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(54) Titre : PROCEDE POUR STIMULER LA PHAGOCYTOSE DE CELLULES EXPOSANT LA PHOSPHATIDYLSERINE
(54) Title: METHOD FOR ENHANCING PHAGOCYTOSIS OF PHOSPHATIDYLSERINE-EXPOSING CELLS

(57) Abrégé/Abstract:
An Annexin A5 variant, comprising one or more RGD (Arg-Gly-Asp) sequences, is suitable for use in the treatment of a disease in mammals, including humans, wherein augmentation of phagocytosis is a desired effect of treatment. The Annexin A5 variant can e.g. be used in the treatment of chronic inflammatory diseases such as atherosclerotic plaque or in the treatment of COPD. At least one RGD sequence may substitute sequences of three amino acids within the range 1-19or other regions of the amino acid sequences of Annexin A5; alternatively one or more RGD sequences may be part of an extension at the N-terminal side.

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(54) **Title**: METHOD FOR ENHANCING PHAGOCYTOSIS OF PHOSPHATIDYLSERINE-EXPOSING CELLS

(57) **Abstract**: An Annexin A5 variant, comprising one or more RGD (Arg-Gly-Asp) sequences, is suitable for use in the treatment of a disease in mammals, including humans, wherein augmentation of phagocytosis is a desired effect of treatment. The Annexin A5 variant can e.g. be used in the treatment of chronic inflammatory diseases such as atherosclerotic plaque or in the treatment of COPD. At least one RGD sequence may substitute sequences of three amino acids within the range 1-19or other regions of the amino acid sequences of Annexin A5; alternatively one or more RGD sequences may be part of an extension at the N-terminal side.

Method for enhancing phagocytosis of phosphatidylserine-exposing cells

The invention pertains to means and methods for use in enhancing phagocytosis of phosphatidylserine-exposing cells, for example for the treatment of diseases that are characterized by chronic inflammation such as atherosclerosis and chronic autoimmune diseases such as systemic lupus erythematodes (SLE).

Background

Apoptosis plays an important role in atherogenesis. A positive correlation exists between the number of apoptotic cells in the plaque and plaque instability. Phagocytosis of apoptotic cells is relevant to progression of atherosclerosis towards the unstable phenotype. Impaired phagocytosis is associated with accelerated progression of the atherosclerotic plaque towards the unstable phenotype. Atherosclerosis, hence, can be treated with methods that enhance phagocytosis of apoptotic cells.

Apoptotic cells expose phosphatidylserine (PS) at their cell surface. Annexin A5 (anxA5) binds to PS expressed on the apoptotic cell surface. AnxA5 bound to the apoptotic cell inhibits its phagocytosis by shielding the PS, which PS is recognized by the phagocyte as an 'eat me' signal (Kenis et al. *Exp. Cell Res.* 312 (2006) 719-726; and references cited therein).

Asano et al. *J. Exp. Med.* 200(4), 459-467 (2004) show that a milk fat globule mutant containing a point mutation in its RGD motif, inhibits phagocytosis of apoptotic cells and induces production of auto-antibodies upon injection in mice.

Bérat et al., *Biointerphases* 2(4), 165-172 (2007) describe a rat annexin A5 variant wherein Thr-163 is replaced by Cys, and a synthetic oligopeptide containing the RGD (arginine-glycine-aspartate) motif and a Cys residue is coupled to the Cys residue of the annexin mutant through a disulfide bond. Matrices of these annexin-RGD conjugates were found to promote adhesion of human saphenous vein endothelial cells. The authors suggest that these results may open cell-transporting techniques and the like. The annexin variants in which the RGD motif is attached to a central part of the molecule via a Cys-Cys (disulfide) bridge may have the disadvantage of insufficient stability in various environments.

Description of the invention

This invention describes methods to enhance phagocytosis by using anxA5 variants that bind to PS on the apoptotic cell and activate phagocytes to engulf the apoptotic cell instead of inhibiting phagocytosis.

The tertiary structure of AnxA5 is arranged in four domains and an N-terminal tail at the concave side of the molecule connecting domain I and IV. The N-terminal tail is opposite to the convex side of the anxA5 molecule that harbours the Ca^{2+}/phospholipids binding sites. Hence, the concave side, and especially the N-terminal tail can be mutated and conjugated to different molecules (e.g. proteins or sugars) without interfering with the PS-binding capacity.

The anxA5 variants of the invention can thus carry a phagocytosis-inducing signal in or as an extension of its N-terminal tail or elsewhere at the concave side. The phagocytosis-inducing signal can be one or more, up to twenty RGD (arginine-glycine-aspartate) motifs. The variants can be anxA5 derivatives having an RGD motif in their N-terminal tail that activates integrin receptors of the phagocyte. Also anxA5 derivatives that carry the Fc-domain of IgG or parts thereof activate IgG-receptors of the phagocytes.

