawn into the tube, adding mammalian blood to said tube, separating the red blood cells from the mammalian plasma, and collecting the mammalian plasma.

[0124] Blood collection tubes include stoppered test tubes having a vacuum therein as a means to draw blood obtained by venipuncture into the tubes. Such test tubes include those which are made of borosilicate glass, and have the dimensions of, for example, 10x47 mm, 10x50 mm, 10x64 mm, 10x82 mm, 13x75 mm, 13x100 mm, 16x75 mm, 16x100 mm or 16x125 mm. Stoppers include those which can be easily punctured by a blood collection needle and which when placed onto the test tube provide a seal sufficient to prevent leaking of air into the tube.

[0125] The polypeptides of the present invention, including portions thereof having anticoagulant activity, are added to the blood collection tubes in variety of forms well known in the art, such as a liquid composition thereof, a solid composition thereof, or a liquid composition which is lyophilized to a solid in the tube. The amount added to such tubes is that amount sufficient to inhibit the formation of a clot when mammalian blood is drawn into the tube. The polypeptides of the present invention are added to blood collection tubes in such amounts that, when combined with 2 to 10 ml of mammalian blood, the concentration of such polypeptides will be sufficient to inhibit clot formation. Typically, this effective

concentration will be about 1 to 10,000 nM, with 10 to 1000 nM being preferred. Alternatively, the polypeptides of the present invention may be added to such tubes in combination with other clot-inhibiting additives, such as heparin salts, EDTA salts, citrate salts or oxalate salts.

[0126] After mammalian blood is drawn into a blood collection tube containing either a polypeptide of the present invention or the same in combination with other clot-inhibiting additives, the red blood cells are separated from the mammalian plasma by centrifugation. The centrifugation is performed at g-forces, temperatures and times well known in the medical arts. Typical conditions for separating plasma from red blood cells include centrifugation at a centrifugal force of about 100 xg to about 1500 xg, at a temperatures of about 5° to about 25°C, and for a time of about 10 to about 60 minutes.

[0127] The mammalian plasma may be collected by pouring it off into a separate container, by withdrawing it into a pipette or by other means well known to those skilled in the medical arts.

[0128] In another aspect, the present invention includes methods for preventing or inhibiting thrombosis (clot formation) or blood coagulation in a mammal, comprising administering to said mammal a therapeutically effective amount of a polypeptide or a pharmaceutical composition of the present invention.

[0129] The polypeptides or pharmaceutical compositions of the present invention are administered *in vivo*, ordinarily in a mammal, including a human. In employing them *in vivo*, the polypeptides or pharmaceutical compositions can be administered to a mammal in a variety of ways, including orally, parenterally, intravenously, subcutaneously, intramuscularly, colonically, rectally, nasally or intraperitoneally, employing a variety of dosage forms.

[0130] In practicing the methods of the present invention, the polypeptides or pharmaceutical compositions of the present invention are administered alone or in combination with one another, or in combination with other therapeutic or *in vivo* diagnostic agents.

[0131] As is apparent to one skilled in the medical art, a therapeutically effective amount of the anticoagulant polypeptides or pharmaceutical compositions of the present invention will vary depending upon the age, weight and mammalian species treated, the particular polypeptide(s) employed, the particular mode of administration and the desired affects and the therapeutic indication. Because these factors and their relationship to determining this amount are well known in the medical arts, the determination of therapeutically effective dosage levels, the amount necessary to achieve the desired result of preventing thrombosis, will be within the ambit of one skilled in these arts.

[0132] Typically, administration of the polypeptides or pharmaceutical composition of the present invention is commenced at lower dosage levels, with dosage levels being increased until the desired

effect of preventing *in vivo* thrombosis is achieved which would define a therapeutically effective amount. For the polypeptides of the present invention, alone or as part of a pharmaceutical composition, such doses are between about 0.01 mg/kg and 100 mg/kg body weight, typically between about 0.01 and 10 mg/kg, body weight.

[0133] Polypeptides of the present invention when made and selected as disclosed are useful as potent inhibitors of blood coagulation *in vitro* and *in vivo*. As such, these polypeptides are useful as *in vitro* diagnostic reagents to prevent the clotting of blood and are also useful as *in vivo* pharmaceutical agents to prevent or inhibit thrombosis or blood coagulation in mammals.

[0134] The polypeptides of the present invention are useful as *in vitro* diagnostic reagents for inhibiting clotting in blood drawing tubes. The use of stoppered test tubes having a vacuum therein as a means to draw blood obtained by venipuncture into the tube is well known in the medical arts. Kasten, B. L., "Specimen Collection", Laboratory Test Handbook, 2nd Edition, Lexi-Comp Inc., Cleveland pp. 16-17 (Edits. Jacobs, D. S. et al. 1990). They may contain clot-inhibiting additives (such as heparin salts, EDTA salts, citrate salts or oxalate salts). Some polypeptides of the present invention are potent inhibitors of blood clotting and as such, can be incorporated into blood collection tubes to prevent clotting of the mammalian blood drawn into them.

[0135] The polypeptides of the present invention are used alone, in combination of other polypeptides of the present invention, or in combination with other known inhibitors of clotting, in the blood collection tubes, for example, with heparin salts, EDTA salts, citrate salts or oxalate salts.

[0136] The amount to be added to such tubes, or effective amount, is that amount sufficient to inhibit the formation of a blood clot when mammalian blood is drawn into the tube. The polypeptides of the present invention are added to blood collection tubes in such amounts that, when combined with 2 to 10 ml of mammalian blood, the concentration of such polypeptides will be sufficient to inhibit the formation of blood clots. Typically, this effective amount is that required to give a final concentration in the blood of about 1 to 10,000 nM, with 10 to 1000 nM being preferred.

[0137] The polypeptides of the present invention may also be used to prepare diagnostic compositions. In one embodiment, diagnostic compositions are prepared by dissolving the polypeptides of the present invention into diagnostically acceptable carriers, which carriers include phosphate buffered saline (e.g., 0.01M sodium phosphate+ 0.15M sodium chloride, pH 7.2) or Tris buffered saline (e.g., 0.05M Tris-HCl+0.15M sodium chloride, pH 8.0). In another embodiment, the polypeptides of the present invention may be blended with other solid diagnostically acceptable carriers

by methods well known in the art to provide solid diagnostic compositions. These carriers include buffer salts.

[0138] The addition of the polypeptides of the present invention to blood collection tubes may be accomplished by methods well known in the art, which methods include introduction of a liquid diagnostic composition thereof, a solid diagnostic composition thereof, or a liquid diagnostic composition which is lyophilized in such tubes to a solid plug of a solid diagnostic composition.

[0139] The use of blood collection tubes containing the diagnostic compositions of the present invention comprises contacting an effective amount of such diagnostic composition with mammalian blood drawn into the tube. Typically, when a sample of 2 to 10 ml of mammalian blood is drawn into a blood collection tube and contacted with such diagnostic composition therein; the effective amount to be used will include those concentrations of the polypeptides formulated as a diagnostic composition which in the blood sample are sufficient to inhibit the formation of blood clots. Effective concentrations can be about 1 to 10,000 nM, such as 10 to 1000 nM.

[0140] According to an alternate aspect of our invention, the polypeptides of the present invention are also useful as pharmaceutical agents for preventing or inhibiting thrombosis or blood coagulation in a mammal. This prevention or inhibition of thrombosis or blood coagulation includes preventing or inhibiting abnormal thrombosis.

[0141] Conditions characterized by abnormal thrombosis are well known in the medical arts and include those involving the arterial and venous vasculature of mammals. With respect to the coronary arterial vasculature, abnormal thrombosis (thrombus formation) characterizes the rupture of an established atherosclerotic plaque which is the major cause of acute myocardial infarction and unstable angina, and also characterizes the occlusive coronary thrombus formation resulting from either thrombolytic therapy or percutaneous transluminal coronary angioplasty (PTCA). With respect to the venous vasculature, abnormal thrombosis characterizes the condition observed in patients undergoing major surgery in the lower extremities or the abdominal area who often suffer from thrombus formation in the venous vasculature resulting in reduced blood flow to the affected extremity and a predisposition for pulmonary embolism. Abnormal thrombosis further characterizes disseminated intravascular coagulopathy which commonly occurs within both vascular systems during septic shock, certain viral infections and cancer, a condition wherein there is rapid consumption of coagulation factors and systemic coagulation which results in the formation of life-threatening thrombi occurring throughout the microvasculature leading to widespread organ failure.

[0142] The pharmaceutical compositions of this invention may be in a variety of conventional depot forms. These include, for example, solid, semi-solid and liquid dosage forms, such as tablets, pills, powders, liquid solutions or suspensions, liposomes, capsules, suppositories, injectable and infusible solutions. The form chosen depends upon the intended mode of administration and prophylactic application.

[0143] Such dosage forms may include pharmaceutically acceptable carriers and adjuvants which are known to those of skill in the art. These carriers and adjuvants include, for example, RIBI, ISCOM, ion exchangers, alumina, aluminum stearate, lecithin, serum proteins, such as human serum albumin, buffer substances, such as phosphates, glycine, sorbic acid, potassium sorbate, partial glyceride mixtures of saturated vegetable fatty acids, water, salts or electrolytes such as protamine sulfate, disodium hydrogen phosphate, sodium chloride, zinc salts, colloidal silica, magnesium trisilicate, polyvinyl pyrrolidone, cellulose-based substances, and polyethylene glycol. Adjuvants for topical or gel-base forms may be selected from the group consisting of sodium carboxymethylcellulose, polyacrylates, polyoxyethylene-polyoxypropylene-block polymers, polyethylene glycol, and wood wax alcohols.

[0144] The vaccines and compositions of this invention may also include other components or be subject to other treatments during preparation to enhance their immunogenic character or to improve their tolerance in patients.

[0145] Compositions comprising an antibody of this invention may be administered by a variety of dosage forms and regimens similar to those used for other passive immunotherapies and well known to those of skill in the art. Generally, the polypeptides, portions or fragments may be formulated and administered to the patient using methods and compositions similar to those employed for other pharmaceutically important polypeptides (e.g., the vaccine against hepatitis).

[0146] Any pharmaceutically acceptable dosage route, including parenteral, intravenous, intramuscular, intralesional or subcutaneous injection, may be used to administer the polypeptide or antibody composition. For example, the composition may be administered to the patient in any pharmaceutically acceptable dosage form including those which may be administered to a patient intravenously as bolus or by continued infusion over a period of hours, days, weeks or months, intramuscularly — including paravertebrally and periarticularly — subcutaneously, intracutaneously, intra-articularly, intrasynovially, intrathecally, intralesionally, periostally or by oral or topical routes. The compositions of the invention are generally packaged in the form of a unit dose and will usually be administered to the patient intramuscularly.

[0147] The polypeptides, portions or fragments or corresponding antibodies of this invention may be administered to the patient at one time or over a series of treatments. The most effective mode of administration and dosage regimen will depend upon the level of immunogenicity, the particular composition and/or adjuvant used for treatment, the severity and course of the expected infection, previous therapy, the patient's health status and response to immunization, and the judgment of the treating physician.

[0148] For example, in an immunocompetent patient, the more highly immunogenic the polypeptide, the lower the dosage and necessary number of immunizations. Similarly, the dosage and necessary treatment time will be lowered if the polypeptide is administered with an adjuvant. Generally, the dosage will consist of 10 µg to 100 mg of the purified polypeptide, and preferably, the dosage will consist of 10-1000 µg. Generally, the dosage for an antibody will be 0.5 mg-3.0 g.

[0149] In yet another embodiment, *E. coli* expressing proteins comprising a polypeptide, portion or fragment of the invention are administered orally to non-human animals according to methods known in the art, to confer tick immunity and to prevent or reduce the transmission of tick-borne pathogens. For example, a palatable regimen of bacteria expressing a polypeptide, portion or fragment, alone or in the form of a fusion protein or multimeric protein, may be administered with animal food to be consumed by wild mice or other animals that act as alternative hosts for *I. scapularis* ticks.

[0150] Ingestion of such bacteria may induce an immune response comprising both humoral and cell-mediated components. See J.C. Sadoff et al., "Oral *Salmonella typhimurium* Vaccine Expressing Circumsporozoite Protein Protects Against Malaria," Science 240: 336-38 (1988) and K.S. Kim et al., "Immunization of Chickens with Live *Escherichia coli* Expressing *Eimeria acervulina* Merozoite Recombinant Antigen Induces Partial Protection Against Coccidiosis," Infect. Immun. 57: 2434-40 (1989); M. Dunne et al., "Oral Vaccination with an Attenuated *Salmonella typhimurium* Strain Expressing *Borrelia burgdorferi* OspA Prevents Murine Lyme Borreliosis," Infect. Immun. 63: 1611-14 (1995); E. Fikrig et al., "Protection of Mice from Lyme Borreliosis by Oral Vaccination with *Escherichia coli* Expressing OspA," J. Infect. Dis. 164: 1224-27 (1991).

[0151] Moreover, the level of pathogens in ticks feeding on such animals may be lessened or eliminated, thus inhibiting transmission to the next animal.

[0152] According to yet another embodiment, the polypeptides, portions or fragments of this invention, and the nucleic acid molecules encoding them, are useful as diagnostic agents for detecting tick immunity and tick bite. The polypeptides are capable of binding to antibody molecules produced in animals, including humans, that have been exposed to *I. scapularis* antigens as a result of a tick bite.

The detection of *I. scapularis* antigens is evidence of tick attachment and at least some feeding. Such information is an important aid in the early diagnosis of *I. scapularis*-borne diseases.

[0153] Such diagnostic agents may be included in a kit which may also comprise instructions for use and other appropriate reagents, preferably a means for detecting when the polypeptide or antibody is bound. For example, the polypeptide or antibody may be labeled with a detection means that allows for the detection of the polypeptide when it is bound to an antibody, or for the detection of the antibody when it is bound to *I. scapularis* or an antigen thereof.

[0154] The detection means may be a fluorescent labeling agent such as fluorescein isocyanate (FIC), fluorescein isothiocyanate (FITC), and the like, an enzyme, such as horseradish peroxidase (HRP), glucose oxidase or the like, a radioactive element such as ^{125}I or ^{51}Cr that produces gamma-ray emissions, or a radioactive element that emits positrons which produce gamma rays upon encounters with electrons present in the test solution, such as ^{11}C , ^{15}O , or ^{13}N . Binding may also be detected by other methods, for example via avidin-biotin complexes.

[0155] The linking of the detection means is well known in the art. For instance, monoclonal antibody molecules produced by a hybridoma can be metabolically labeled by incorporation of radioisotope-containing amino acids in the culture medium, or polypeptides may be conjugated or coupled to a detection means through activated functional groups.

[0156] The diagnostic kits of the present invention may be used to detect the presence of anti-*I. scapularis* antibodies in a body fluid sample such as serum, plasma or urine. Thus, in preferred embodiments, an *I. scapularis* polypeptide or an antibody of the present invention is bound to a solid support typically by adsorption from an aqueous medium. Useful solid matrices are well known in the art, and include cross-linked dextran; agarose; polystyrene; polyvinylchloride; cross-linked polyacrylamide; nitrocellulose or nylon-based materials; tubes, plates or the wells of microtiter plates. The polypeptides or antibodies of the present invention may be used as diagnostic agents in solution form or as a substantially dry powder, e.g., in lyophilized form.

[0157] The polypeptides, portions and fragments and antibodies directed against them provide much more specific diagnostic reagents than whole ticks preparations and thus may reduce or eliminate false positive or false negative results.

[0158] One skilled in the art will realize that it may also be advantageous in the preparation of detection reagents to utilize epitopes from more than one *I. scapularis* polypeptide and antibodies directed against such epitopes.

[0159] The skilled artisan also will realize that it may be advantageous to prepare a diagnostic kit comprising diagnostic reagents to detect *I. scapularis* polypeptides or antibodies as well as polypeptides or antibodies from pathogens found in the same tick vector, for example, *Borrelia burgdorferi*, *Babesia microti*, aHGE (the agent of human granulocytic ehrlichiosis), and some arboviruses, such as the Eastern equine encephalitis virus.

[0160] The polypeptides, portions and fragments and corresponding antibodies of the present invention, and compositions and methods comprising them, may also be useful to prevent tick bites by ticks other than *I. scapularis*. Such ticks may express polypeptides that share amino acid sequence or conformational similarities with the *I. scapularis* polypeptides of the present invention.

[0161] Throughout this specification and claims, the word "comprise," or variations such as "comprises" or "comprising," will be understood to imply the inclusion of a stated integer or group of integers but not the exclusion of any other integer or group of integers.

[0162] In order that this invention may be better understood, the following examples are set forth. These examples are for purposes of illustration only, and are not to be construed as limiting the scope of the invention in any manner. The publications and patents cited herein are incorporated by reference.

[0163] EXAMPLE I - Cloning Nucleic Acid Molecules Encoding *I. scapularis* Salivary Gland Proteins

[0164] A. Preparing cDNA Libraries

[0165] To obtain *I. scapularis* salivary glands for preparation of a cDNA expression library we fed 1,000 *I. scapularis* nymphs on naive 5-6 week old C3H/HeJ mice. After 72 hours, we pulled off the ticks and kept them under humidified conditions until dissection, which was within 24 hours of being pulled.

[0166] For dissection, we placed the ticks over a drop of PBS on a cover slip and cut them in half using a spear and sharp-pointed tweezers. We transferred the upper half of the body to a second drop of PBS within the cover slip and cut lengthwise. We scooped the interior contents of the upper segment from the shell and recovered the pair of salivary glands. We kept the salivary glands under guanidium/ β -mercaptoethanol until all dissections were complete to prevent degradation by RNases.

[0167] We isolated RNA using Stratagene's Micro RNA Isolation Kit. Briefly, we added 30 μ l of 2M sodium acetate, 300 μ l of water-saturated phenol and 60 μ l of chloroform:isoamyl alcohol to a 300 μ l aliquot of salivary gland in GITC/ β -mercaptoethanol. We capped the tube, vortexed and microfuged for 5 min at maximum speed.

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[0168] We transferred the upper phase containing the RNA to a new tube, added glycogen carrier and isopropanol and microfuged for 30 min in the cold to precipitate RNA. We washed the pellet in 75% ethanol and dried in a vacuum for 5 min. We resuspended the RNA in water and read an aliquot in a spectrophotometer at 260 nm. Our yield was 0.1-0.27 µg total RNA per tick. We sent the isolated RNA to Clontech Laboratories, Inc. where a Lambda ZAP® II expression library was made after initial amplification of the message.

[0169] B. Screening *I. scapularis* Libraries With Hyperimmune and Immune Sera

[0170] To identify antigens recognized by tick-immune sera, we screened the cDNA libraries as follows. We prepared whole-tick immune sera by infesting rabbits with 10 adult *I. scapularis* ticks 3 times at 21-day intervals. We sacrificed the animals 15 days after the final tick feeding and collected blood by heart puncture. We left the blood at room temperature for 2 hours for clot formation, then isolated the immune sera by centrifugation at 1000xg for 5 min. We stored the sera at -20°C until further use.

[0171] To confirm that these animals had developed tick-immunity, we challenged groups of 3 tick-sensitized animals with 50 *I. scapularis* nymphs and monitored the progress of the tick infestation. We recorded the duration of tick attachment, the weight of the recovered ticks and the appearance of erythema at the bite sites. We observed that ticks did not readily attach and engorge on tick-sensitized animals compared with naive (control) animals (Figure 1). Furthermore, we observed erythema at the tick-bite sites of tick-sensitized, but not control, animals (Figure 1).

[0172] We grew approximately 100,000 Lambda phage plaques containing the *I. scapularis* salivary gland cDNA (prepared as in part A) on *E. coli* XL1-Blue cell lawns in 90 mm culture plates. We then induced expression of the cDNA with 10 mM IPTG in a soaked nitrocellulose membrane for 3 hours and probed the membranes with tick-immune rabbit sera in 2-10 fold dilutions. As controls, we probed replica plates with normal rabbit sera.

[0173] After washing, we incubated the filters with alkaline phosphatase conjugated goat anti-rabbit antibody to detect clones. The tick-immune sera recognized 47 different clones from the salivary gland cDNA library. We plated individual plaques for secondary and tertiary screening with immune sera and purified to homogeneity.

