

conservative substitutions. Another indication that two nucleic acid sequences are substantially identical is that the two molecules hybridize to each other under stringent conditions, as described below.

[0022] An “immunoglobulin” is a serum protein which functions as an antibody in vertebrate organisms. Each immunoglobulin comprises a light chain and a heavy chain. Each chain comprises a constant domain and a variable domain. There are five types of heavy chain, denoted by α , β , ϵ , γ , and μ , which defines the class of antibody, IgA, IgD, IgE, IgG, and IgM, respectively. In humans, IgG consists of four subclasses, designated IgG1, IgG2, IgG3, and IgG4. DNA sequences encoding human and non-human immunoglobulin chains are well known in the art.

[0023] The term “immunoglobulin heavy chain constant domain polypeptide” denotes a wild-type immunoglobulin heavy chain constant domain or a variant thereof. An IgG constant domain comprises the C_{H1} , C_{H2} , and C_{H3} domains and the hinge region.

[0024] An “Fc fragment” is a fragment of the heavy chain constant domain corresponding to the region of the immunoglobulin that interacts with Fc receptors. In IgG, IgA and IgD, the Fc region corresponds to C_{H2} , and C_{H3} domains and the hinge region. In IgM and IgE, the Fc regions contain three heavy chain constant domains (C_{H2} , C_{H3} , and C_{H4}).

[0025] “Operably linked” indicates that two or more DNA segments are joined together such that they function in concert for their intended purposes. For example, coding sequences are operably linked to promoter in the correct reading frame such that transcription initiates in the promoter and proceeds through the coding segment(s) to the terminator.

[0026] A “polynucleotide” is a single- or double-stranded polymer of deoxyribonucleotide or ribonucleotide bases typically read from the 5' to the 3' end. Polynucleotides include RNA and DNA, and may be isolated from natural sources, synthesized *in vitro*, or prepared from a combination of natural and synthetic molecules. When the term is applied to double-stranded molecules it is used to denote overall length and will be understood to be equivalent to the term “base pairs”.

[0027] A “polypeptide” is a polymer of amino acid residues joined by peptide bonds, whether produced naturally or synthetically. Polypeptides of less than about 50 amino acid residues are commonly referred to as “oligopeptides”.

[0028] The term “promoter” is used herein for its art-recognized meaning to denote a portion of a gene containing DNA sequences that provide for the binding of RNA polymerase and initiation of transcription of an operably linked coding sequence. Promoter sequences are typically found in the 5' non-coding regions of genes.

[0029] A “protein” is a macromolecule comprising one or more polypeptide chains. A protein may also comprise non-peptidic components, such as carbohydrate groups. Carbohydrates and other non-peptidic substituents may be added to a protein by the cell in which the protein is produced, and will vary with the type of cell. Proteins are defined herein in terms of their amino acid backbone structures; substituents such as carbohydrate groups are generally not specified, but may be present nonetheless.

[0030] The term “substantially similar” in the context of the fusion proteins of the invention indicates that a polypeptide comprises a sequence with at least 90%, preferably at least 95% sequence identity to the reference sequence (e.g., the GP-Fc fusion exemplified here) over a comparison window of

10-20 amino acids. Percentage of sequence identity is determined by comparing two optimally aligned sequences over a comparison window, wherein the portion of the polynucleotide sequence in the comparison window may comprise additions or deletions (i.e., gaps) as compared to the reference sequence (which does not comprise additions or deletions) for optimal alignment of the two sequences. The percentage is calculated by determining the number of positions at which the identical nucleic acid base or amino acid residue occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the window of comparison and multiplying the result by 100 to yield the percentage of sequence identity.

BRIEF DESCRIPTION OF THE DRAWINGS

[0031] FIG. 1 is a schematic representation and purification of the ZEBOVGP-Fc and FLAG-Fc proteins. A) Schematic representation of the fusion proteins. The ZEBOVGP-Fc fusion protein contains the ectodomain of ZEBOV GP tagged at its C terminus with a FLAG peptide and fused to the hinge and Fc regions of human IgG1. The FLAG-Fc fusion protein contains a FLAG tag fused to the hinge and Fc regions of IgG 1. B) SDS-PAGE analysis of fusion proteins. Protein A-purified ZEBOVGP-Fc and FLAG-Fc preparations were analyzed by denaturing SDS-PAGE in a 4-12% gradient gel and stained with Coomassie blue. C) Western blot analysis of ZEBOVGP-Fc and FLAG-Fc. Proteins were resolved by SDS-PAGE under denaturing conditions, transferred to PVDF membranes, and probed with ZEBOV-specific anti-GP1 mAb 13F6-1-2, anti-Flag M2 mAb, or goat anti-human Fc Ab. ZEBOV GP 1, GP2-FLAG-Fc, and FLAG-Fc bands are indicated with arrows. Positions and size of molecular weight markers are indicated in kDa.

[0032] FIG. 2 is a characterization of ZEBOVGP-Fc fusion protein. A) FPLC analysis of protein A-purified ZEBOVGP-Fc. Undigested (gray) or enterokinase-digested (blue) ZEBOVGP-Fc was run on a Superdex 200 size exclusion column under non-denaturing conditions, and absorbance at 280 nm was recorded for each of the 38 collected fractions. Peaks representing ZEBOVGP-Fc, ZEBOV GP, and Fc are marked with arrows. A peak of approximately 150 kDa represents a carrier protein in the enterokinase preparation. The migration of molecular weight standards is shown as arrowheads and their molecular weight is expressed in kDa. B) Western blot analysis of enterokinase-digested ZEBOVGP-Fc protein. Gel filtration peak fractions (20 and 32) were fractionated in denaturing SDS-PAGE, transferred to a PVDF membrane, probed with ZEBOV-specific anti-GP 1 mAb 13F6-1-2 or goat anti-human anti-Fc antibody. Undigested (U) or enterokinase digested (D) ZEBOVGP-Fc, and FLAG-Fc (Fc) were included in the gel as markers. Arrows indicate the migration of GP1, GP2-FLAG-Fc, and FLAG-Fc. The migration of the molecular weight markers and their sizes are indicated in kDa.

[0033] FIG. 3 shows analysis of anti-ZEBOV GP antibodies in vaccinated C57BL/6 mice. A) Analysis of anti-ZEBOV GP specific antibodies by viral particle ELISA. Mice were vaccinated with ZEBOVGP-Fc or FLAG-Fc and sera samples were obtained 2 weeks after the final vaccination. Sera were titrated on 96-well plates coated with sucrose-purified irradiated ZEBOV particles, rVSV-ZEBOVGP, or control wt VSV. The endpoint dilution titer for each mouse sera is represented as a dot. B) FACS analysis of binding of mouse sera to ZEBOV GP expressed at the cell surface of HEK293-ZE-