It is preferred that at least one or preferably each RGD motif is flanked by a neutral amino acid, in particular an amino acid selected from A, G, I, L, M, P, S, T and V.

The anxA5 variant of the invention contains at least one RGD motif within the Annexin chain, i.e. in the linear polypeptide chain constituting the annexin molecule. Thus, the RGD motif may be inserted within the annexin polypeptide chain or replace one or more, amino acids of the annexin molecule, or be attached to the N-terminal side of the annexin molecule. Thus, the annexin variant can be, and preferably is, produced by recombinant production using host cell which are transformed with the suitably modified DNA.

For example, the anxA5 variant of the invention can have one or more RGD sequences which substitute sequences of three amino acids in the concave surface of the anxA5 protein. The amino acids at the concave surface anxA5 are shown in italics in the sequence illustrated in Table 1 (SEQ ID No. 1) and include amino acid positions 1-19, 24, 28, 46-64, 86-89, 118-135, 150, 157-170, 202-219, 245-248, and 280-294. Thus the one or more RGD motifs are preferably located in the range 1-19, 46-64, 86-89, 118-135, 157-170, 203-219, 245-248 and 280-294, more preferably within the range 1-15, 46-58, 86-87, 118-134, (170), 245-248 and 280-294, indicated in bold italics in Table 1.

In particular, the anxA5 variants according to the invention have at least one RGD sequence which substitutes a sequence of three amino acids within the range 1-15, preferably within the range 1-10 of Table 1 (SEQ ID No. 1). Suitable examples of this embodiment include sequences 49-51 (substitution of QE by GD), 17-19 (A by G), 13-15 (GF by RG) by suitable replacement of the native sequences; in the latter two it is preferred that E at 16 is additionally substituted by a neutral amino acid. More suitable examples include 10-12 (DFP by RGD), 8-10 (VT by RG), 1-3 (AQV by RGD) and especially 5-7 (T by D).

Table 1

Amino acid sequences of Human annexin A5 (without the original methionine). Amino acids in italics are located on the concave side. Amino acids in bold italics are preferred amino acids for substitution at the concave side. Underlined regions are regions where amino acids can be substituted to inhibit internalization.

AQVLRGTVTD FPGFD*ERADA* ETL*R*KAM*K*GL GTDEESILTL 40

LTSRS***NAQRQ EISAAFKT****LF* *GRDL*LDDLKS ELTGKFEKLI 80

VALMK***PS****RLY* DAYELKHALK GAGTNEKVLT EIIASRT***PEE*** 120

LRAIKQVYEE EYGS*SLEDDV* VGDTSGYYQR MLVVL*QANR* 160

*DPDAGIDEA****Q*** VEQDAQALFQ AGELKWGTDE EKFITIFGTR 200

SV*SHLRKVFD* *KYMTISGFQ*I EETIDRETSG NLEQLLLAVV 240

KSIR***SIPA***YL AETLYYAMKG AGTDDHTLIR VMVSRSEID***L*** 280

FNIRKEFRKN FATSLYSMIK GDTSGDYKKA LLLL**C**GEDD 319

Alternatively or additionally, the anxA5 variant of the invention can have an extension at the N-terminus of the molecule, which extension contains one or more RGD sequences. The extension may for example be X^1RGDX^2 or $(X^1RGDX^2)_n$, wherein X^1 and X^2 can be any series of amino acids, the terminal ones being selected from A, G, I, L, M, P, S, T and V, preferably from A, G, P and S, and n can be from 1-19, preferably 2-9, e.g. 2 or 3. Preferably however, X^1 and X^2 each are one or two amino acids selected from A, G, I, L, M, P, S, T and V, preferably from A, G, P and S. Thus, an example is a sequence ARGD attached to the N-terminus (A) of the sequences of Table 1 (SEQ ID No.1). Further examples include RGDAARGD, RGDAARGDAARGDA (SEQ ID NO's 3, 4) etc. extended at the N-terminus of the anxA5 molecule. Thus, when the anxA5 variant contains more than one RGD sequence, each pair of RGD sequences is preferably separated by 1-3 amino acids selected from A, G, I, L, M, P, S, T and V.