[0174] We excised the inserts from the clones using the R408 helper phage and digested the vectors with the inserts with *EcoRI* endonuclease.

[0175] To identify additional *I. scapularis* antigens, we rescreen the expression libraries with immune sera from other mammals, for example mice and humans, according to the methods described herein.

[0176] C. Sequencing the Inserts

[0177] The inserts of the clones were sequenced by the Sanger method in the HHMI Biopolymer/Keck Foundation Biotechnology Resource Laboratory, Yale University School of Medicine, 333 Cedar Street, New Haven, CT. A clone containing Salp14 and a clone containing Salp9A were isolated by this protocol.

[0178] EXAMPLE 2 - Purification and Characterization of Additional Anticoagulant Polypeptides

[0179] We chromatographed saliva from adult ticks on a C-18 reverse phase column and developed the chromatogram as follows. We collected *I. scapularis* saliva (500 µl) from partially engorged adult females using the pilocarpine induction method (Tatchell, "A Modified Method for Obtaining Tick Oral Secretions," J. Parasitol. 53:1106-1107 (1967)). We allowed ticks to engorge on the ears of New Zealand white rabbits, harvested them, rinsed them in distilled water, and fixed them to glass slides with tape. We placed a sterile glass micropipette around the tick hypostome to collect saliva. We applied Pilocarpine (50 mg/ml in 95% ethanol) to the scutum of the tick to induce salivation and incubated at 35°C in a humid chamber for 2-3 h. Each tick yielded approximately 5-10 µl of saliva. We dried the saliva in a Speed-Vac-Sc 110 (Savant Instruments, Inc., Holbrook, NY) and resuspended in 450 µl of HPLC-grade water containing 0.1% trifluoroacetic acid (TFA). We subjected the saliva to reverse-phase HPLC using a Vydac C-18 column equilibrated with water/0.1% TFA. We eluted the bound protein under a gradient of acetonitrile (0-50% over 90 min) in 0.1% TFA. We collected individual peaks of protein detected by absorbance at 210 nm (Figure 2). We dried aliquots of each of the HPLC column fractions (20 µl), resuspended in 20 µl of Phosphate Buffered Saline (PBS) and assayed for anticoagulant activity by the activated partial thromboplastin time (APTT) assay as follows. We added HPLC column fractions or purified recombinant proteins in a maximum volume of 20 µl to 20 µl of APTT-FS reagent (Sigma-Aldrich, St Louis, MO) and then added 50 µl normal human plasma, in a 96 well microtiter plate (Harrison et al., "Molecular characterization of Ancylostoma inhibitors of coagulation factor Xa. Hookworm anticoagulant activity *in vitro* predicts parasite blood feeding *in vivo*," J. Biol. Chem. 277: 6223-29 2002). After incubation for 15 min at 37°C, we added 20 µl 50 nM calcium chloride to each of the wells and measured the time to thrombus formation over 3 min at 630 nm using a kinetic microplate reader (MRX HD; Dynex Laboratories, Shantilly, VA) (Figure 3). The clotting time was defined as the time (in seconds) following the addition of CaCl₂ at which the rate of increase in OD₆₃₀ (OD/min) reached its maximum value. This value was determined using the computer software program (Revelation 2.3) included with the microplate reader.

[0180] We observed that the protein peak eluting between 42.97 and 44.57 min (peak 19) contained the predominant anticoagulant activity, as evidenced by its ability to prolong the clotting time of human plasma in the APTT assay (Figure 3). The active peak (fraction 19) eluted in a total volume of 200 μ l and 10 % of this peak was required per reaction to observe inhibitory activity in the APTT assay. We analyzed the protein in the active peak by Matrix Assisted Laser Desorption Ionization-Mass Spectrometry (MALDI-MS) and N-terminal sequencing. The anticoagulant protein in fraction 19 showed a major MALDI-MS peak corresponding to a mass of 9878.77 (Figure 4) and N-terminal Amino Acid Sequence analysis of fraction 19 revealed the following primary structure: X D C Q V G T R P A S E E K R E (SEQ ID NO:). We performed a homology search of this N-terminal sequence against the GenBank™ database, which revealed identity to Salp14, an immunodominant antigen from *I. scapularis* saliva (Das et al., "Salp25D, an *Ixodes scapularis* antioxidant, is 1 of 14 immunodominant antigens in engorged tick salivary glands," *J. Infect. Dis.* 184: 1056-64 (2001); United States patent application 09/728,914).

[0181] The open reading frame of Salp14 cDNA encodes a predicted mature protein with a molecular mass of 11.5 kD, which is larger than the molecular mass of the anticoagulant protein in the HPLC fraction 19. We concluded that Salp14 and the protein in fraction 19 might represent homologues of a family of related proteins. We identified and cloned the *I. scapularis* gene encoding the 9.8 kD anticoagulant in fraction 19 using a PCR-based cDNA cloning approach as follows. We designed a degenerate 5' primer corresponding to the 7th through the 13th amino acid residues of the N-terminal sequence of Salp9A and a 3' primer corresponding to the T3 promoter sequence flanking the insert in the lambda ZapII® vector (Stratagene, La Jolla, CA) which was synthesized at the W.M. Keck Yale oligonucleotide facility. We performed PCR with these primers and amplified a product encoding the gene for Salp9A from an *I. scapularis* salivary gland cDNA library constructed in a Lambda Zap II® expression vector (Das et al., *supra*). The PCR reaction conditions were as follows: 35 cycles of 95°C for 1 min, 50°C for 1 min and 72 °C for 1 min, followed by a one cycle extension at 72 °C for 10 min. We cloned the PCR product into a pGEM®-T vector system (Promega Corporation, Madison, WI) and sequenced the insert DNA. We used the sequence to design Salp9A specific 5' primers which we used to amplify by PCR the full-length Salp9A cDNA from the salivary gland cDNA library.

[0182] The deduced amino acid sequence of Salp9A (Figure 5; SEQ ID NO:) contained the N-terminal sequence obtained by amino acid sequencing of the HPLC fraction 19. The encoded protein is 102 amino acids with a pI of 4.8 and a 21 amino acid long signal sequence motif identified by the Lasergene DNA and protein analysis software. The molecular mass of the mature Salp9A protein was

9583 daltons, 310 daltons smaller than that obtained by MALDI-MS. The protein has two predicted N-glycosylation sites (positions 26 and 87) and two predicted N-myristoylation sites (positions 27 and 70) as determined using the Prosite database. A homology search of the Salp9A protein sequence against the GenPep, Protein Data Bank and SwissProt databases showed 70% identity to Salp14 (Das et al. *supra*; Figures 5 and 6).

[0183] We performed RT-PCR analysis on Salp9A and Salp14 transcripts to determine expression as follows. We dissected salivary glands and midguts from unfed and engorged *I. scapularis* nymphs, suspended them in RNAWIZ™ (Ambion, Austin, TX), and isolated RNA according to the manufacturer's protocol. We used the isolated RNA to prepare cDNA using the Thermoscript™ RT-PCR synthesis kit (Invitrogen, Carlsbad, CA). We then used cDNA from unfed and fed tick salivary glands and midguts as template in PCR experiments. Salp14 and Salp9A PCR products were amplified using gene specific primers. We used *I. scapularis* β -actin (GenBank™ Accession # AF-426178) gene expression levels in control RT-PCR experiments to normalize the amount of cDNA used in each reaction. We observed that both Salp9A and salp14 transcripts were induced upon tick feeding (Figure 7). We also observed that while the expression of salp14 was specific to the salivary glands, Salp9A transcripts were observed both in the mid-guts and salivary glands upon engorgement (Figure 7).

[0184] EXAMPLE 3 - Active Immunization

[0185] To test the tick polypeptides of the invention for the ability to confer tick immunity, we immunize naive guinea pigs with the polypeptide in incomplete Freund's adjuvant (IFA). We boost twice at 15-day intervals. Fourteen days after the last boost, we place 50 *I. scapularis* ticks on the shaved backs of the animals immunized with the polypeptides. For a positive control, we challenge animals made tick-immune as described in Example I. As a negative control, we challenge animals that had been immunized with IFA. We judge tick immunity by the duration of tick attachment, engorgement weight and mortality during engorgement.

[0186] Results of one such immunization with tick polypeptides described in United States application 09/728,914 are depicted graphically in Figures 8 and 9. As shown in Figure 8, the average duration of stay of the ticks on guinea pigs immunized with the combination of Salp25C and Salp14B was significantly diminished compared to control animals. Likewise, as shown in Figure 9, the engorgement weight of ticks feeding on those animals also was significantly reduced and most of the ticks died on the guinea pig. These data indicate that immunization with tick salivary polypeptides can confer tick immunity in an immunized animal.

[0187] EXAMPLE 4 - Passive Immunization

[0188] We obtained tick-immune sera from rabbits and guinea pigs by exposure to tick feeding as described in Example 1. We confirmed that both tick-immune rabbits and guinea pigs developed antibodies to *I. scapularis* antigens, which were detectable by ELISA at a serum dilution of 1:1,000 using tick saliva as a substrate. We then transferred sera from tick-immune animals (rabbits or guinea pigs) to naive animals. We then infested these animals with *I. scapularis* nymphs and monitored the progress of tick infestation. We observed that passive transfer of immune sera partially protected either guinea pigs or rabbits from tick infestation (Figure 1, $P < 0.05$, Students' t-test). This effect was not as strong as seen with active immunization with either the tick antigens of this invention or following tick infestation. We also observed that animals that were passively administered immune sera also developed erythema at the sites of tick attachment. The erythema at these sites was similar in intensity to the erythema observed following active sensitization (Figures 1C and 1D).

[0189] We also passively immunize animals using antiserum from animals immunized with polypeptides of the invention. We prepare antiserum by immunizing C3H/He mice with polypeptides of the invention and boost twice. Fourteen days after the last boost, we sacrifice the immunized animals and collected the antiserum. We immunize guinea pigs or mice with the antiserum, challenge the passively immunized animals with ticks and evaluate tick immunity as described above.

[0190] EXAMPLE 5 - Preparation of Fab Fragments of Immune Serum

[0191] To obtain Fab fragments of immune serum for use in screening a salivary gland expression library, we first make rabbit and/or guinea pig anti-tick antiserum. We repeatedly infest rabbits and/or guinea pigs with larval or nymphal ticks, preferably *I. scapularis* ticks. We determine if the animals are tick-immune if the site of tick attachment becomes red or if tick feeding is less than 48 hours (see Examples 3 and 4). We bleed tick-immune animals to collect tick-immune serum.

[0192] We also prepare guinea pig anti-tick salivary gland antiserum by immunizing guinea pigs subcutaneously with salivary-gland extract prepared as described above, in incomplete Freund's adjuvant. We boost twice with the same amount of crude extract.

[0193] To prepare the Fab fragment, we precipitate the antiserum with ammonium sulfate and isolate the IgG fraction using DEAE chromatography. We digest the IgG preparation using a solid-phase papain column. We purify Fab fragments from the papain digestion using a protein A affinity column to remove Fc and intact IgG molecules.

[0194] EXAMPLE 6 - Prevention of Tick Pathogen Transmission

[0195] We test the effect of immunization with tick polypeptides of the invention on the transmission of tick-borne pathogens, including but not limited to *B. burgdorferi*, the agent of human granulocytic ehrlichiosis (aoHGE), *Babesia microti*, or various Rickettsiae.

[0196] Before testing the transmission of *B. burgdorferi*, the agent of Lyme Disease, we determined whether guinea pigs could be infected by challenge with *B. burgdorferi* infected ticks. We challenged naive guinea pigs with 5 B31- or N40-strain-infected *I. scapularis* nymphs. Skin punches at the site of tick attachment and elsewhere 2, 4 and 7 weeks after tick challenge were consistently positive for spirochetes by culture.

[0197] To confirm infection, we determined that guinea pigs develop an immune response against *B. burgdorferi*. Western blots of cloned N40 spirochetes probed with serum from the challenged animals showed antibodies to flagellin, P39 and OspC antigens. Sera from animal exposed to uninfected ticks and those exposed to infected ticks but that were not culture positive failed to develop such antibodies.

[0198] We demonstrated, thus, that guinea pigs become infected with *B. burgdorferi* by tick challenge.

[0199] We then determine if immunization with tick polypeptides or antibodies of the invention affect the transmission of *B. burgdorferi*. We immunize guinea pigs with tick polypeptides or antibodies of the invention as described above and five weeks later, we challenge the immunized animals with ticks from a pool with an approximately 80% infection rate of *B. burgdorferi*. We obtain skin-punch biopsies at the tick attachment site and serum samples at 2, 4 and 7 weeks after tick challenge. At 8 weeks after challenge we sacrifice the animals and collect blood, bladder and spleen for culture.

[0200] One of skill in the art will appreciate that the ability of the polypeptides or antibodies of the invention to prevent or lessen the transmission of other tick born pathogens can be determined using the method described herein.

[0201] EXAMPLE 7 - Preparation of Antibodies to Tick Polypeptides

[0202] To prepare antibodies to a polypeptide of the invention, we immunize C3H/He mice subcutaneously with the polypeptide in complete Freund's adjuvant and boost with the same amount in incomplete Freund's adjuvant at 14 and 28 days. We immunize control animals in the same manner with bovine serum albumin (BSA).

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[0203] Ten days after the last boost, we collect sera from the immunized animals and use it to hybridize to western blots of SDS-PAGE gels of *I. scapularis* tick extract or to the polypeptide. We detect binding with alkaline phosphatase goat-anti-mouse antibody developed with nitroblue tetrazolium and 5-bromo-4-chloroindolyl phosphate. Alternatively, we use the ECL™ kit (Amersham, Arlington Heights, IL) in which the secondary antibody, horseradish peroxidase-labeled goat anti-mouse antibody, can be detected.

[0204] Alternatively, we immunize BALB/c mice with polypeptides of the invention for production of neutralizing antibodies. We emulsify five micrograms of polypeptide in complete Freund's adjuvant and injected subcutaneously in four sites. Ten days later we boost the animals with a similar amount of protein in incomplete Freund's adjuvant and two weeks later we boost a final time with aqueous protein. We bleed animals from the tail and determine antibody titers to the immunizing polypeptide by ELISA assays.

[0205] The ability of the antibody in mouse sera to neutralize anticoagulant activity is determined by addition of varying amounts of diluted antibody to the polypeptide and testing in the APTT assay. We also synthesize overlapping fragments of the polypeptide and use these fragments as competitive inhibitors of antibody binding to the polypeptide to determine where the antibody binds.

[0206] To prepare a monoclonal antibody, we recover antibody-producing cells from the spleens of the immunized animals and fuse the antibody-producing cells with immortalized cells to produce hybridomas according to the methods of Kohler and Milstein. We screen the resulting hybridomas for specific binding to the polypeptide. Those of skill in the art will appreciate that polyclonal and monoclonal antibodies specific for all polypeptides of the invention, including portions, immunogenic fragments and fusion proteins, may be prepared using the methods described herein.

[0207] EXAMPLE 8 - *I. scapularis* Saliva Contains Multiple Proteins Related to Salp14

[0208] We chromatographed saliva from adult ticks on a C-18 reverse phase column and developed the chromatogram as follows. We collected *I. scapularis* saliva (500 µl) as described in Example 2, dried it in a Speed-Vac-Sc 110 (Savant Instruments, Inc., Holbrook, NY) and resuspended in 450 µl of HPLC-grade water containing 0.1% trifluoroacetic acid (TFA). We subjected the saliva to reverse-phase HPLC using a Vydac C-18 column equilibrated with water/0.1% TFA. We eluted the bound protein under a gradient of acetonitrile (0-50% over 90 min) in 0.1% TFA. We collected individual peaks of protein detected by absorbance at 210 nm (Figure 2). We identified and collected 26 individual peaks of protein as detected by absorbance at 210 nm. We obtained N-terminal amino acid

sequence of Fractions 17, 18, 19, and 20 which are shown in Table 1 along with the mass of the predominant protein in the fraction.

Table 1. MALDI-MS and N-terminal amino acid sequence data from tick salivary proteins

rpHPLC Fraction	Mass of predominant protein	N-terminal sequence	SEQ ID NO
Fraction 19	9878	XDXQVGTRPASEEKRXGXDY	
Fraction 17	8704	HNXQXGTRPASEEKREGXDYYXXNAE	
Fraction 18	8761	HNXQXGTRPASEEKREGXDYY	
Fraction 20	8700	HNXQXGTR	

[0209] We compared the N-terminal sequences obtained from the polypeptide in Fractions 17 and 19 to the amino acid sequences of Salp9A and Salp14. We used a highly conserved region to design a first PCR primer (5' GA ACG AGA CCC GCC TCG 3'; SEQ ID NO:) for use with a second primer complementary to the T7 polymerase transcription initiation site in the cloning vector for amplification of DNA sequences belonging to the Salp14/Salp9A gene family by PCR from a cDNA library containing inserts derived from adult salivary glands. We amplified the sequences for 35 cycles with Taq polymerase. We separated the amplification products by agarose gel electrophoresis, eluted them from the gel, and ligated them into the pGEM-T Easy cloning vector (Promega). We purified 10 plasmids from colonies selected at random and determined the nucleotide sequence of their inserts. We then compared these nucleotide sequences to Salp14 and Salp9A. As shown in Tables 2-3, the sequences of the ten inserts were all distinct from one another and, while related to Salp9A and Salp14, none was identical to either Salp14 or to Salp 9. Lengths of overlaps varied from 130 to 296 nucleotides. Table 2 shows the sequences in a ClustalW alignment and Table 3 shows the percentage of nucleotide differences between each insert and the Salp14 and Salp9A nucleotide sequences.

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Table 2. ClustalW alignment of Salp9A, Salp14 and insert sequences

Salp14	1 EQDREGCDYYCWNAETKSWDQFFFGNGEKCFYNSGDHGTQCQNGECHLTNN
Salp9A	1 EEKREGCDYYCWNTETKSWDKFFFGNGERCFYNNNGDEGLCQNGECHLTDD
H1	1 EEKREGCDYYCWNTETKSWDKFFFGNGERCFYNNNGDEGLCQNGECHLTDD
L8	1 EEKREGCDYYCWNAETKSWDQFFFGNGERCFYNNNGDEGLCQNGECHLTDD
M1	1 EEKREGCDYYCWNTETKSWDKFFFGNGERCFYNNNGDEGLCQNGECHLTDD
L3	1 EEKREGCDYYCWNTETKSWDKFFFGNGERCFYNNNGDEGLCQNGECHLTDD
H2	1 EEKREGCDYYCWNTETKSWDKFFFGNGERCFYNNNGDEGLCQNGECHLTDD
L2	1 EEKREGCDYYCWNTETKSWDKFFFGNGERCFYNNNGDEGLCQNGECHLTRD
H3	1 EKNREGCDYYCWNEVTNSWDQFFFGNGERCFYNNNGDEGLCQNGECHLTDD
L1	1 EENREGCDYYCWNEVTNSWDQFFFGNGERCFYNTGENGKCKQNG
H7	1 EQNREGCDYYCWTAETKSWDQFFFGNGEKCFYNSGDHGTQCQNGECHLTNN
L4	1 EENREGCDYYCWDDGTNKWDQFFFGNGEICFYNSGEKGICQNGECHLTNN
Salp14	51 SGGPNETDDYTPAPTEKPKQKKKKTKKTKKPKRKSKKDQEKNL*
Salp9A	51 SGVPNDTDAKIEETEEEELEA*
H1	51 SGVPNDTDAKKEETEEEELEA*
L8	51 SGVPNDTDANKEETEEEELEA*
M1	51 SGVPNDTDAKIEETEEEELEA*
L3	51 SGVPNDTDAKIEETEEEELEA*
H2	51 SGMPNDTDAKIEETEEEELEA*
L2	51 SGVPNDTD
H3	51 SGVPNDTDAKIEETEEEELEA*
L1	
H7	51 SGGPNETDDYTAAPTEKPKQKKKEEN*
L4	51 SGGPNETDDNTPATTEKPK*

Table 3. Nucleotide differences between inserts and Salp14 and Salp9A nucleotide sequences

cDNA	Alignment to Salp9A			Alignment to Salp14		
	Length of overlap	Number of differences	% Differences	Length of overlap	Number of differences	% Differences
Salp9A				250	38	15
H2	296	5	2	250	39	15
H1	295	9	3	250	40	16
M1	296	4	1	250	37	15
L2	176	3	2	176	23	13
L3	277	10	4	250	38	15
L8	295	12	4	250	36	14
H3	295	14	5	250	39	16
L1	130	18	14	130	16	12
L4	176	33	19	211	26	12
H7	295	49	17	281	9	3
Salp14	250	38	15			

[0210] We also used these inserts to screen the original cDNA library from which these sequences were identified to obtain full-length clones. We also use a clone corresponding to the region of highest homology between Salp 9A and 14 and the other polypeptides to screen the cDNA library to identify additional family members. The amino acid and nucleic acid sequences for these full-length clones and other inserts are in Tables 4 and 5.

Table 4. Amino acid sequences for HOMO and BEKO clones.

Protein	Amino acid sequence	SEQ ID NO:
128-HOMO	MGLTEITLVLSLAFFGTAVAHNCQNGTRPASEENREGCDYYCWNAE TKSWDKFFFGNGERCIFYNDGGEGLCQNGECHLTTDSSVPNDTDV*	
13-HOMO	MGLTGTTLVLCVAFFGTAAAHNCNGTRPASEENREGCDYYCWNE VTNSWDQFFFNGNERCFYNTGENGKQCQNGECHLTTNSDGPNETDD NTPPTEKPKQKKEET*	
14-HOMO	MGLTGTTLVLSLAFFGSAAAHNCNGTRPASEENREGCDYYCWND GTNSWDQFFFNGEICFYNSGEKGICQNGECHLTNNSGGPNETDEN TPATTEKPKQKKKTKKPKPKRKSCKDQ*	
154-HOMO	MGLTGTTLVLCVAFFGSAAAHNCQNGTRPASEENREGCDYYCWNT DTNSWDQFFFNGEKCIFYNTGEKGKCLNGECHLTTSSGGPDDTGD NTPPTEKPKQKKKKPKKTKKPKRKSCKDQKENF*	
155-HOMO	MGLTEITLVLSLAFVATAAAHDCQNGTRPASEEKREGCDYYCWNEE TKSWDKFFFGNGERCIFYNDGGEGLCQNGECHLTTDSSVPNDTDV*	
156-HOMO	MGLTEMLVLVSPAFVATAAAHDCQNGTRPASEEKREGCDYYCWNT TETKSWDKFFFGNGERCIFYNNGDEGLCQNGECHLTTDSGVPNDTD AKKEETEELEA*	
159-HOMO	MGLTGTTLVLCVXFFXSAAAHNCQNGTRPASEENRXGCDYYCWNE VTXSWDQFFFNGXRCFXNTGENGKCQ	
168-HOMO	MGLTEIMLVLSLAFVATAAAHDCQNGTRPASEEKREGCDYYCWNT ETKSWDKFFFGNGERCIFYNNGDEGLCQNGECHLTTDSGVPNDTDA KIEETEELEA*	
188-HOMO	MGLTGTTLVLCVAFFGSAAAHNCQNGTRPASEENREGCDYYCWNE VTNSWDQFFFNGNERCFYNTGENGKQCQNGECHLTTNSDGPNETDD NTPPTEKPKQKKKKPKKPKPKRKSCKDQ*	
190-HOMO	MGLTGTTLVLCVAFFGSAAAHNCQNGTRPASEENREGCDYYCWNE VTNSWDQFFFNGNERCFYNTGENGKQCQNGECHLTTNSDGPNETDD NTPPTEKPKQKKKKPKKPKPKRKSCKDQ	
191-HOMO	MGLTGTTLVLCVAFFGSAAAHNCQNGTRPASEKNREGCGYYCWNT AETKSWDQFFFGDGERCFYNTGENGTCRNGECHLTTSSGGPNETD DNTPPTEKPKQKKKKPKKTKKPKRKSCKDQ*	
199-HOMO	MGLTGTTLVLSLAFFGSAAAHNCQNGTRPASEQNIEGCDYYCWNA ETKSWDQFFFNGEKCIFYNSGDHGTGTCQNGECHLTTNSGGPNETDD YTAAPTEKPKQKKEEN*	
19-HOMO	MGLTGTTLVLCVAFFGSAAAHNCQNGTRPASEENREGCDYYCWNT DTNSWDQFFFNGEKCIFYNTGEKGTCLNGECHLTTSSGGPDDTGD NTPPTEKPKQKKKKPKKTKKPKRKSCKDQKENF*	
203-HOMO	MGLTGTMVLAVSLAFFGSAAAHNCQNGTRPASEQNREGCDYYCW NAETKSWDQFFFNGEKCIFYNSGDHGTGTCQNGECHLTTNSGGPNET DDYTPAPTEKPKQKKKKTKKTKKPKRKSCKDQKENF*	
209-HOMO	MGLTEITLVLSLAFIATAAAHNCQNGTRPASEEKREGCDYYCWNAE TNSWDKFFFGNGERCIFYSDGSEGLRQNGECHLTTDSSVPNDSDV*	
210-HOMO	MGLTGTTLVLCVAFFGSAAAHNCQNGTRPASEENREGCDYYCWNS GTNSWDQFFFNGEKCIFYNTGEKGICQNGECHLTTNSGGPNETDD NTPAPTEKPKQKKKKTKKTKKPKRKSCKDQ*	
214-HOMO	MGLTGTMVLAVSLAFFGSAAAHNCQNGTRPASEQNREGCDYYCW	

	NAETKSWDQFFFNGEKCIFYNSGDHGTCCQNGECHLTNNSGGPNET DDYTPAPTEKPKQKKEEN*	
21-HOMO	MGLTGTXLVLVCVAFFGSAAAXNCQNGTRPASEENREGCDYYCWNS GTNSWDQFFFNGEKCIFYNXGAKGICQNGECHLTNNSGGPNETDD NTPAPXEKPK	
220-HOMO	MGLTGTTLVLVSLAFFGSAAAHNCNGTRPASEENREGCDYYCWND GTNSWDQSFNGEICFYNSGEKGICQNGECHLTNNSGGPNETDDN TPATTEKPKQKKKTKKTKKPKRKSCKDQ*	
22-HOMO	MGLTEVMLVLVSLAFVATAAAHDCQNGTRPASEEKREGCDYYCWNT ETKSWDKFFFNGGERCFYNNGDEGLCQNGECHLTDDSSVPNDNDV*	
24-HOMO	MGLTGTMLVLVSLAFFGSAAAHNCQNGTRPASEQNREGCDYYCWN AETKSWDQFFFNGEKCIFYNSGDHGTCCQNGECHLTNNSGGPNETD DYTPAPTEKPKQKKKTKKTKKPKRKSCKDQKNF*	
32-HOMO	MGLTGTTLMLAVSLAFFGSAAAHNCQNGTRPASEQNREGCDYYCW NAETKSWDQFFFNGEKCIFYNSGDHGTCCQNGECHLTNNSGGPNET DDYTAAPTEKPKQKKKTKKTKKTKRKSCKD*	
35-HOMO	MGLTGTTLMLAVSLAFFGSAAAHNCQNGTRPASEQNREGCDYYCW NAETKSWDQFFFNGEKCIFYNSGDHGTCCQNGECHLTNNSGGPNET DDYTAAPTEKPKQKKKTKKTKKTKRKSCKD	
36-HOMO	MGLTGTMLVLAVSLAFFGSAAAHNCQNGTRPASEQNREGCDYYCW NAGTKSWDQFFFNGEKCIFYNSGDHGTCCQNGECHLTNNSGGPNET DDYTPAPTEKPK	
3-HOMO	MGLTGTTLVLVSLAFFGSAAAHNCQNGTRPASEQNREGCDYYCWNA ETKSWDQFFFNGEKCIFYNSGDHGTCCQNGECHLTNNSGGPNETDD YTAAPTEKPKQKKKTKKSKKPKRKSCKD*	
45-HOMO	MGWTGTTLVLVCVAFFGSAAAHNCQNGTRPASEKNREGCDYYCWN EVTNSWDQFFFNGEKCIFYNTGEHGKCQNGECHLTSSDGPNETD DNTPPPTEKPKQKKKPKKPKPKRKSCKDQ*	
46-HOMO	MGLTEITLVLVSLAFFGSVAHNCQNGTRPASEENREGCDYYCWNA ETKSWDQFFFNGEKCIFYNTGEKGICQNGECHLTNNSGGPNETDDN TPAPTEKPKQKKKTKKTKKPKRKSCKDQ*	
55-HOMO	MGLTGTAALVLVSLAFFGSAAAHNCQNGTRPASEENREGCDYYCWND VTNSWDQFFFNGGERCFYXTGENGKCLNGECHLTSSGGPDDTGD NTPPPAEKPXQKKKPKKPKPKRKSCKDQ*	
67-HOMO	MGLTGTAALVLVSLAFFGSAAAHNCQNGTRPASEENREGCDYYCWND VTNSWDQFFFNGGERCFYNTGENGKCLNGECHLTSSGGPDDTGD NTPPPTEKPKQKKKPKKPKPKRKSCKDQ*	
BEKO4891	IMLVLVSLAFVATAAAHDCQNGTRPASDEKREGCDYYCWNTETKSW DKFFFNGGERCFYNNGDEGLCQNGECHLTDSGVPNDTDAKIEETE EELEA*	
BEKO4892	IMLVLVSLAFVATAAAHDCQNGTRPASEEKREGCDYYCWNTETKSW DKFFFNGGERCFYNNGDEGLCQNGECHLTDSGVPNDTGAKIEETE EELEA*	
BEKO4893	IMLVLVSLAFVATAAAHDCQNGTRPASEEKREGCDYYCWNTETKSW DKFFFNGGERCFYNSGDEGLCQNGECHLTDSGVPNDTDAKIEETE EELEA*	
BEKO4894	IMLVLVSLAFVATAAAHDCQNGTRPASEEKREGCDYYCWNTETKSW DKFFFNGGERCFYNNGDEGLCQNGECHLTDSGVPNDTDAKIEETE	

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	EELEA*	
BEKO4895	IMLVLVSLAFVATAAAHDCQNGTRPASEEKREGCDYYCWNTETKSW DKFFFGNGERC FYNNGDEGLCQNGEHLTTDSGVPNDTDAKIEETE EELEA*	
BEKO6691	IMVVLVSLAFVATAAAHDCQNGTRPASEEKREGCDYYCWNTETKSW DKFFFGNGERC FYNNGDGGLCQNGEHLTTDSGVPNDTDAKIEETE EGLEA*	
BEKO6692	EKREGCDYYCWNTETKSWDKFFFGNGERC FYNNGDEGLCQNGECH LTTDSGVPNDTDAKIEETEEEELEV*	
BEKO6693	IMLVLVSLAFVATAAAHDCQNGTRPASEEKREGCDYYCWNTETKSW DKFFFGNGERC FYNNGDEGLCQNGEHLTTDSGVPNDTDAKIEETE EELEA*	
BEKO6694	IMLVLVSLAFVATAAAHDCQNGTRPASEEKREGCDYYCWNTETKSW DKFFFGNGERC FYNNGDEGLCQNGEHLTTDSGVPNDTDAKIEETE EELEA*	
BEKO48142	TRPASEQNREGCDYYCWNAEAKSWDQFFFGNGEKC FYNSGDHGTC QNGEHLTNNSGGPNETDDYTPAPTEKPKQKKKKTKKTKKPKRKSK KD	
BEKO48143	TRPASEQNREGCDYYCWNAETKSWDQFFFGNGEKC FYNSGDHGTC QNGEHLTNNSGGPNETDDYTPAPTEKPKQKKKKTKKTKKPKRKSK KD	
BEKO48144	TRPASEQNREGCDYYCWNAETKSWDQFFFGNGEKC FYNSGDHGTC QNGEHLTNNSGGPNETDDYTPAPTEKPKQKKKKTKKTKKPKRKSK KDQEKNL	
BEKO48145	TRPASEQNREGCDYYCWNAETKSWDQFFFGNGEKC FYNSGDHGTC QNGEHLTNNSGGPNETDDYTPAPTEKPKQKKKKTKKTKKPKRKSK KD	

Table 5. Nucleic acid sequences for HOMO and BEKO clones.

Protein	Nucleic acid sequence	SEQ ID NO:
128-HOMO	ACTCACGCACTCCTTGAGAGCGCTACAAAACTATGGGGTTGACT GAGATCACGCTGGTGTGGTGTCTCTGGCCTTCTTCGGGACCGCT GTGGCCCAACAATTGCCAAAACGGAACGAGACCCGCCTCGGAAGA AAATAGAGAAGGCTGCGACTATTACTGCTGGAACGCTGAGACCAA ATCATGGGACAAATTTTTCTTCGGGAACGGAGAACGATGCTTTTAC AACGATGGTGGTGAGGGACTATGTCAAATGGAGAATGCCATTTG ACAACCGATTCAAGTGTGCCCAATGACACTGATGTCTAAACGCCT GCTCCCACTGAAAAGCCGAAGCAAAAAATGGAGAAACCGAAGAAA AGTAAGAAGCCTAAGGGAAAGTCAAAGAAAGATTAGGAAGGAAAC TTTTGATTAAGACCCGAAACTAAACCAGGCTAAATAAAGCTAAG GCACTGACCGTTTGTAGTGTGTACACMATAA	
13-HOMO	ATTCACATCCTCGTTGAGAACCAGTACACCAAATATGGGGTTGACC GGAACCACGCTGGTGTGGTGTGTGGCCTTCTTCGGGACTGCT GCAGCCCACAATTGCAAGAACGGAACGAGACCCGCATCGGAGGA AAACCGAGAAGGCTGCGACTATTACTGCTGGAACGAAGTCACCAA TTCATGGGACCAATTTTTCTTTGGGAACGGAGAAAGATGCTTTTAC AACACTGGTGAAAATGAAAATGTCAAACGGAGAATGTCATTTGA CAACTAATTCAGATGGGCCCAACGAGACTGATGACAATACTCCTC CGCCCACTGAAAAGCCCAAGCAAAAAAAGAAGAAACCTAAGAAG CCTAAGAAACCAAGCGCAAGTCAAAGAAAGATCAGTAGGAGAAC TTCTCACTAGAAGAAATAAACCTAAACCATATTAATAAAGTTGAGC ACAC	
14-HOMO	ATTCACAACCTCGTTGAGGAGCAGTACACACACTATGGGGTTGAC CGGAACCACGCTGGTGTGGTGTCTCTGGCCTTCTTCGGGAGCG CTGCAGCCCACAATTGCAAGAACGGAACGAGACCCGCATCGGAG GAAAATCGTGAAGGCTGCGACTATTACTGCTGGAACGATGGCACC AATTCATGGGACCAATTTTTCTTTGGGAACGGAGAAATCTGCTTTT ACAACAGTGGTGAAAAGGGAATTTGTCAAACGGAGAATGTCATTT GACAAATAATTCAGGTGGGCCCAACGAGACTGATGAAAATACTCC TGCAGCACTGAAAAGCCTAAGCAAAAAAAGAAGAAACTAAGAA GCCTAAGAAACCTAAGCGCAAGTCAAAGAAAGATCAGTAGGAGAA CTTCTCACTAGAAGAACTAAACCTAAATCATGTAAATAAAGTTGAG CNCNCNNN	
154-HOMO	ATTCACATCCTCGTTGAGAACCAGTACACCAAATATGGGGTTGACC GGAACCACGCTAGTGTGGTGTGTGGCCTTCTTCGGGAGCGCT GCAGCCCACAATTGCCAGAACGGAACGAGACCCGCATCGGAGGA AAATAGAGAAGGCTGCGACTATTACTGCTGGAACACTGACACTAAT TCATGGGACCAATTTTTCTTTGGGAACGGAGAAAAATGCTTTTACA ACACTGGTGAAAAGGGAAAATGTCTAAACGGAGAATGTCATTTGAC AACTAGTTCAGGTGGGCCCGACGACACTGGTGACAATACCCCTCC GCCCACTGAAAAGCCCAAGCAAAAAAAGAAGAAACCTAAGAAGAC TAAGAAACCTAAGCGCAAGTCAAAGAAAGATCAGAAGGAGAACTT CTGACTAAAAGAACTAAACCTAAACCTGGCTAAATAAAGTTGAGAC ACT	
155-HOMO	ACTCAGTACTCGTTGAGAGCACTACACAAGCTATGGGGTTGACT	

	GAGATCACGCTTGTGTTGGTGTCTCTGGCCTTCGTGCGCAACCGCT GCGGCCCACGATTGCCAAAACGGAACGAGACCCGCCTCGGAAGA AAAGAGAGAAGGCTGCGACTATTACTGCTGGAACGAAGAGACCAA ATCATGGGACAAGTTTTTCTTCGGCAACGGAGAACGATGTTTTAT AACGATGGTGGTGAGGGACTATGTCAAACGGAGAATGCCATTTG ACAACCGATTCAAGTGTGCCCAATGACACTGATGTCTAACTCCTG CTCCCACTGGAAAGCCGAAGCAAAAAAAGAAGAAACCGAAGAAGA GTAAGAAGCCTAAGGGGAAGTCAAAGAAAAATTAGGAAGGAACT TCTTATTAAGTCCGGAACTAAACCATGATTAATAAAGCTAAAGC ACTGACTAG	
156-HOMO	ACTCACGTACTCGTTGAGAGCACTACACAACCTATGGGGTTGACT GAGATGATGCTGGTGTGGTGTCTCCGGCCTTCGTGCGCAACCGCT GCGGCCCACGATTGCCAAAACGGAACGAGACCCGCCTCGGAAGA AAAGAGAGAAGGCTGCGACTATTACTGCTGGAACACCGAGACCAA ATCATGGGACAAATTTTTCTTCGGGAACGGAGAACGATGCTTTTAC ACAATGGTGTGATGAGGGATTATGTCAAACGGAGAATGCCATTTGA CAACAGATTCAGGTGTGCCCAATGACACTGATGCAAAAAAAGAAG AAACCGAAGAAGAGTTAGAAGCCTAAGGGCAAGTCAAAGAAAAAT TAGGAAGGAACTTCTTATTAAGTCCCGAACTAAACCAGGCTT AATAAAGATAAAGCACGG	
159-HOMO	ATTCACATCCTCGTTGAGAACCAGTACACCMATATGGGGTTGACC GGAACCACGCTGGTGTGGTGTGTGGMCTTCTTCKGGAGTGCT GCAGCCCACAATTGCCAGAACGGAACGAGACCCGCATCGGAGGA AAACCGASAAGGSTGCGACTATTACTGCTGGAACGAAGTCACCWA TTCATGGGACCAATTTTTCTTTGGGAACGGAKAAAGATGCTTTTRC AACACTGGTGAAAATGGAAAATGTCAAAA	
168-HOMO	ACTCACATACTCGTTGAGAGCACTACACAAGCTATGGGGTTGACT GAGATCATGCTGGTGTGGTGTCTCTGGCCTTCGTGCGCAACCGCT GCGGCCCACGATTGCCAAAACGGAACGAGACCCGCCTCGGAAGA AAAGAGAGAAGGCTGCGACTATTACTGCTGGAACACCGAGACCAA ATCATGGGACAAATTTTTCTTCGGAAACGGAGAACGATGCTTTTAC ACAATGGTGTGATGAGGGATTATGTCAAACGGAGAATGCCATTTGA CAACAGATTCAGGTGTGCCCAATGACACTGATGCAAAAATAGAAG AAACCGAAGAAGAGTTAGAAGCCTAAGGGCAAGTCAAAGAAAAAT TAGGAAGGAACTTCTTATTAAGTCCCGAACTAAACCAGGC	
188-HOMO	ATTCACATCCTCGTTGAGAACCAGTACACCAAATATGGGGTTGACC GGAACCACGCTGGTGTGGTGTGTGGCCTTCTTCGGGAGTGCT GCAGCCCACAATTGCCAGAACGGAACGAGACCCGCATCGGAGGA AAACCGAGAAGGCTGCGACTATTACTGCTGGAACGAAGTCACCAA TTCATGGGACCAATTTTTCTTTGGGAACGGAGAAAGGTGCTTTTAC AACACTGGTGAAAATGGAAAATGTCAAACGGAGAATGTCATTTGA CAACTAATTCAGATGGGCCCAACGAGACTGATGACAATACTCCTC CGCCCACTGAAAAGCCCAAGCAAAAAAAGAAGAAACCTAAGAAGC CTAAGAAACCAAGCGCAAGTCAAAGAAAGATCAGTAGGAGAACT TCTCACTAGAAGAAATAGACCTAAACCATGTAAATAAAGTTGAGC ACACACNNN	
190-HOMO	ACTCACATACTCGTTGAGAGCACTACACAAGCTATGGGGTTGACT GAGATCATGCTGGTGTGGTGTCTCTGGCCTTCGTGCGCAACCGCT	

	GCGGCCCACGATTGCCAAAACGGAACGAGACCCGCCTCGGAAGA AAAGAGAGAAGGCTGCGACTTACTGCTGGAACACCGAGACCAA ATCATGGGACAAATTTTCTTCGGGAACGGAGAACGATGCTTTTAC AACAATGGTGATGAGGGATTATGTCAAACGGAGAGTGCCATTTG ACAACAGATTCAGGTGTGCCAATGACACTGATGCAAAAATAGAA GAAACCG	
191-HOMO	ATTCACATCCTCGTTGGAACCAAGTACACCAATATGGGGTTGAC CGGAACCACGCTGGTGTGTTGGTGTGTGGCCTTCTTCGGGAGCG CTGCAGCACACAATTGCCAGAACGGAACGAGGCCCGCATCGGAG AAAAATAGAGAAGGCTGCGGCTTACTGCTGGAACGCTGAGACC AAATCATGGGACCAATTTTCTTTGGCGACGGAGAAAGATGCTTTT ACAACACTGGTGAATGGAACATGTGAAACGGAGAATGTCATTT GACAAGTGTTCAGGTGGGCCCAACGAGACTGATGACAATACTCC TCCGCCCACTGAAAAGCCCAAGCAAAAAAAGAAGAAACCTAAGAA GACTAAGAAACCAAGCGCAAGTCAAAGAAAGATCAGTAGGAGAA CTTCTCACTAGAAGAACTAAACCTAAACCAGACTAAATAAAGTTGA GCACACTG	
199-HOMO	TTCACATCCTCGTTGAGAACCAGTACACCAACAATGGGGTTGACC GGAACCACGCTGGTGTGTTGGTATCTCTGGCCTTCTTCGGGAGCGCT GCAGCCCACAATTGCCAGAACGGAACGAGACCCGCATCGGAGCA AAATATAGAAGGCTGCGACTTACTGCTGGAACGCTGAGACCAA ATCATGGGACCAATTTTCTTTGGGAACGGAGAAAAATGCTTTTAC AACAGTGGTGATCACGGAACATGTCAAACGGAGAATGTCATTTG ACAAATAATTCGGGTGGGCCCAACGAACTGATGACTATACTGCT GCGCCCACTGAGAAGCCGAAGCAAAAAAAGAAGAAACTAAGAA GTCTAAGAAACCTAAGCGCAAGTCAAAGAAAGATTAGGAGGAAAA CTTCTGATTAAACGCCCTAAAAATAAACCCAGGCTAAATAAATTTGA GACACCG	
19-HOMO	TCACATCCTCGTTGAGAACCAGTACACCAATATGGGTTTGACCG GAACCACGCTAGTGTTGGTGTGTGTGGCCTTCTTCGGGAGCGCTG CAGCCCACAATTGCCAGAACGGAACGAGACCCGCATCGGAGGAA AATAGAGAAGGCTGCGACTTACTGCTGGAACACTGACACTAATT CATGGGACCAATTTTCTTTGGGAACGGAGAAAAATGCTTTTACAA CACGGGTGAAAAGGGAACATGTCTAAACGGAGAATGTCATTTGAC AACTAGTTCAGGTGGGCCCGACGACACTGGTGACAATACTCCTCC GCCCACTGAAAAGCCCAAGCAAAAAAAGAAGAAACCTAAGAAGAC TAAGAAACCTAAGCGCAAGTCAAAGAAAGATCAGAAGGAGAACTT CTGACCAAAAGAACTAAACCTAAACCTGGCTAAATAAAGTTGAGAC ACTG	
203-HOMO	ATTCACATCCTCGTTGAGAACCAGTACACCAACAATGGGGTTGAC CGGAACCATGCTGGTGTGTTGGCAGTATCTCTGGCCTTCTTCGGGAG CGCTGCAGCCCACAATTGCCAGAACGGAACGAGACCCGCATCGG AGCAAAATAGAGAAGGCTGCGACTTACTGCTGGAACGCTGAGA CCAAATCATGGGACCAATTTTCTTTGGGAACGGAGAAAAATGCTT TTACAACAGTGGTGATCACGGAACATGTCAAACGGAGAATGTCAT TTGACAAATAATTCAGGTGGGCCCAACGAACTGATGACTATACTC CTGCGCCCACTGAGAAGCCGAAGCAAAAAAAGAAGAAACTAAGA AGACTAAGAAACCTAAGCGCAAGTCAAAGAAAGATCAGGAGAAAA	

	ACTTCTGATTAAACGCCCTAAAAATAAACAGGCTAAATAAATTTGA GACACC	
209-HOMO	ACTCACGTA CTGTTGAGAGCACTACACAAGCTATGGGGTTGACT GAGATCACGCTGGTGTGTTGGTGTCTCTGGCCTTCATCGCAACAGCT GCGGCCCAACAATTGCCAAAACGGAACGAGACCCGCCTCGGAGGA AAAACGAGAAGGCTGCGACTATTACTGCTGGAACGCTGAGACCAA CTCATGGGACAAATTTTTCTTCGGGAACGGAGAACGATGCTTTTAC AGCGATGGTAGTGAGGGATTGCGTCAAAACGGAGAATGCCATTTG ACAACCGATTCAAGTGTGCCCAATGACAGTGATGTCTAAACTCCTG CTCCCACTGGAAAGCCGAAGCAAAAAAAGAAACCGAAGAAGAGT AAGAAGCCTAAGGACAAGTCAAAGAAAAATTAGGAAGGGAATTTCT TATTAAACTCCCGAACTAAACCATGATTAATAAAGCTAAAGCMC GGT	
210-HOMO	ATTCACATCCTCGTTGAGAAGTAGTACACCAAGTATGGGGTTGACC GGAACCACGCTGGTGTGTTGGTGTGTGGCCTTCTTCGGCAGCGCT GCAGCCCACAATTGCCAGAACGGAACGAGACCCGCATCGGAGGA AAACCGAGAAGGCTGCGACTATTACTGCTGGAACCTGCGACCAA TTCATGGGACCAATTTTTCTTTGGGAACGGAGAAAAATGCTTTTAC AACACTGGCGAAAAGGGAATTTGTCAAAACGGAGAATGTCATTTGA CAAATAACTCAGGTGGGCCCAACAAGACTGATGACAATACTCCTG CGCCCACTGAAAAGCCTAAACAAAAAAGAAAGAACTAAGAAGA CTAAGAAACCAAAGCGCAAGTCAAAGAAAGATCAGTAGGAGAACT TCTCACTAGAAGAACTAAACCGAAACAGACTAAATAAAGTTGAGC ACACTGACAT	
214-HOMO	ACTTCACATCCTCGTTGAGAACCAGTACACCAACAATGGGGTTGA CCGGAACCATGCTGGTGTGTTGGCAGTATCTCTGGCCTTCTTCGGGA GCGCTGCAGCCCACAATTGCCAGAACGGAACGAGACCCGCATCG GAGCAAAATAGAGAAGGCTGCGACTATTACTGCTGGAACGCTGAG ACCAAATCATGGGACCAATTTTTCTTTGGGAACGGAGAAAAATGCT TTTACAACAGTGGTGATCACGGAACATGTCAAAACGGAGAATGTCA TTTGACAAATAATTCAGGTGGGCCCAACGAAACTGATGACTATACT CCTGCGCCCACTGAGAAGCCGAAGCAAAAAAAGAAAGAACTAA GAAGACTAAGAAACCTAAGCGCAAGTCAAAGAAAGATCAGGAGAA AAACTTCTGATTAAACGCCCTAAAAATAAACAGGCTAAATAAATTT GAGACACTG	
21-HOMO	GTTGAGAAGTAGTACCCCAAATATGGGGTTGACCGGAACCMCGCT GGTGTGTTGGTGTGTGTGGCCTTTTTTCGGCAGCGCTGCAGCCCMCA ATTGCCAGAACGGAACGAGACCCGCATCGGAGGAAAACCGAGAA GGCTGCGACTATTACTGCTGGAACCTGCGACCAATTCATGGGAC CAATTTTTYTTTGGGAACGGAGAAAAATGCTTTTACAACAMTGGCG CAAAGGGAATTTGTCAAAACGGAGAATGTCATTTGACAAATAATTC AGGTGGGCCCAACGAGACTGATGACAACACTCCTGCGCCCMCTG AAAAGCCCAAG	
220-HOMO	TTCACAACCTCGTTGAGGAGCAGTACACACACTATGGGGTTGACC GGAACCACGCTGGTGTGTTGGTGTCTCTGGCCTTCTTCGGGAGCGCT GCAGCCCACAATTGCAAGAACGGAACGAGACCCGCATCGGAGGA AAATCGTGAAGGCTGCGACTATTACTGCTGGAACGATGGCACCAA TTCATGGGACCAATCTTTCTTTGGGAACGGGGAAATCTGCTTTTAC	

	AACAGTGGTGAAAAGGGAATTTGTCAAACGGAGAATGTCATTTGACAAATAATTCAGGTGGGCCCAACGAGACTGATGACAATACTCCTGCGACCACTGAAAAGCCTAAGCAAAAAAAGAAGAAACTAAGAAGACTAAGAAACCTAAGCGCAAGTCAAAGAAAGATCAGTAGGAGAACTTCTCACTAGAAGAACTAAACCTAAATCATGTAAATAAAGTTGAGCACACCN	
22-HOMO	ACATACTCGTTGAGAGCACTACACAAGCTATGGGGTTGACTGAGGTCATGCTGGTGTGGTGTCTCTGGCCTTCGTCGCAACCGCTGCGGCCACGATTGCCAAAACGGAACGAGACCCGCCTCGGAAGAAAAGAGAGAAGGCTGCGACTATTACTGCTGGAACACCGAGACCAAATCATGGGACAAATTTTTCTTCGGGAACGGAGAACGATGCTTTTACAACAATGGTGATGAGGGATTATGTCAAATGGAGAATGCCATTTGACAACCGATTCAAGTGTACCCAATGACAACGATGTCTAACTCCTGCTCCCCTGAAAAGCCGAAGCAAAAAAAGAAGAAACCGAAGAAAAGTAAGAAGCCTAAGGGAAAAGTCAAAGAAAGATTAGGAAGGAACTTTTGATTAAAGACCCGAAACTAAACCAGGCTAAATAAAGCTAAGGCACTGACTGC	
24-HOMO	ATTCACATCCTCGTTGAGAACCAGTACACCAACAATGGGGTTGACCGGAACCATGCTGGTGTGGTATCTCTGGCCTTCTTCGGGAGCGCTGCAGCCCACAATTGCCAGAACGGAACGAGACCCGCATCGGAGCAAAATAGAGAAGGCTGCGACTATTACTGCTGGAACGCTGAGACCAATCATGGGACCAATTTTTCTTTGGGAACGGAGAAAAATGCTTTTACAACAGTGGTGATCACGGAACATGTCAAACGGAGAATGTCATTTGACAAATAATTCAGGTGGGCCCAACGAACTGATGACTATACTCCTGCGCCCACTGAGAAGCCGAAGCAAAAAAAGAAGAAACTAAGAAGACTAAGAAACCTAAGCGCAAGTCAAAGAAAGATCAGGAGAAAAACTTCTGATTAAACGCCCTAAAAATAAACCAGGCTAAATAAATTTGAGACAC	
32-HOMO	ATTCACATCCTCGTTGGGAACCAGTACACCAACAATGGGGTTGACCGGAACCACGCTGATGTTGGCAGTGTCTCTGGCCTTCTTCGGGAGCGCTGCAGCCCACAATTGCCAGAACGGAACGAGACCCGCATCGGAGCAAAATAGAGAAGGCTGCGACTATTACTGCTGGAACGCTGAGACCAAATCATGGGACCAATTTTTCTTTGGGAACGGAGAAAAATGCTTTTACAACAGTGGTGATCACGGAACATGTCAAACGGAGAATGTCATTTGACAAATAATTCAGGTGGGCCCAACGAACTGATGACTATACTGCTGCGCCCACTGAGAAGCCGAAGCAAAAAAAGAAGAAACTAAGAAGACTAAGAAGACTAAGCGCAAGTCAAAGAAAGATTAGGAGGAAAACCTTCTGATTAAACGCCCTAAAAATAAACCAGGCTAAATAAATTTGAGACTGACTGTAAAG	
35-HOMO	ATTCACATCCTCGTTGAGAACCAGTACACCAACAATGGGGTTGACCGGAACCACGCTGGTGTGGTGTCTCTGGCCTTCTTCGGGAGCGCTGCAGCCCACAATTGCCAGAACGGAACGAGACCCGCATCGGAGCAAAATAGAGAAGGCTGCGACTATTACTGCTGGAACGCTGAGACCAAATCATGGGACCAATTTTTCTTTGGGAACGGAGAAAAATGCTTTTACAACAGTGGTGATCGGGGGACATGTCAAATGGAGAATGTCATTGACAACTACTTCAGGTGGGCCCAACGAACTGATGACTATACTGCTGCGCCCACTGAGAAGCCGAAGC	
36-HOMO	ATTCACATCCTCGTTGAGAACCAGTACACCAACAATGGGGTTGAC	

	CGGAACCATGCTGGTGTGGCAGTATCTCTGGCCTTCTTCGGGAG CGCTGCAGCCCACAATTGCCAGAACGGAACGAGACCCGCATCGG AGCAAAATAGAGAAGGCTGCGACTATTACTGCTGGAACGCTGGGA CCAAATCATGGGACCAATTTTTCTTTGGGAACGGAGAAAAATGCTT TTACAACAGTGGTGATCACGGAACATGTCAAAACGGAGAATGTCAT TTGACAAATAATTCAGGTGGGCCCAACGAAACTGATGACTATACTC CTGCGCCCACTGAGAAGCCGAAAC	
3-HOMO	TTCACATCCTCGTTGAGAACCAGTACACCAACAATGGGGTTGACC GGAACCACGCTGGTGTGGTATCTCTGGCCTTCTTCGGGAGCGCT GCAGCCCACAATTGCCAGAACGGAACGAGACCCGCATCGGAGCA AAATAGAGAAGGCTGCGACTATTACTGCTGGAACGCTGAGACCAA ATCATGGGACCAATTTTTCTTTGGGAACGGAGAAAAATGCTTTTAC AACAGTGGTGATCACGGAACATGTCAAAACGGAGAATGTCATTG ACAAATAATTCGGGTGGGCCCAACGAAACTGATGACTATACTGCT GCGCCCACTGAGAAGCCGAAGCAAAAAAGAAGAAACTAAGAAG TCTAAGAAACCTAAGCGCAAGTCAAAGAAAGATTAGGAGGAAACT TCTGATTAAACGCCCTAAAAATAAACAGGCTAAATAAATTTGAAAA NG	
45-HOMO	ACATCCTCGTTGAGAAGTACACCAATATGGGGTGGACCGGA ACCACACTGGTGTGGTGTGTGGCCTTCTTCGGGAGTGCTGCA GCCACAATTGCCAGAACGGAACGAGACCCGCATCGGAGAAAAA CCGAGAAGGCTGCGACTATTACTGCTGGAACGAAGTCACCAATTC ATGGGACCAATTTTTCTTTGGGAACGGAGAAAAATGCTTTTACAAC ACTGGAGAACATGGAAAATGTCAAAACGGAGAATGTCATTTGACAA CTAGTTCAGATGGGCCCAACGAGACTGATGACAATACTCCTCCGC CCACTGAAAAGCCCAAGCAAAAAAGAAGAAACCTAAGAAGCCTA AGAAACCCAAGCGCAAGTCAAAGAAAGATCAGTAGGAGAACTTCT CACTAGAAGAAATAAACCTAAACCATGTAAATAAAGTTGAGCAACN CCG	
46-HOMO	CTCACGCACTCCTTGAGAGCGCTACAAAACTATGGGGTTGACTG AGATCACGCTGGTGTGGTGTCTCTGGCCTTCTTCGGGAGCGTTG TGGCCCAACAATTGCCAAACGGAACGAGACCCGCCTCGGAAGAAA ATAGAGAAGGCTGCGACTATTACTGCTGGAACGCTGAGACCAAAT CATGGGACCAATTTTTCTTTGGGAACGGAGAAAAATGCTTTTACAA CACTGGCGAAAAGGGAATTTGTCAAAACGGAGAATGTCATTTGAC AAATAATTCAGGTGGGCCCAACGAGACTGATGACAATACTCCTGC GCCCACTGAAAAGCCTAAGCAAAAAAGAAGAAACTAAGAAGAC TAAGAAACCTAAGCGCAAGTCAAAGAAAGATCAGTAGGAGAACTT CTCACTAGAAGAACTAAACCTAAACCAGGCTAAATAAAGTTGAGCA CACCNNN	
55-HOMO	ATTCACATCCTCGTTGAGAAGCCGCACACGCACTATGGGGTTGAC CGGAACCGCGCTGGTGTGGTGTCTCTGGCCTTCTTCGGGAGCG CTGCAGCCCACAATTGCCAGAACGGAACGAGACCCGCATCGGAG GAAAACCGAGAAGGCTGCGACTATTACTGCTGGAACGATGTCACC AATTCATGGGACCAATTTTTCTTTGGAAACGGAGAAAGATGCTTTT ACMACACTGGTGAAAATGGAAAATGTCTAAACGGAGAATGTCATTT GACAACCAAGTTCAGGKGGGCCCGACGACACTGGTGACAATACTCC TCCACCCGCTGAAAAGCCCMAGCAAAAAAGAAGAAACCTAAGAA	

	GCCTAAGAAACCTAAGCGCAAGTCAAAGAAAGATCAGTAGAGAAC TTCTGCCTAAAAAACTAAACCTATTCAGGCTAAATAAAGTTGAGC ACACTG	
67-HOMO	ACATCCTCGTTGAGAAGCTGCACACGCACTATGGGGTTGACCGGA ACCGCGCTGGTGTGGTGTCTCTGGCCTTCTTCGGGAGCGCTGCA GCCCACAATTGCCAGAACGGAACGAGACCCGCATCGGAGGAAAA CCGAGAAGGCTGCGACTATTACTGCTGGAACGATGTCACCAATTC ATGGGACCAATTTTTCTTTGAAACGGAGAAAGATGCTTTTACAAC ACTGGTGAAAATGGAATGTCTAAACGGAGAATGTCATTTGACAA CCAGTTCAGGTGGGCCCGACGACACTGGTGACAATACTCCTCCGC CCTACTGAAAAGCCCAAGCAAAAAAGAAGAAACCTAAGAAGCCTA AGAAACCTAAGCGCAAGTCAAAGAAAGATCAGTAGAGAACTTCTG CCTAAAAAACTAAACCTATTCAGGCTAAATAAAGTTGAGCACAC TGACTCTT	
BEKO4891	ATCATGCTGGTGTGGTGTCTCTGGCCTTCGTGCAACCGCTGCG GCCCACGATTGCCAAAACGGAACGAGACCCGCCTCGGATGAAAA GAGAGAAGGCTGCGACTATTACTGCTGGAACACCGAGACCAAATC ATGGGACAAATTTTTCTTCGGGAACGGAGAACGATGCTTTTACAAC AATGGTGATGAGGGATTATGTCAAACGGAGAGTGCCATTTGACA ACAGATTCAGGTGTGCCCAATGACACTGATGCAAAAATAGAAGAAA CCGAAGAAGAGTTAGAAGCCTAAGGGCAAGTCAAAGAAAAATTAG GAAGGAAACTTCTTATTAAGTCCCGAAACTAAACCAGG	
BEKO4892	ATCATGCTGGTGTGGTGTCTCTGGCCTTCGTGCAACCGCTGCG GCCCACGATTGCCAAAACGGAACGAGACCCGCCTCGGAAGAAAA GAGAGAAGGCTGCGACTATTACTGCTGGAACACCGAGACCAAATC ATGGGACAAATTTTTCTTCGGGAACGGAGAACGATGCTTTTACAAC AATGGTGATGAGGGATTATGTCAAACGGAGAGTGCCATTTGACA ACAGATTCAGGTGTGCCCAATGACACTGGTGCAAAAATAGAAGAA ACCGAAGAAGAGTTAGAAGCCTAAGGGCAAGTCAAAGAAAAATTA GGAAGGAACTTCTTATTAAGTCCCGAAACTAAACCAGG	
BEKO4893	ATCATGCTGGTGTGGTGTCTCTGGCCTTCGTGCAACCGCTGCG GCCCACGATTGCCAAAACGGAACGAGACCCGCCTCGGAAGAAAA GAGAGAAGGCTGCGACTATTACTGCTGGAACACCGAGACCAAATC ATGGGACAAATTTTTCTTCGGGAACGGAGAACGATGCTTTTACAAC AGTGGTGATGAGGGATTATGTCAAACGGAGAGTGCCATTTGACA ACAGATTCAGGTGTGCCCAATGACACTGATGCAAAAATAGAAGAAA CCGAAGAAGAGTTAGAAGCCTAAGGGCAAGTCAAAGAAAAATTAG GAAGGAACTTCTTATTAAGTCCCGAAACTAAACCAGG	
BEKO4894	ATCATGCTGGTGTGGTGTCTCTGGCCTTCGTGCAACCGCTGCG GCCCACGATTGCCAAAACGGAACGAGACCCGCCTCGGAAGAAAA GAGAGAAGGCTGCGACTATTACTGCTGGAACACCGAGACCAAATC ATGGGACAAATTTTTCTTCGGGAACGGAGAACGATGCTTTTACAAC AATGGTGATGAGGGCTTATGTCAAACGGAGAATGCCATTTGACA ACAGATTCAGGTGTGCCCAATGACACTGATGCAAAAATAGAAGAAA CCGAAGAAGAGTTAGAAGCCTAAGGGCAAGTCAAAGAAAAATTAG GAAGGAACTTCTTATTAAGTCCCGAAACTAAACCAGG	
BEKO4895	ATCATGCTGGTGTGGTGTCTCTGGCCTTCGTGCAACCGCTGCG GCCCACGATTGCCAAAACGGAACGAGACCCGCCTCGGAAGAAAA	

	GAGAGAAGGCTGCGACTATTACTGCTGGAACACCGAGACCAAATC ATGGGACAAATTTTTCTTCGGGAACGGAGAACGATGCTTTTACAAC AATGGTGATGAGGGATTATGTCAAACGGAGAGTGCCATTTGACA ACAGATTCAGGTGTGCCCAATGACACTGATGCAAAAATAGAAGAAA CCGAAGAAGAGTTAGAAGCCTAAGGGCAAGTCAAAGAAAAATTAG GAAGGAACTTCTTATTAAGTCCCGAACTAAACCAGG	
BEKO6691	ATCATGGTGGTGTGGTGTCTCTGGCCTTCGTGCGCAACCGCTGCG GCCCACGATTGCCAAAACGGAACGAGACCCGCCTCGGAAGAAAA GAGAGAAGGCTGCGACTATTACTGCTGGAACACCGAGACCAAATC ATGGGACAAATTTTTCTTCGGGAACGGAGAACGATGCTTTTACAAC AATGGTGATGAGGGATTATGTCAAACGGAGAGTGCCATTTGACA ACAGATTCAGGTGTGCCCAATGACACTGATGCAAAAATAGAAGAAA CCGAAGAAGGTTAGAAGCCTAAGGGCAAGTCAAAGAAAAATTAG GAAGGAACTTCTTATTAAGTCCCGAACTAAACCAGG	
BEKO6692	GGAAGAAAAGAGAGAAGGCTGCGACTATTACTGCTGGAACACCGA GACCAAATCATGGGACAAATTTTTCTTCGGGAACGGAGAACGATG CTTTTACAACAATGGTGATGAGGGATTATGTCAAACGGAGAGTGC CATTTGACAACAGATTCAGGTGTGCCCAATGACACTGATGCAAAA TAGAAGAAACCGAAGAAGAGTTAGAAGTCTAAGGGCAAGTCAAAG AAAAATTAGGAAGGAACTTCTTATTAAGTCCCGAACTAAACC AGGCTTAATAAAGATACGGTACCGT	
BEKO6693	ATCATGCTGGTGTGGTGTCTCTGGCCTTCGTGCGCAACCGCTGCG GCCCACGATTGCCAAAACGGAACGAGACCCGCCTCGGAAGAAAA AAGAGAAGGCTGCGACTATTACTGCTGGAACACCGAGACCAAATC ATGGGACAAATTTTTCTTCGGGAACGGAGAACGATGCTTTTACAAC AATGGTGATGAGGGATTATGTCAAACGGAGAGTGCCATTTGACA ACAGATTCAGGTGTGCCCAATGACACTGATGCAAAAATAGAAGAAA CCGAAGAAGAGTTAGAAGCCTAAGGGCAAGTCAAAGAANAATTAG GAAGGAACTTCTTATTAAGTCCCGAACTAAACCAGG	
BEKO6694	ATCATGCTGGTGTGGTGTCTCTGGCCTTCGTGCGCAACCGCTGCG GCCCACGATTGCCAAAACGGAACGAGACCCGCCTCGGAAGAAAA GAGAGAAGGCTGCGACTATTACTGCTGGAACACCGAGACCAAATC ATGGGACAAATTTTTCTTCGGGAACGGAGAACGATGCTTTTACAAC AATGGTGATGAGGGATTATGTCAAACGGAGAGTGCCATTTGACA ACAGATTCAGGTGTGCCCAATGACACTGATGCAAAAATAGAAGAAA CCGAAGAAGAGTTAGAAGCCTAAGGGCAAGTCAAAGAAAAATTAG GAAGGAACTTCTTATTAAGTCCCGAACTAAACCAGG	
BEKO48142	GAACGGAACGAGACCCGCATCGGAGCAAAATAGAGAAGGCTGCG ACTATTACTGCTGGAACGCTGAGGCCAAATCATGGGACCAATTTTT CTTTGGGAACGGAGAAAAATGCTTTTACAACAGTGGTGATCACGG AACATGTCAAACGGAGAAATGTCATTTGACAAATAATTCAGGTGGG CCCAACGAACTGATGACTATACTCCTGCGCCCACTGAGAAGCCG AAGCAAAAAAGAAGAACTAAGAAGACTAAGAAACCTAAGCGCA AGTCAAAGAAAGATCAGGAGAAAACTTATG	
BEKO48143	GAACGGAACGAGACCCGCATCGGAGCAAAATAGAGAAGGCTGCG ACTATTACTGCTGGAACGCTGAGACCAAATCATGGGACCAATTTTT CTTTGGGAACGGAGAAAAATGCTTTTACAACAGTGGTGATCACGG GACATGTCAAACGGAGAAATGTCATTTGACAAATAATTCAGGTGGG	

	CCCAACGAAACTGATGACTATACTCCTGCGCCCACTGAGAAGCCG AAGCAAAAAAAGAAGAAAATAAGAAGACTAAGAAACCTAAGCGCA AGTCAAAGAAAGATCAGGAGAAAAACTTATG	
BEKO48144	GAACGGAACGAGACCCGCATCGGAGCAAAATAGAGAAGGCTGCG ACTATTACTGCTGGAACGCTGAGACCAAATCATGGGACCAATTTTT CTTTGGGAACGGAGAAAAATGCTTTTACAACAGTGGTGATCACGG AACATGTCAAACGGGAGAATGTCATTTGACAAATAATTCAGGTGGG CCCAACGAAACTGATGACTATACTCCTGCGCCCACTGAGAAGCCG AAGCAAAAAAAGAAGAAAATAAGAAGACTAAGAAACCTAAGCGCA AGTCAAAGAAAGATCAGGAGAAAAACTTATG	
BEKO48145	GAACGGAACGAGACCCGCATCGGAGCAAAATAGAGAAGGCTGCG ACTATTACTGCTGGAACGCTGAGACCAAATCATGGGACCAATTTTT CTTTGGGAACGGAGAAAAATGCTTTTACAACAGTGGTGATCACGG AACATGTCAAACGGGAGAATGTCATTTGACAAATAATTCAGGTGGG CCCAACGAAACTGATGACTATACTCCTGCGCCCACTGAGAAGCCG AAGCAAAAAAAGAAGAAAATAAGAAGACTAAGAAACCTAAGCGCA AGTCAAAGAAAGATCAGGAGAAAAACTTATG	

[0211] EXAMPLE 9 - Purification of Polypeptides

[0212] Salivary gland extract (1.5 µl of salivary gland extract; 2 µg total protein) lengthens APTT from 59 to 171 seconds. Fractionation of salivary gland extract on a MonoQ chromatography column indicates that significant anticoagulant activity is in the unbound fraction that is expected to contain Salp14. Anticoagulant activity is also found in the bound fraction where Salp9A is expected to elute. Buffer and buffer plus 1 M NaCl do not affect coagulation. Immunoblotting showed shows that Salp14-related proteins are present in both the low-salt fractions (<0.25 M NaCl) and in high-salt fractions (>0.25 M NaCl).

[0213] In order to fractionate basic proteins such as Salp14, fractions containing proteins that do not bind to the MonoQ column are dialyzed against 50 mM HEPES pH 8 to replace the buffer. This material is then be applied to a HR5/5 MonoS cation exchange chromatography column (Amersham Pharmacia), and eluted with a NaCl gradient in 50 mM HEPES buffer.

[0214] In order to further purify proteins of interest, ion-exchange chromatography fractions are applied to a C4 reverse phase chromatography column (Vydac). Proteins are eluted with a 0-50 % gradient of acetonitrile in 0.1 % trifluoroacetic acid (TFA). Absorbance of the eluate is monitored at 280 nm or at 230 nm if a greater sensitivity is necessary. All fractions are lyophilized to remove the organic solvent and redissolved in 20 mM Tris-HCl pH 8 for further analysis.

[0215] Alternatively, we separate polypeptides by denaturing and non-denaturing gel electrophoresis using denaturing and non-denaturing gels. We concentrate tick saliva and separate 100 µg in a SDS PAGE gel at pH 7.8. The entire gel is minced and triturated prior to injection into five 500 g female

Hartley guinea pigs (20 µg salivary protein per guinea pig each). Concurrently, 20 µg of tick salivary protein is injected in incomplete Freund's adjuvant into each of five guinea pigs to serve as a positive control. After two weeks, animals are boosted with the same respective antigens. Ten days later, test bleeds from animals from both groups are collected and sera tested for antibodies by western blot. Two groups of negative controls are also maintained: the two groups of guinea pigs described above, and two negative control groups of animals. The gel control is guinea pigs immunized with tritured SDS PAGE gel with no antigen (the gel control) in it and the tick control consists of naive guinea pigs (the tick control). Each animal in the four groups is challenged with 40 nymphal ticks. The duration of attachment and weight of recovered ticks is measured.

[0216] EXAMPLE 10 - Anticoagulant Activity of Recombinant Salp9A and Salp14.

[0217] We expressed recombinant Salp9A and Salp14 in the pMAL fusion system with maltose binding protein (MBP) as a fusion partner. We constructed the Salp9A and Salp14 MBP fusions as follows. We PCR amplified the coding region of Salp14 using a forward primer corresponding to the predicted N-terminal end of the mature Salp14 (5' GAATTCGCCCCACAATTGCCAGAAC 3' (SEQ ID NO:) and a reverse primer (5' AAGCTTTCATAAGTTTTCTCCTG 3'; SEQ ID NO:) corresponding to the C-terminal end of Salp14 including the stop codon. We engineered EcoRI and HindIII restriction enzyme digestion sites into the 5' and 3' primers, respectively, to enable cloning into the pMAL-c2X expression vector (New England Biolabs, USA). We subcloned the PCR product into the EcoRI-HindIII digested pMAL-c2X plasmid vector and transformed into *E. coli* strain TB1. We selected transformants on LB-Ampicillin plates. The gene encoding the mature Salp9A protein was similarly cloned into the pMAL-c2X expression vector at the EcoRI and HindIII multiple cloning site, using 5' AGAATTCGCCCCACGATTGCC 3' (SEQ ID NO:) and 5' AAGCTTTTAGGCTTCTAAC 3' (SEQ ID NO:) as forward and reverse primers, respectively.

[0218] We purified the Salp9A and Salp14 MBP fusions as follows. We inoculated recombinant *E. coli* cells (500 µl) from an overnight culture into one liter of Luria broth (LB) medium supplemented with 100 µl (w/v) ampicillin and 2 mg/ml (w/v) glucose at 37°C for 2 hours on a laboratory shaker. We induced expression of the fusion protein by the addition of 100 mM isopropyl thiogalactoside (IPTG) to a final concentration of 0.3 mM and shaking at 37°C for 4 hours. We harvested the *E. coli* cells by centrifugation at 4,000xg for 20 min and suspended the pellet in 50 ml column buffer (20 mM Tris-HCl pH 7.4, 200 mM NaCl, 1 mM EDTA). We prepared lysates containing the expressed fusion protein by sonicating the *E. coli* followed by centrifugation at 10,000xg for 30 min. We chromatographed the

supernatant on an amylose affinity resin according to the manufacturer's protocol (New England Biolabs, MA). We eluted the bound fusion protein in twenty 2 ml fractions with column buffer containing 10 mM maltose. The recombinant proteins MBP-Salp9A and MBP-Salp14 were purified to near homogeneity as estimated by SDS-PAGE (Figure 10). The yield of purified recombinant proteins averaged about 3-5 mg/liter.

[0219] We tested various concentrations of recombinant Salp9A and Salp14 in the APTT assay as described above. We observed that recombinant MBP-Salp14 was able to prolong the clotting time of human plasma in a concentration dependent manner (Figure 11). We observed that MBP-Salp9A did not show any significant inhibition of the intrinsic coagulation pathway at similar concentrations. We observed that whole tick saliva was able to prolong coagulation time significantly even at a total protein concentration of 100 ng/100 μ l.

[0220] EXAMPLE 11 - Salp14 Inhibits Factor Xa Activity

[0221] We evaluated the inhibitory activity of recombinant MBP-Salp14 against a spectrum of proteases including thrombin as follows. We preincubated recombinant Salp14 (50 nM) with a 100 fold molar excess of several different enzymes for 15 min at room temperature followed by the addition of the appropriate chromogenic substrate. We monitored the kinetic rate of substrate hydrolysis (mA/min) at 405 nm was monitored over a period of 5 min as described above for the chromogenic assays for factor Xa. We evaluated the following enzyme/substrate combinations: bovine pancreatic trypsin (3 nM; Sigma, MO)/S-2302 (300 μ M; Diapharma, OH); bovine pancreatic α -chymotrypsin (3 nM; Sigma, MO)/Suc-Ala-Ala-Pro-Phe-p-nitroanilide (200 μ M; Bachem, Torrence, CA); human Factor XIIa (5 nM; Haematologic Technologies, Burlington, VT)/S-2302 (250 μ M; Diapharma, OH), α -thrombin (1 nM; Enzyme Research laboratories, IN)/S-2238 (250 μ M; Diapharma, OH) and human kallikrein (5 nM; Haematologic Technologies, VT)/S-2302 (400 μ M; Diapharma, OH). We expressed the initial results as % inhibition = $1 - (\text{inhibited rate} / \text{uninhibited rate}) \times 100$ (Cappello et al. 1995). We observed that at a 100 fold molar excess of inhibitor over the enzyme there was no significant inhibition of trypsin, chymotrypsin, Factor XIIa, kallikrein, plasmin or thrombin.

[0222] We monitored the inhibition of factor Xa by recombinant MBP-Salp14 by measuring the rate of hydrolysis of the chromogenic substrate S-2765 in the presence of increasing molar concentrations of recombinant Salp14 (10-300 nM) as follows. We diluted clotting factor Xa to 200 pM in 10 mM HEPES (pH 7.5) containing 0.1% bovine serum albumin and 150 mM NaCl (HBSA). We then incubated 5 μ l of Salp14 solution at room temperature for 15 min with 100 μ l of factor Xa in individual wells of a 96-well

microtiter plate. We then added 50 μ l of 1 mM S-2765 and measured substrate hydrolysis at 405 nm over a period of 5 min using a Vmax kinetic microplate reader. We calculated the results as percent inhibition of factor Xa activity using the formula: % inhibition = $[1 - (\text{inhibited rate}/\text{uninhibited rate})] \times 100$. To monitor the kinetics of Salp14A-dependent inhibition of factor Xa activity, we measured the rate of hydrolysis of the chromogenic substrate S-2765 in the presence of increasing molar concentrations of Salp14A. We observed that recombinant Salp14A and Salp9A inhibited factor Xa activity suggesting that the anticoagulant activity is mediated by factor Xa inhibition. The results were expressed as a ratio of substrate hydrolysis in the presence (V_i) or absence (V_0) of MBP-Salp14 or MBP-Salp9A or adult tick saliva and plotted to evaluate the relative inhibition. We observed that recombinant Salp14 was a specific inhibitor of Factor Xa (Figure 12). MBP-Salp9A did not show inhibition of factor Xa in the chromogenic assay (data not shown).

[0223] We monitor inhibition of multiple coagulation related proteins by the polypeptides of the invention as follows. We use a single-stage chromogenic assay to measure the inhibition of thrombin. Briefly, 500 pM human α -thrombin (Haemotologic Technologies, Burlington, VT) in 100 μ l HBSA (25 mM Hepes, pH 7.5) is added to polypeptide (1 pM to 1 μ M) in individual wells of a microtiter plate. After incubating at 25°C for 15 min, 50 μ l of chromogenic substrate, S2238 (Diapharma, West Chester, OH) at a final concentration of 250 μ M is added. The rate of hydrolysis of the chromogenic substrate is measured (OD_{405}/min) over 5 min using a kinetic microplate reader (Dynex, Chantilly, VA). A curve of the inhibited rate of thrombin mediated hydrolysis of the chromogenic substrate is plotted over the range of inhibitor (polypeptide) concentrations used. These data are used to derive the apparent K_i for the polypeptides. *I. scapularis* saliva (0.5-2 μ g) is also tested for its ability to inhibit thrombin activity.

[0224] We also monitor inhibition of Factor XIIa, factor VIIa and Kallikrein. We use a single stage chromogenic assay to monitor the inhibitory activity of polypeptides of the invention on Kallikrein, factor VIIa and factor XIIa essentially as described for thrombin inhibition assay. Chromogenic substrates S2288 and S2302 and Spectrozyme XIIa are from American Diagnostica (Greenwich, CT). Kallikrein and factor XIIa is obtained from Enzyme Research Laboratories (South Bend, IN). Recombinant human factor VIIa is purchased from BioPacifica (Emeryville, CA). Kallikrein (500 pM), factor VIIa (1000 pM) or factor XIIa (250 pM) is incubated with varying concentrations of polypeptide (1 pM-1 μ M) at 25°C for 15 min. The chromogenic substrate S2302 (350 μ M) or S2288 (250 μ M) or Spectrozyme XIIa (100 μ M) is added and its rate of hydrolysis monitored over 5 min using a kinetic microplate reader (Dynex, Chantilly, VA). *I. scapularis* saliva (0.5-2 μ g) is also simultaneously compared for its inhibitory effect on factor XIIa, factor VIIa and Kallikrein.

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[0225] EXAMPLE 12 - Antibodies to MBP-Salp14 Recognize Proteins in Tick Saliva

[0226] We analyzed adult *I. scapularis* saliva (0.5 ug) on a western blot for the presence of antigens reacting with recombinant MBP-Salp14 antisera as follows. Guinea pigs were immunized subcutaneously with either 20 mg of recombinant MBP-Salp14 in complete Freund's adjuvant followed by two boosts in incomplete Freund's adjuvant at 2 and 4 weeks. Guinea pig antisera against recombinant MBP-Salp14 was collected 10 days after the final boost. Antisera from tick-immune guinea pigs were generated as described earlier (Das et al. *supra*). Briefly, 3 guinea pigs were sensitized to ticks by three infestations with 100 nymphs/animal, with a resting period of 21 days between challenges. To obtain tick-immune guinea pig sera, the animals were sacrificed 2 weeks after the final challenge and blood collected by cardiac puncture.

[0227] Recombinant MBP-Salp14 (0.5 mg), recombinant MBP-Salp9A (0.5 mg), the MBP fusion tag (0.5 mg) and adult tick saliva (1 mg) were electrophoresed on a 12% SDS-PAGE, and proteins transferred to duplicate nitrocellulose membranes. The nitrocellulose membranes were separately incubated with tick-immune guinea pig serum and naive guinea pig serum. The bound antibodies were detected using horseradish peroxidase-conjugated goat anti-rabbit antibodies (Sigma-Aldrich, St Louise, MO). The blot was developed using the Western Lightening Chemiluminescence kit (PerkinElmer Life Sciences, Boston, MA). A predominant protein corresponding to a molecular mass of 28 kD hybridized to anti-recombinant MBP-Salp14 (Figure 13C). A protein band corresponding to a molecular mass of approximately 18 kD also reacted weakly with anti-MBP-Salp14 antisera (Figure 13C). Tick immune guinea pig sera also predominantly recognized a 28 kD protein in tick saliva in addition to other cross hybridizing antigens (compare Figure 13B to Figure 13A). Tick immune guinea pig sera reacted avidly with recombinant Salp14, and weakly with recombinant Salp9A (Figure 13B to Figure 13A).

[0228] EXAMPLE 13 - Antibodies to Recombinant MBP-Salp14 Recognize Predominant Salivary Anticoagulants

[0229] We separately incubate adult tick saliva (1 mg) with tick-immune or MBP-Salp14 at a dilution of 1:50 for 1h at room temperature. The saliva was separated from antigen-antibody complex using Protein A/G coated Sepharose beads (Pierce, Rockford, IL). The depleted saliva was evaluated for inhibition of coagulation by the APTT assay as described elsewhere herein.

[0230] Absorption of salivary proteins with anti-recombinant Salp14 sera abolished 87 % of the salivary anticoagulant activity (Figure 14). Absorption with tick immune guinea pig sera reduced the anticoagulant activity of tick saliva in the APTT assay to background levels (i.e., plasma incubated with PBS) (Figure 14). Absorption with naive guinea pig or anti MBP guinea pig sera did not reduce the anticoagulant activity of saliva significantly (Figure 14).

[0231] EXAMPLE 14 - Expression and Purification of Polypeptides

[0232] We express the polypeptides in both prokaryotic and eukaryotic systems. In prokaryotic systems, we typically express and purify the polypeptides as fusion proteins. For example, we use the pBAD/TOPO ThioFusion (pBAD vector), Glutathione S-transferase (GST) fusion expression system (pMX vector) and Maltose-binding protein (MBP) fusion system (pMAL c2X vector) because of their simplicity in cloning, expression and purification of recombinant proteins. For each polypeptide, we synthesize 5' and 3' primers specific for the encoding gene with appropriate restriction endonuclease sites for specific expression systems. We generally use pBluescript vectors with the genes of interest, excised from the Lambda ZAP[®] II expression library, as the DNA templates for these reactions. For expression in bacterial systems, we typically design primers to amplify DNA sequences encoding the polypeptides without any secretory signal sequences. We gel-purify the DNA bands using a Qiagen gel-purification kit (Qiagen). For expression in the pBAD/TOPO thioredoxin protein expression system, 10 ng of PCR products in 4 µl of sterile water are mixed with 1 µl pBAD/Thio-TOPO vector and incubated for 5 minutes at room temperature. 2 µl of the TOPO Cloning reaction are added into a vial of 50 µl One Shot cells (Invitrogen) and transformed by heat shock for 30 seconds at 42°C. We spread 10 µl from each transformation on a prewarmed plate and incubate overnight at 37°C. For expression of polypeptide-encoding genes as GST fusion proteins, the PCR product for each gene and a modified pJEX2T vector (pMX vector) is digested with 2 appropriate restriction enzymes, fragments are gel-purified and then ligated at 14°C overnight. We transform *E. coli* DH5α cells by heat shock with 1 µl of ligation mixture. To generate MBP fusion proteins, pMAL C2X vector (New England Biolab) and the polypeptide-encoding gene fragments are double digested with specific endonucleases, ligated and then used to transform competent *E. coli* cells.

[0233] We also express the polypeptides in *Drosophila* expression systems because both Salp9A and Salp14 contain predicted N-linked glycosylation and myristylation sites. These posttranslational modifications may have a role in protein folding and catalytic activity and these eukaryotic posttranslational modifications will not be incorporated in the proteins expressed in a bacterial

expression system. Gene-specific primers containing NcoI and XhoI sites are used to generate a PCR product corresponding to the polypeptide using fed salivary gland cDNA as template. The PCR product is gel purified as described elsewhere and digested with NcoI and XhoI. The double digested product is ligated with NcoI and XhoI digested pMT/BIP/V5/His vector (Invitrogen, Carlsbad, CA) at 16°C overnight. Transformants containing the recombinant plasmid are selected on LB/Amp plates. Plasmid DNA is isolated and the DNA sequence is confirmed. Transfection of S2 cells with the recombinant pMT/BIP/V5/His-polypeptide is carried out essentially according to the manufacturer's protocol (Invitrogen, CA) and hygromycin resistant stable transfectants selected. The expression of recombinant polypeptide is induced by the addition of copper sulphate. Recombinant protein expression in the medium is monitored by western blot using V5 antibody. Purification of the protein is carried out by affinity chromatography on Probond™ using the manufacturer's protocol.

[0234] We purify the fusion proteins by affinity chromatography. We use nickel resins to purify thioredoxin fusion proteins from *E. coli* and the recombinant proteins made in the *Drosophila* expression system. We use glutathione sepharose resins and Amylose resins to purify GST fusion proteins and MBP fusion proteins, respectively. Since the MBP fusion tag is 42 kD, this large fusion tag may interfere with the anticoagulant activity of recombinant polypeptides. Therefore, we also cleave the fusion partner away from the recombinant anticoagulant proteins. An enterokinase site is engineered between the multiple cloning site and the MBP fusion tag of pMAL-p2E vectors. Recombinant MBP-polypeptide (~ 50 µg) is incubated with 0.5 µg or 0.5 units of enterokinase at room temperature or 4°C for varying amounts of time. We monitor cleavage efficiency by SDS-PAGE electrophoresis. We adjust cleavage conditions to obtain optimal yield of cleaved recombinant polypeptides. The cleaved protein is purified away from enterokinase and the cleaved MBP fusion partner by DEAE ion exchange chromatography, according to the manufacturers protocol (New England Biolabs, Beverly, MA).

[0235] EXAMPLE 15 - DNA Vaccines

[0236] We prepare DNA vaccines to the polypeptides of the invention. Such vaccines are potent immunogens producing good titers of antibodies as well as cell mediated reactions. The DNA vaccine can also be tailored to contain several polypeptides, parts or fragments as well as antigens from other pathogen from which we wish to protect the host.

[0237] We prepare a DNA vaccine using Salp14. We amplify the gene encoding Salp14 by PCR using specific primers carrying BamHI restriction sites. We clone the PCR product into the TOPO TA cloning vector PCRII (Invitrogen) following the manufacturer's specifications. We transform TOP 10

cells (Invitrogen) with the ligation mixture and incubate the cells overnight at 37°C. We pick several colonies and mix with 10 µl of sterile water. We transfer 5 µl of each sample to Luria broth with ampicillin (100 µg/ml) and grow at 37°C. We use the other 5 µl as a template for a PCR reaction using two vector-specific primers to confirm the presence of the insert and for sequencing analysis. After visualization of the PCR product on a 1.0% agarose gel, we completely sequence the PCR products.

[0238] We choose a sample that contains the sequence from the amino terminus to the stop codon of the Salp14 gene including the incorporated BamHI sites. We grow cells containing the plasmid carrying the Salp14 gene overnight at 37°C in Luria broth with ampicillin (100 µg/ml), and we isolate the plasmid isolation using the Wizard Miniprep kit (Promega). The plasmid containing the Salp 14 gene with incorporated BamHI sites is digested with BamHI and then ligated with BamHI predigested VR1020 DNA plasmid vector (V1CAL). The V1020 plasmid contains a kanamycin resistance gene, the human cytomegalovirus promoter, and the tissue plasminogen activator signal peptide upstream of the BamHI cloning site. The ligation reaction between the BamHI digested Salp14 gene and similarly digested VR1020 DNA vector is done overnight at 16°C and used to transform TOP 10 cells (Invitrogen). Cells are incubated on a Luria broth kanamycin (30 µg/ml) plate overnight at 37°C. We pick colonies and mix with 10 µl of sterile water. 5 µl of each sample are transferred to Luria broth with kanamycin (100 µg/ml) and grown at 37°C. The other 5 µl are used as a template for a PCR reaction using two vector-specific primers from the VR1020 vector to confirm the presence of the insert and for sequencing analysis. After visualization of the PCR product on a 1.0% agarose gel, we sequence the PCR products. For the vaccine construct, we choose a sample that contains the sequence from the amino terminus to the stop codon in the right orientation and in the correct open-reading frame after the tissue plasminogen activator signal peptide.

[0239] The concentrations of the samples are measured by UV absorbance, and then stored at -70°C before immunization experiments. Control plasmids consisting of the vector alone or vector with an insert of the *Ehrlichia* HGE 44 kD gene are used as controls. In the present instance, the use of the *Ehrlichia* gene is as a non cross-reactive control for the *Borrelia* and polypeptides which we are studying.

[0240] Guinea pigs are immunized with Salp14 plasmids and the development of antibodies are ascertained. Subsequent to successful immunization, hosts are challenged with five ticks infected with *B. burgdorferi* and 2 hrs later an additional 30 uninfected ticks are placed on the same animal. This allows us to study both tick immunity and effects on transmission in the same experiment.

[0241] EXAMPLE 16 - Identification of Portions of Polypeptides with Anticoagulant Activity

[0242] We compare the sequences of the polypeptides to each other and to the primary structures of TAP, a factor Xa inhibitor from the tick *Ornithodoros moubata* and a factor Xa inhibitor from the tick *Ornithodoros savignyii* using the alignment Clustal W alignment program available at EMBL, European Bioinformatics Institute (www2.ebi.ac.uk). For example, we observed that Salp-9A and Salp-14 show limited conservation in amino acid residues with TAP and factor Xa inhibitor from *O. savignyii* (Figure 6). All four tick anticoagulants contain 6 cysteine residues and could thus potentially form three disulphide bridges. The three native disulphide bonds in TAP are cys5-cys59, cys15-cys39, cys33-cys55 (Sardana et al., 1991).

[0243] Salp-9 and Salp-14 are about 90% identical in their first 52 residues, but diverge dramatically in their C-terminal residues. This similarity indicates that the first 52 residues are likely to contain the anticoagulant activity. We perform deletion analysis of Salp-9 and Salp-14 and assay the resulting portions for anticoagulant activity as described earlier.

[0244] We prepare a deletion of Salp-9 as follows. We synthesize a 5' primer specific to the N-terminal end of Salp9A, encoding amino acid residue 1-13 and a 3' primer encoding residues 59-48. Restriction sites compatible for cloning into the pMAI-p2E bacterial expression vector (EcoRI and XbaI) or pMT/BIP/V5/His Drosophila expression vector (NcoI and XhoI) are engineered into the primers for expression in the different cells. The primers are used to PCR amplify the region of Salp9A encoding residues 1-59 using the recombinant plasmid encoding the full-length Salp9A as template. The amplicon generated, is gel purified as described earlier. The PCR product is restriction digested with the appropriate enzymes and ligated into the expression vector of choice as described above. The transformants are selected and recombinant protein expression induced and purified in the bacterial or Drosophila expression vector following the manufacturers protocol as described above. The purified Salp9A deletion polypeptide is stored appropriately and tested in the anticoagulant and inhibition assays as described.

[0245] We prepare a deletion of Salp-14 as follows. We synthesize a 5' primer specific to the N-terminal end of IS AP-14, encoding amino acid residue 1-13 and a 3' primer encoding residues 59-48. Restriction sites are engineered into the primers appropriately as described for the construction of the deletion in Salp-9A. The construct, rSalp-141-59 differs from rSalp-91-59 at amino acid residues 2, 13, 14, 25, 32, 40, 45, 48 and 50 (Figure 15, indicated by circles). The primers are used to PCR amplify the region of IS AP-14 encoding residues 1-59 using the recombinant plasmid encoding the full-length Salp14 as template. The ligation of the amplicon into the expression vector and recombinant protein

expression is carried out as described above. The purified Salp-14 deletion polypeptide is purified and assayed as described above.

[0246] Disulphide bridges play a crucial role in the biological activity of many small molecular weight anticoagulants. We identify the cysteines involved in critical disulphide bridges in the polypeptides of the invention. A comparison of the primary sequence of Salp9A and Salp14 with known tick anticoagulants did not reveal information on the potential disulphide bridges (Figure 6). When we used the 3D-PSSM Protein Fold Recognition (Threading) Web Server V 2.0(www.bmm.icnet.uk/servers/3dpssm) to derive a structural model of Salp9A, we observed a similarity to the *Ancylostoma caninum* anticoagulant protein NAPc2 at the secondary structure level (Duggan et al, 1999). NAPc2 contains 10 cysteines and 5 disulphide bridges. Salp9A and Salp14 contain only 6 cysteines and can potentially form three disulphide bridges. A structural model of Salp9A was derived based on the three-dimensional position-specific scoring matrix (3D-PSSM) (Kelley et al., "Enhanced Genome Annotation Using Structural Profiles in the Program 3D-PSSM," J. Molec. Biol. 299:499-520 (2000)). The three native disulphide bridges on Salp9A and Salp14 are likely to be Cys3, Cys41, Cys18, Cys51, Cys22, Cys56. Cysteines at positions 3 and 41, 18 and 51, 22 and 56 of the pMT/BIP/V5/His-Salp9A/Salp14 or pMAL-p2E-Salp9A /Salp14 are systematically replaced with glycine in 6 different constructs. Exchange of glycine for cysteine is carried out using the QuikChange™ XL site-directed mutagenesis kit (Stratagene, CA) following the manufacturer's protocol. The DNA constructs are sequenced to confirm the exchange of cysteine to glycine. Each of these constructs is then transformed into the appropriate expression system and recombinant protein purified as described above. The recombinant proteins are assayed as described above.

[0247] EXAMPLE 17- *in vivo* Gene Silencing Inhibits Anticoagulation Activity

[0248] We demonstrated that this family of proteins has anticoagulant activity and their importance in tick feeding using RNA interference (RNAi) methods. We obtained cDNA from adult salivary glands and used it as template to amplify DNA encoding the full-length salp14, full-length salp9A (also designated as "salp9pac" or "9pac"), and actin (GenBank™ Accession No. AF426178). We purified and cloned the resultant amplicons and synthesized double-stranded RNA (dsRNA) complementary to the DNA insert by *in vitro* transcription using the Megascript™ RNAi kit (Ambion Inc, TX). We injected approximately 0.5 µl of dsRNA (approximately 5x10¹⁰ molecules) corresponding to ds salp14 and ds salp9A individually or in combination or ds actin into the idiosum of adult *I. scapularis* females using 10 µl Drummond microdispensers (Drummond, PA) drawn to fine point needles using a micropipette puller

(Sutter Instruments, CA). We loaded the needles onto a micromanipulator (Narishige, Japan) connected to a Nanojet microinjector (Drummond, PA). We injected control ticks with 1 μ l of injection buffer (10 mM Tris-HCl pH 7.5, 1 mM EDTA). We used about 20 ticks in each group. The ticks were allowed to rest for a day prior to placement on the ears of New Zealand white rabbits along with uninjected male ticks. Ticks that fell off upon repletion or those that remained attached after 6 days were collected and weighed on a digital balance.

[0249] We confirmed that the microinjections silenced expression by northern blot analysis using probes to Salp9A, Salp14 and by western blot analysis using antibodies raised against Salp14 as described in Example 7. Because of the sequence similarity between Salp9A, Salp14 and the other members of the family, there is cross-hybridization between them at the nucleic acid level and antibodies raised against Salp14 also recognize other members of the family. Accordingly, we were unable to determine whether the effect was specific to any particular member of the family. However, we detected expression of mRNA corresponding to Salp25D in both experimental and mock-injected groups, which confirmed that the silencing effect was specific to the Salp14 family of anticoagulant proteins described herein. We observed that injection of ticks with dsRNA for Salp9A, Salp14, Salp9A and Salp14, or actin resulted in impaired feeding (Figure 16).

[0250] We performed Factor Xa inhibition assays to determine whether the impaired feeding was due to inhibition of anticoagulant activity *in vivo*. We diluted clotting factor Xa (Enzyme Research Laboratories, Southbend, IN) to 2 nM in 10mM HEPES (pH 7.5) containing 0.1% bovine serum albumin and 150 mM NaCl (HBSA). We separately incubated duplicate samples of salivary gland extracts from experimental and mock-injected ticks (0.5- 10 μ g of protein) with 100 μ l of factor Xa for 15 min at room temp in a 96-well microtiter plate. We added 50 μ l of 1mM S-2765 (DiaPharma, West Chester, OH) and measured substrate hydrolysis at 405 nm over a period of 5 min using a kinetic microplate reader. The results are expressed as a ratio of substrate hydrolysis in the presence (V_i) or absence (V_0) of adult tick salivary gland extract and plotted to evaluate the relative inhibition. We observed that 10 μ g of mock-injected salivary gland extract inhibited 80% of factor Xa activity in this assay, whereas salivary gland extracts from ticks lacking Salp14 family proteins inhibited only 10-40% of factor Xa activity (Figure 17). Thus, the absence or reduction of expression of the Salp14 family reduced the ability of tick salivary gland extracts to inhibit factor Xa, demonstrating that they have anticoagulant activity.

What is Claimed is:

1. An isolated polypeptide comprising an amino acid sequence selected from the group consisting of:

- (a) Salp9A (SEQ ID NO: _);
- (b) Salp14 (SEQ ID NO: _);
- (c) H1 protein (SEQ ID NO: _);
- (d) H2 protein (SEQ ID NO: _);
- (e) H3 protein (SEQ ID NO: _);
- (f) H7 protein (SEQ ID NO: _);
- (g) L1 protein (SEQ ID NO: _);
- (h) L2 protein (SEQ ID NO: _);
- (i) L3 protein (SEQ ID NO: _);
- (j) L4 protein (SEQ ID NO: _);
- (k) L8 protein (SEQ ID NO: _);
- (l) M1 protein (SEQ ID NO: _);
- (m) 128-HOMO protein (SEQ ID NO: _);
- (n) 13-HOMO protein (SEQ ID NO: _);
- (o) 14-HOMO protein (SEQ ID NO: _);
- (p) 154-HOMO protein (SEQ ID NO: _);
- (q) 155-HOMO protein (SEQ ID NO: _);
- (r) 156-HOMO protein (SEQ ID NO: _);
- (s) 159-HOMO protein (SEQ ID NO: _);
- (t) 168-HOMO protein (SEQ ID NO: _);
- (u) 188-HOMO protein (SEQ ID NO: _);
- (v) 190-HOMO protein (SEQ ID NO: _);
- (w) 191-HOMO protein (SEQ ID NO: _);
- (x) 199-HOMO protein (SEQ ID NO: _);
- (y) 19-HOMO protein (SEQ ID NO: _);
- (z) 203-HOMO protein (SEQ ID NO: _);
- (aa) 209-HOMO protein (SEQ ID NO: _);
- (ab) 210-HOMO protein (SEQ ID NO: _);
- (ac) 214-HOMO protein (SEQ ID NO: _);

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(ad) 21-HOMO protein (SEQ ID NO: _);
(ae) 220-HOMO protein (SEQ ID NO: _);
(af) 22-HOMO protein (SEQ ID NO: _);
(ag) 24-HOMO protein (SEQ ID NO: _);
(ah) 32-HOMO protein (SEQ ID NO: _);
(ai) 35-HOMO protein (SEQ ID NO: _);
(aj) 36-HOMO protein (SEQ ID NO: _);
(ak) 3-HOMO protein (SEQ ID NO: _);
(al) 45-HOMO protein (SEQ ID NO: _);
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(ax) BEKO6694 protein (SEQ ID NO: _);
(ay) BEKO48142 protein (SEQ ID NO: _);
(az) BEKO48143 protein (SEQ ID NO: _);
(ba) BEKO48144 protein (SEQ ID NO: _);
(bb) BEKO48145 protein (SEQ ID NO: _); and
(bc) an amino acid sequence having at least 75% sequence identity to any one of (a)-(bb).

2. The polypeptide of claim 1, wherein the polypeptide has anticoagulant activity.
3. The polypeptide of claim 2, wherein the polypeptide is capable of inhibiting Factor Xa activity.
4. A portion of the polypeptide of claim 1, wherein the portion has anticoagulant activity.

5. The portion of claim 4, wherein the portion is capable of inhibiting Factor Xa activity.
6. A fragment of the polypeptide of claim 1 comprising at least 5 amino acids taken as a block.
7. The fragment of claim 6, wherein the fragment is immunogenic.
8. A fusion protein comprising a component selected from the group consisting of: the polypeptide of any one of claims 1-3; the portion of claim 4 or 5; and the fragment of claim 6 or 7.
9. A nucleic acid molecule comprising a nucleic acid sequence encoding a component selected from the group consisting of: the polypeptide of any one of claims 1-3; the portion of claim 4 or 5; the fragment of claim 6 or 7; and the fusion protein of claim 8.
10. A host cell comprising the nucleic acid molecule of claim 9.
11. The host cell of claim 10, wherein the host cell is selected from the group consisting of: a bacterial cell, a fungal cell, a plant cell and an animal cell.
12. The host cell of claim 11, wherein the host cell is an *Escherichia coli* cell.
13. The host cell of claim 11, wherein the host cell is an insect cell.
14. The host cell of claim 13, wherein the host cell is a *Drosophila* cell.
15. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and a component selected from the group consisting of: the polypeptide of any one of claims 1-3; the portion of claim 4 or 5; the fragment of claim 6 or 7; and the fusion protein of claim 8.
16. The pharmaceutical composition of claim 15, wherein the component is cross-linked to an immunogenic carrier.

17. The pharmaceutical composition of claim 15 or 16, further comprising a non-*I. scapularis* polypeptide.
18. The pharmaceutical composition of any one of claims 15-17, further comprising an adjuvant.
19. The pharmaceutical composition of claim 15, further comprising a non-*I. scapularis* anticoagulant.
20. The pharmaceutical composition of claim 19, wherein the non-*I. Scapularis* anticoagulant is selected from the group consisting of: unfractionated heparin, low molecular weight heparin, aspirin, hirudin, bivalirudin, antistasin, lefaxin, DX9065a, TFPI, and NAPc2.
21. A method for conferring tick immunity comprising the step of administering to a subject the polypeptide of any one of claims 1-3; the portion of claim 4 or 5; the fragment of claim 6 or 7; or the fusion protein of claim 8.
22. A method for conferring tick immunity comprising the step of administering to a subject the pharmaceutical composition of any one of claims 15-18.
23. A method for preventing infection by a tick-borne pathogen or a tick-borne disease comprising the step of administering to a subject the polypeptide of any one of claims 1-3; the portion of claim 4 or 5; the fragment of claim 6 or 7; or the fusion protein of claim 8.
24. A method for preventing infection by a tick-borne pathogen or a tick-borne disease comprising the step of administering to a subject the pharmaceutical composition of any one of claims 15-18.
25. A method for treating a condition where anticoagulant therapy is indicated comprising the step of administering to a subject the polypeptide of any one of claims 1-3 or the portion of claim 4 or 5.
26. A method for treating a condition where anticoagulant therapy is indicated comprising the step of administering to a subject the pharmaceutical composition of any of claims 15 and 19-20.

27. A method for detecting tick immunity or tick bite comprising the step of contact a body fluid of a subject with a component selected from the group consisting of: the polypeptide of any one of claims 1-3; the portion of claim 4 or 5; the fragment of claim 6 or 7; and the fusion protein of claim 8.
28. A kit comprising a component selected from the group consisting of: the polypeptide of any one of claims 1-3; the portion of claim 4 or 5; the fragment of claim 6 or 7; and the fusion protein of claim 8.
29. The kit of claim 28 further comprising a means for detecting binding of said component to an antibody.
30. The kit of claim 28 or 29 further comprising instructions for performing the method of claim 27.
31. An antibody, or antigen-binding portion thereof, that binds to the polypeptide of claim 1.
32. The antibody of claim 31, wherein the antibody is polyclonal.
33. The antibody of claim 31, wherein the antibody is monoclonal.
34. A kit comprising the antibody of any one of claims 31-33.
35. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and the antibody of any one of claims 31-33.
36. A vaccine comprising the antibody of any one of claims 31-33.
37. A method for conferring tick immunity comprising administering to a subject the antibody of any one of claims 31-33, the pharmaceutical composition of claim 35 or the vaccine of claim 36.

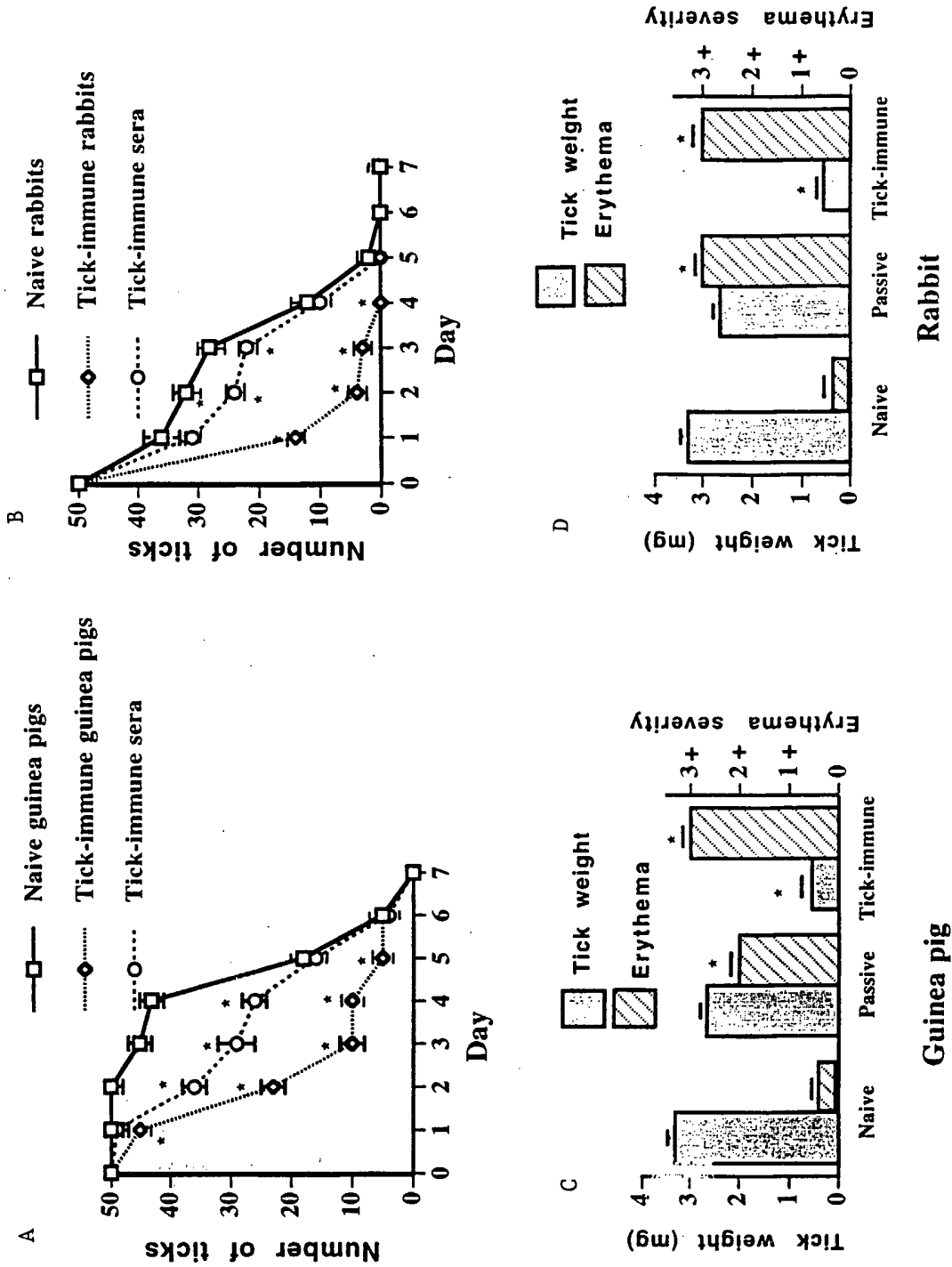


Figure 1

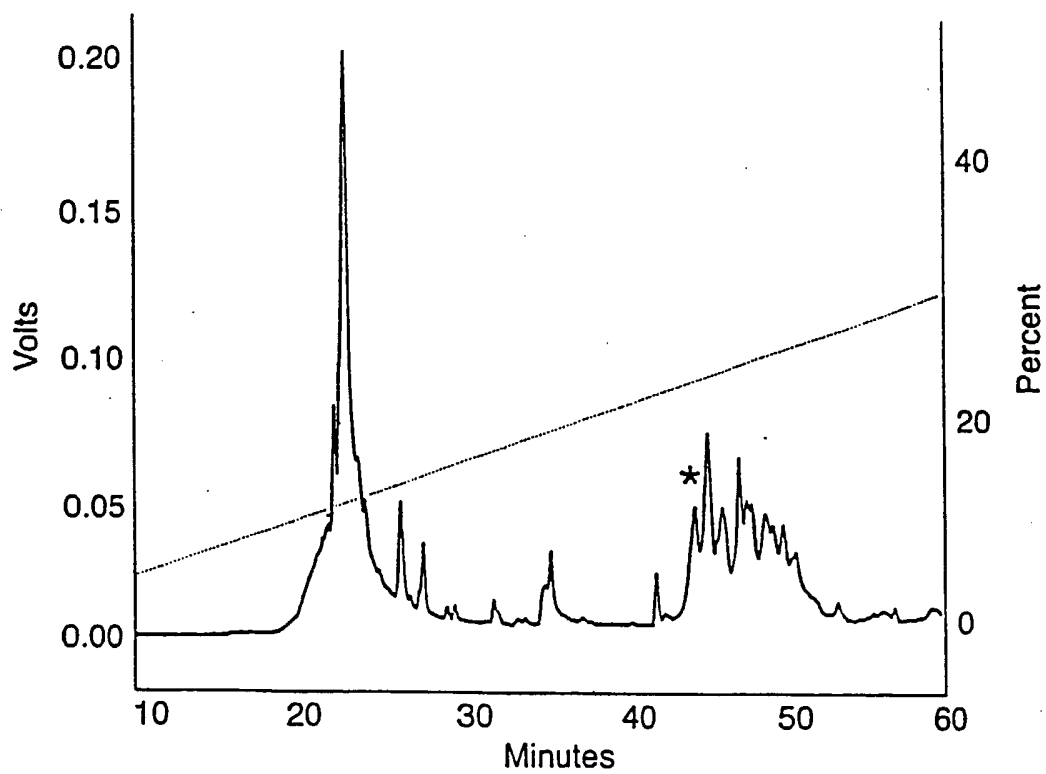
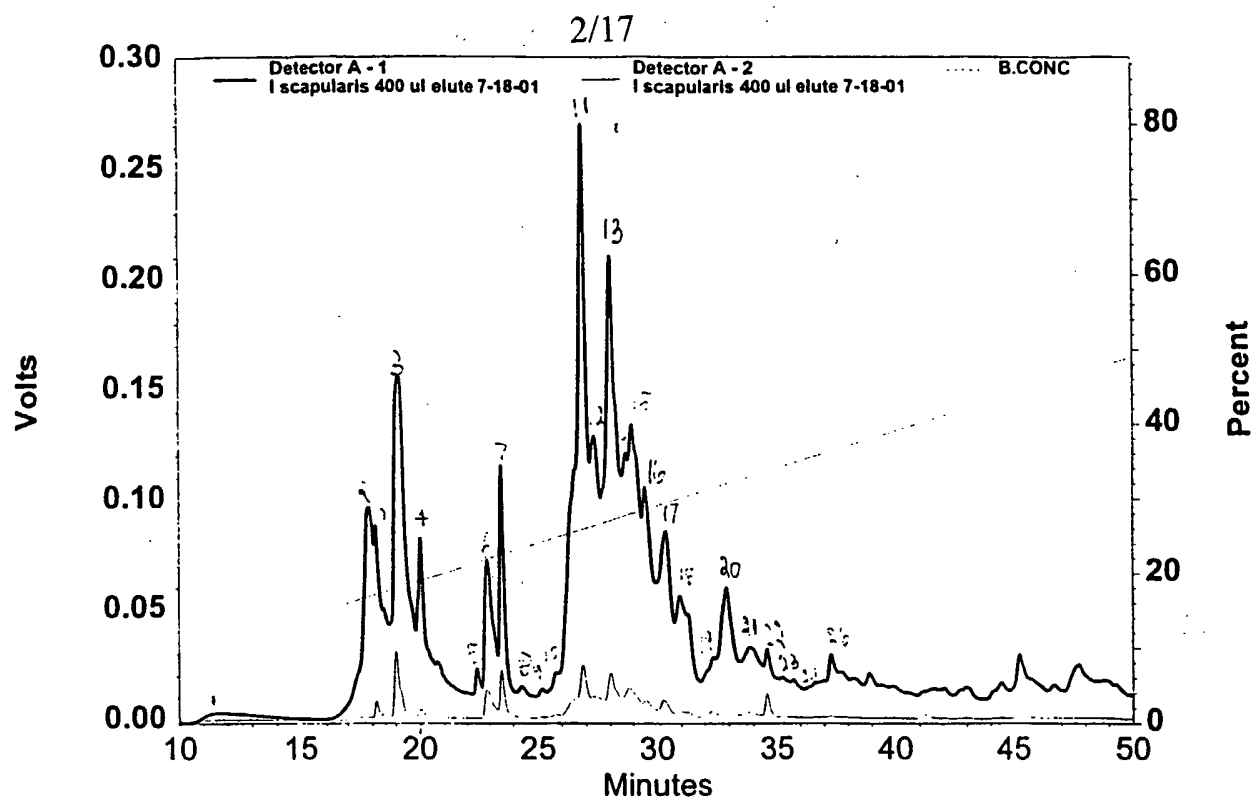


Figure 2

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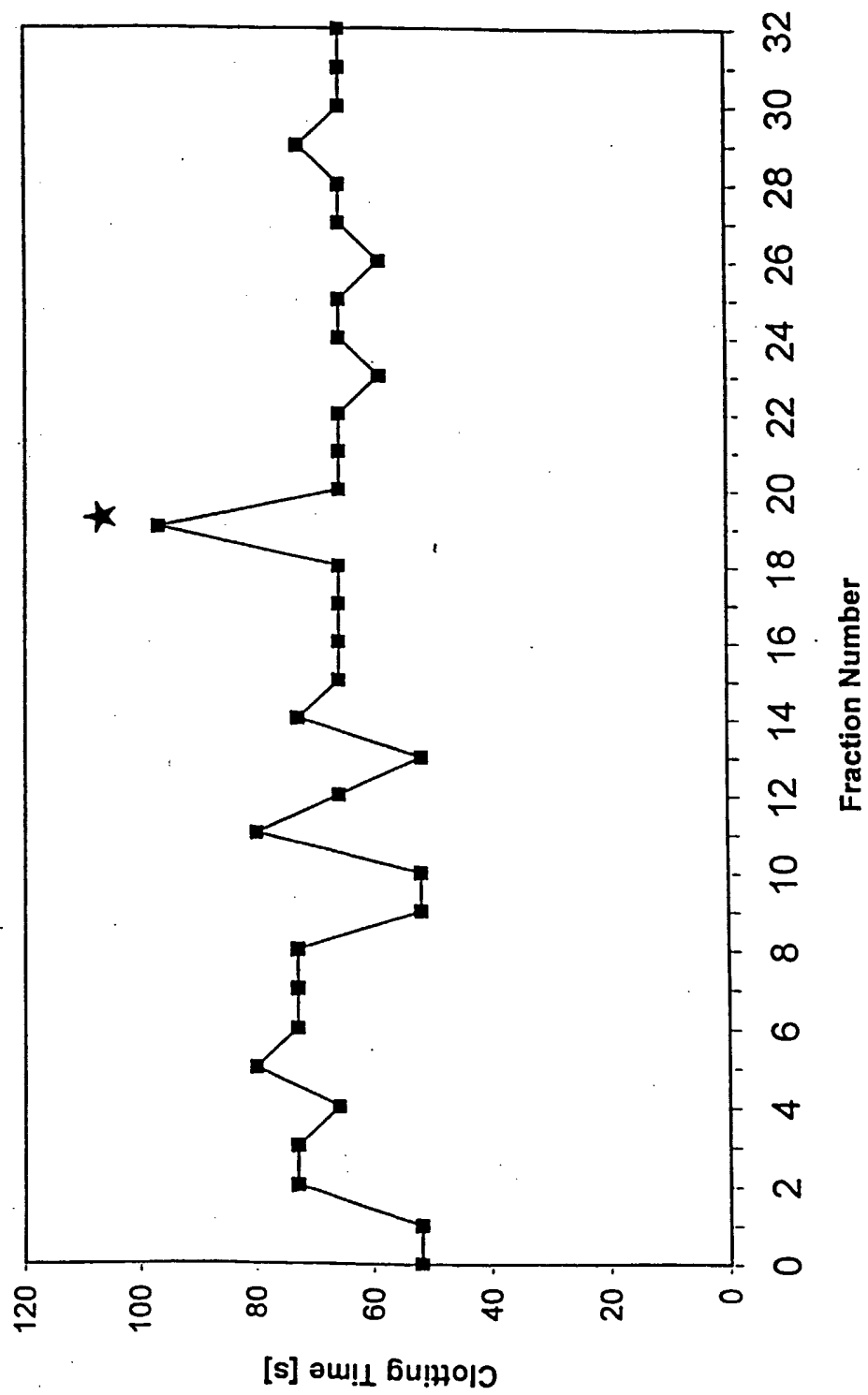


Figure 3

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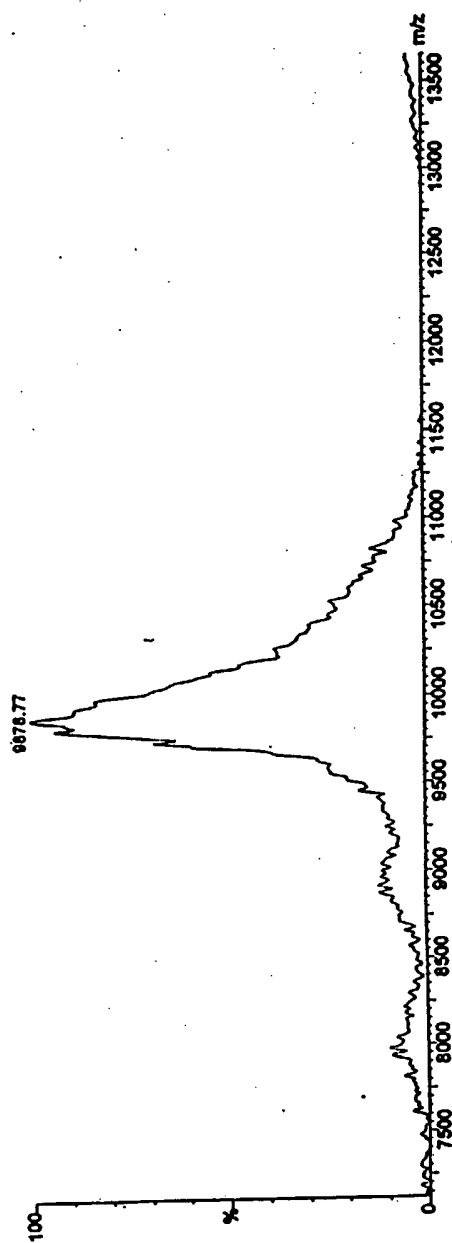


Figure 4.

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Fraction 17      HNXOXGTRPASEEKGXDYXXNAE
Fraction 19      XDQVQTRPASEEKRXGXDY
Salp9A           MGLTEIMLVLSLAFVATAAAHDCQNGTRPASEEKGCDYVCWNTETKSWDKFFFGNGE 60
Salp14           MGLTGTMVLVLSLAFFGSAAAHNCQNGTRPASEQDREGCDYVCWNAETKSWDQFFFGNGE 60
                **** ***** ..:****:*****:..:*****:*****:*****:*****:
                RCFYNNGDEGLCQNGECHLTDSGVNDTD---AKIETEETEELEA-----102
Salp9A           RCFYNSGDHGTCQNGECHLTNNSSGGNETDDYTPAPTEKPKQKKKTKTKPKRKSKD 120
Salp14           ***** * *****:..:****:*****:*****:*****:*****:
                -----
                QEKNL 125
Salp9A
Salp14

```

Figure 5

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Salp14	H N C Q N G T R P A S E Q D R E G C D Y	20
Salp9Pac	H D C Q N G T R P A S E E K R E G C D Y	20
TAP	Y N - R L C I K P R - - D W I D E C C D -	16
fXal	Y N - R L C V K P Q - - D F T K Q C S -	16
Salp14	Y C W N A E T K S W D Q F F F G N G E E K	40
Salp9Pac	Y C W N T E T K S W D K F F F G N G E E R	40
TAP	- - - S N E - - - - - - - G G E E R	23
fXal	- - - D D E - - - - - - - D A V K	23
Salp14	C F Y N S G D H G T C Q N G E C H L T N	60
Salp9Pac	C F Y N N G D E G L C Q N G E C H L T T	60
TAP	A Y F R N - G K G G C D C F W I C P E E D	42
fXal	V F F P H - D K K S C E P F W I C P E E	42
Salp14	N S G G P N E T D D Y T P A P T E K P K	80
Salp9Pac	D S G V P N D T - D - - - A K I E E T E	76
TAP	H T G A D Y Y S - - - - - - - S Y R	53
fXal	D N G V D Y Y S - - - - - - - T K E	53
Salp14	Q K K K K T K K T K K P K R K S K K D Q	100
Salp9Pac	E E L E A .	82
TAP	D C F N A C I	60
fXal	D C L S A C F	60
Salp14	E K N L .	105
Salp9Pac		82
TAP		60
fXal		60

Figure 6

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Figure 7

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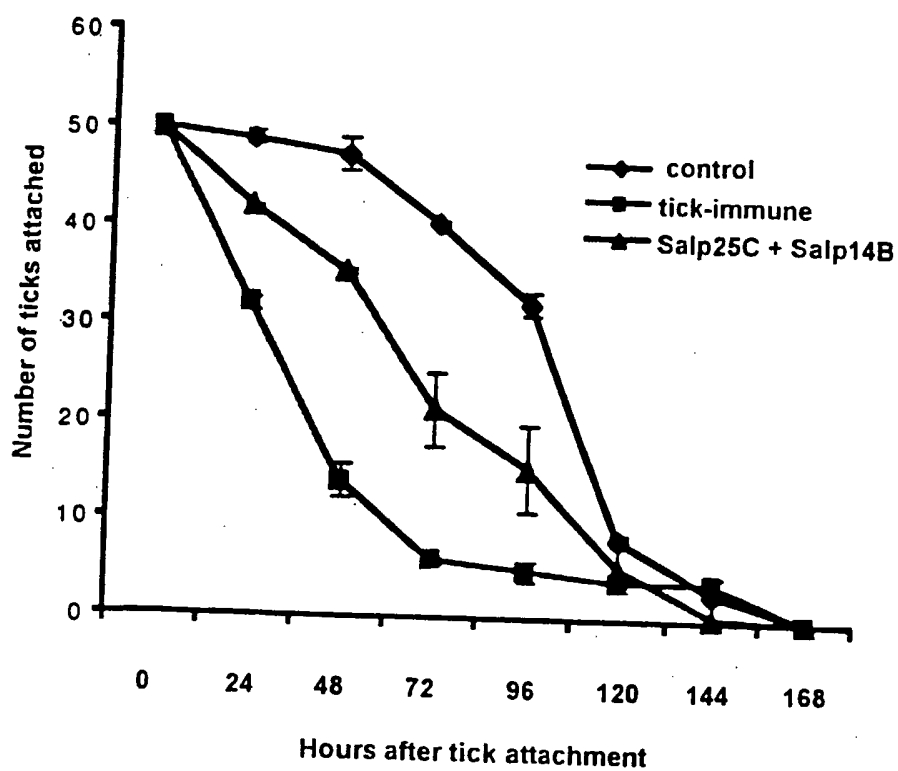


Figure 8

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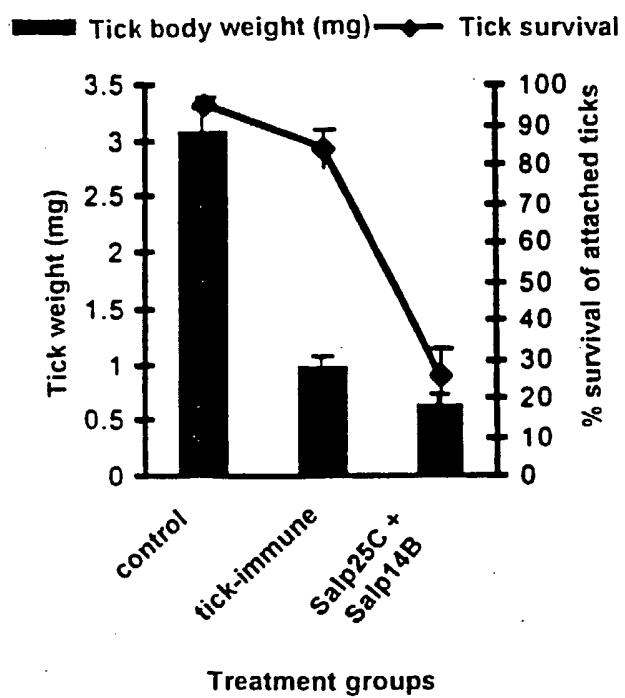


Figure 9

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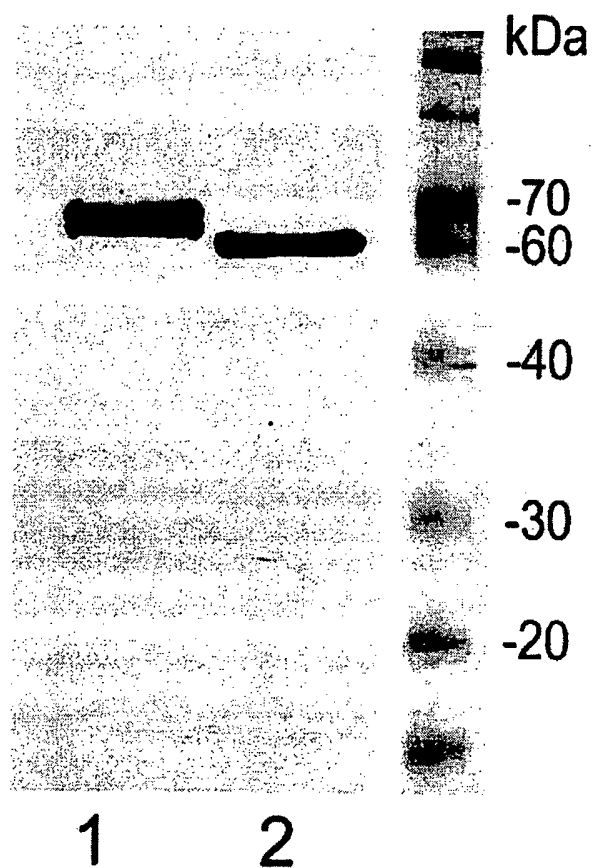


Figure 10

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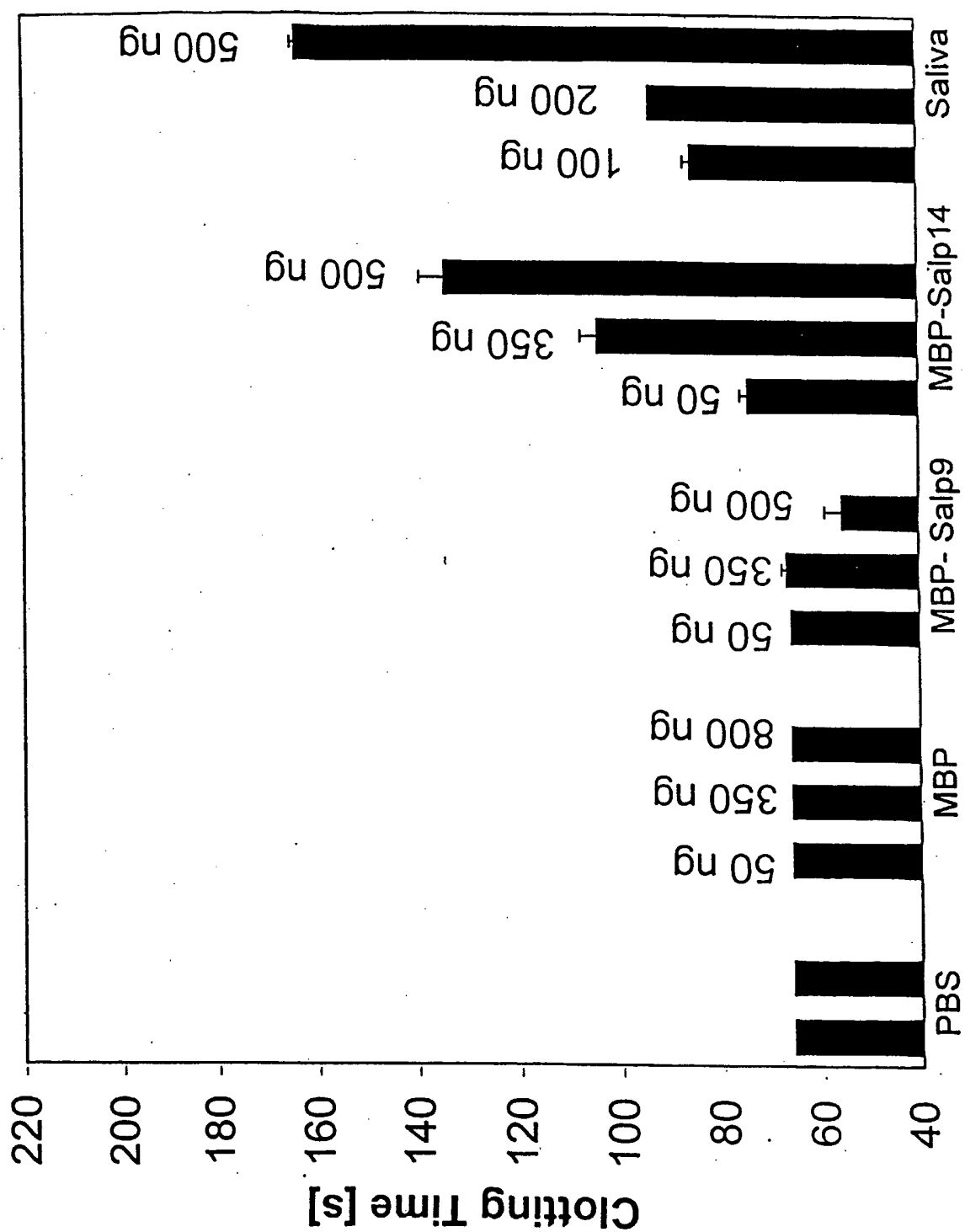


Figure 11

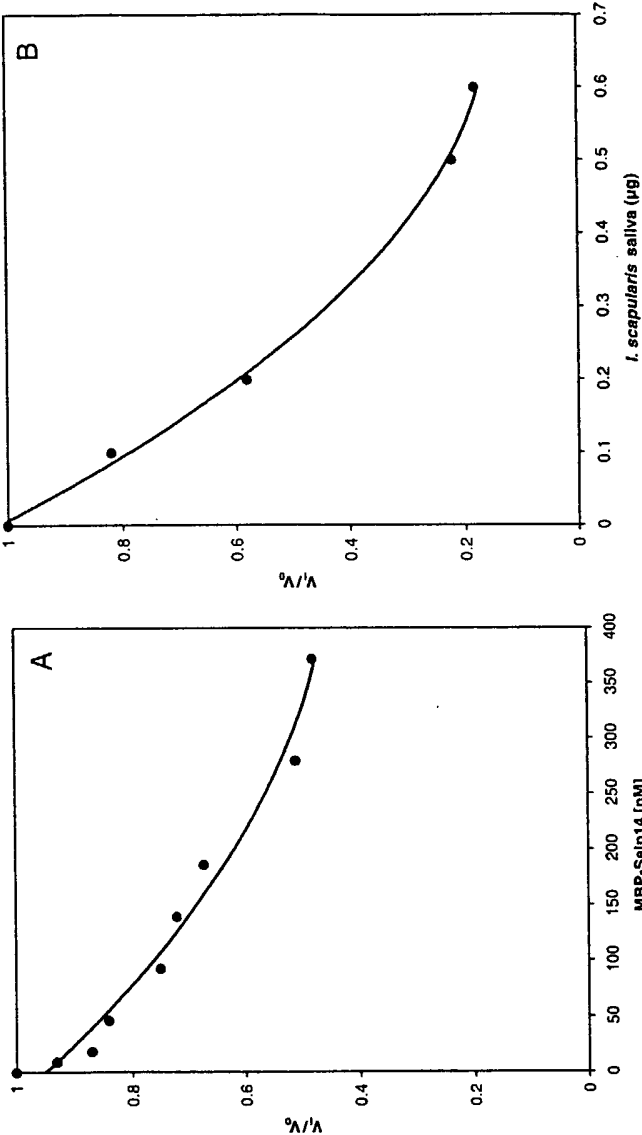


Figure 12

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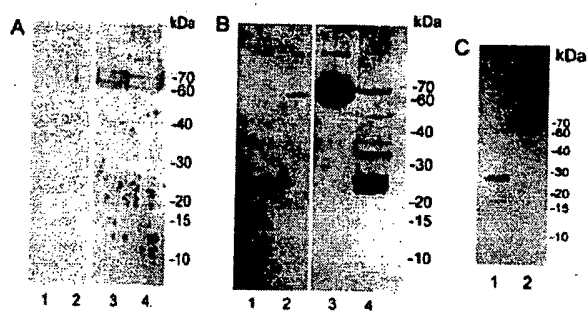


Figure 13

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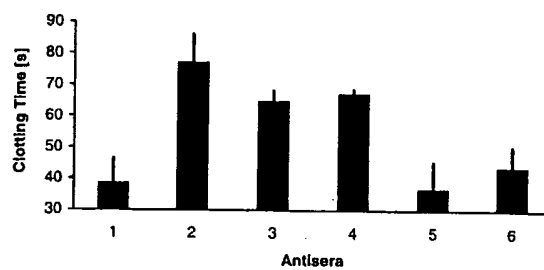


Figure 14

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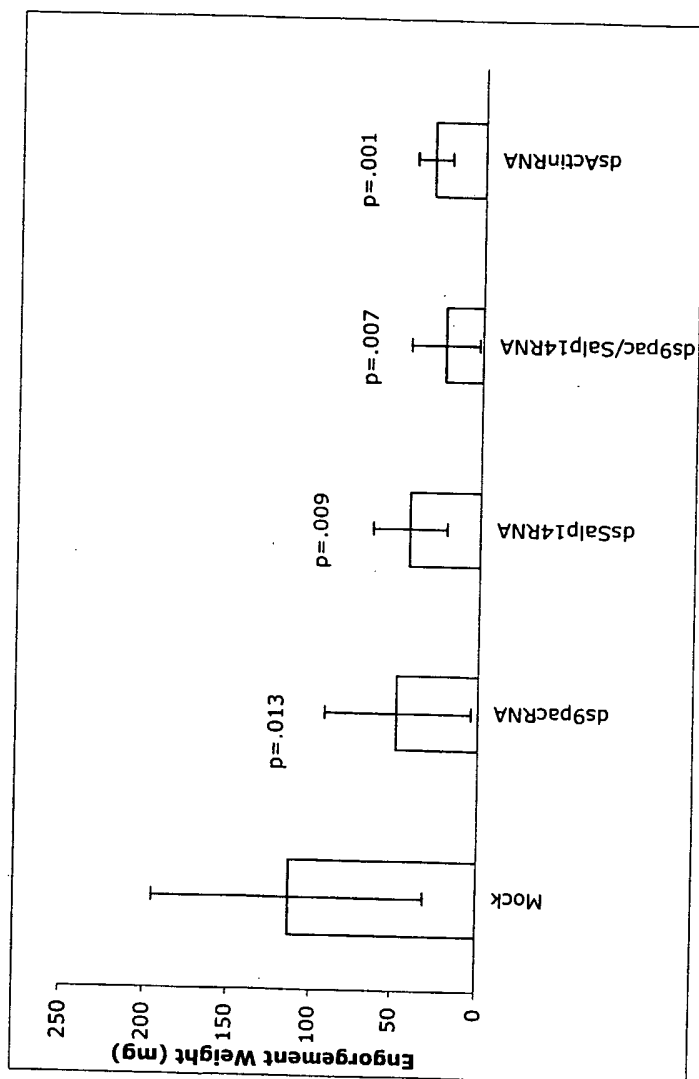


Figure 16

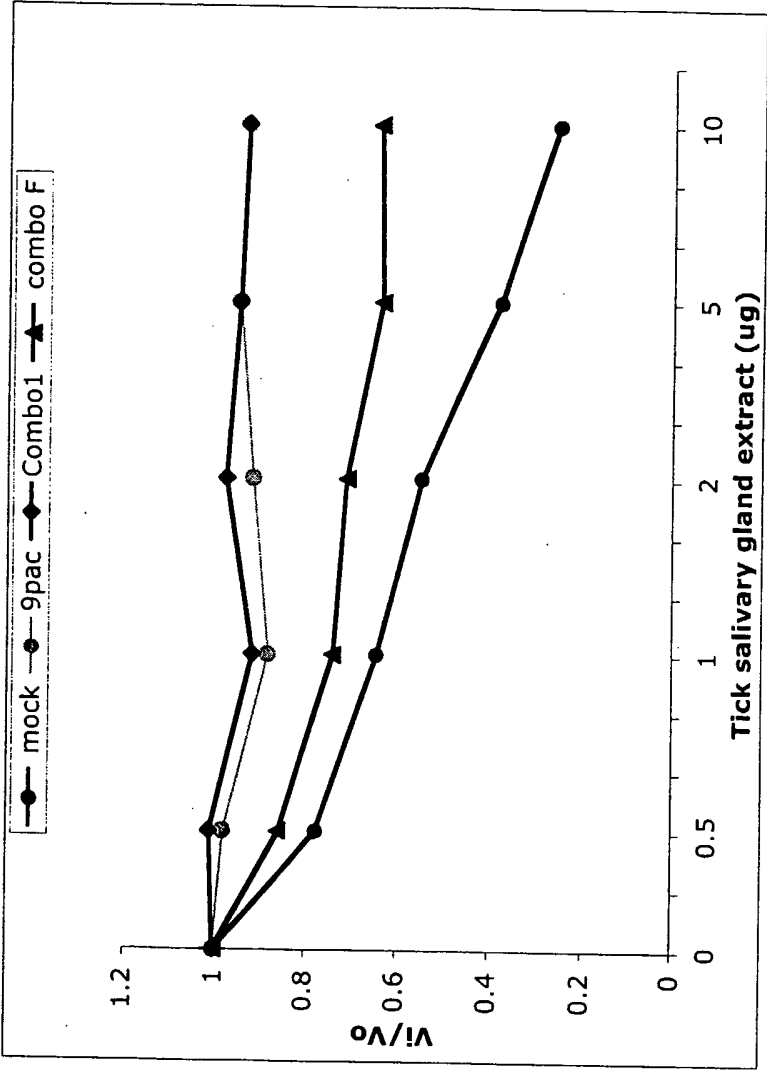


Figure 17