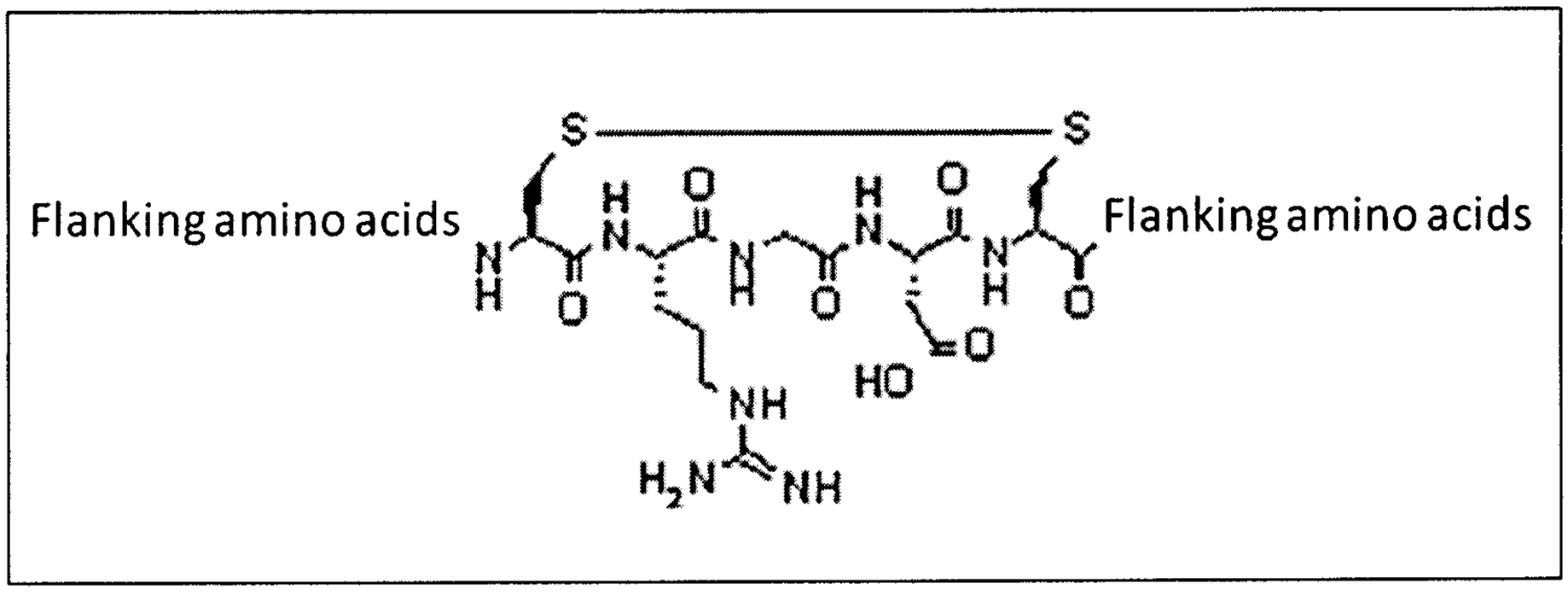
As a further alternative, an anxA5 variant according to the invention may contain one or more RGD sequences which are attached to a cysteine residue which substitutes an amino acid at the N-terminal side of the annexin molecule, i.e. at one of the positions 1-19 of SEQ ID No.1 (Table 1). To this end, a cysteine residue is introduced at one of the above positions by methods known in the art, for example as described in WO 2006/003488, which is incorporated herein by reference. The one or more RGD motifs can be coupled to the sulfhydryl group of the cysteine residue e.g. by a linker. The linker can e.g. be selected from maleimide containing groups, halogen containing groups, an isothiocyanate, an isocyanate, an imido ester, and a succinimidyl ester group, to which e.g. ethylene diamine is bound. Thus in particular, the linker contains an amino group, to which the RGD motif, or X^1RGDX^2 or $(X^1RGDX^2)_n$ motifs (see above) can be bound.

It is preferred that in such Cys derivatives, any cysteine originally present at a non-concave position, in particular at a site buried within the molecule as indicated by underlining in Table 1, is substituted by another amino acid, such as an alanine or serine residue; see WO 2006/003488 and WO 2007/069895. This applies specifically to the cysteine residue originally present at position 315 of the anxA5 molecule. Hence, an anxA5 variant in which Cys-315 has been replaced by another amino acid, or has been deleted, is preferred.

Alternatively or additionally to a straight RGD-containing sequence, the anxA5 variant may contain a cyclic RGD sequence or a plurality thereof. Such cyclic RGD sequence may have the cyclic structure c-X^3RGD, wherein X^3 may represent one, two or three amino acids, other than R and D, which contain one linking amino acid such as K, N, Q, or a triazole-containing or other synthetic amino acid allowing coupling to the anxA5 molecule, e.g. to a cysteine residue thereof. The synthesis of cyclic RGD derivatives is described e.g. by Kuijpers et al. *Bioconjugate Chem.* 2007, *18*, 1847-1854 and references cited therein. RGD can be made cyclic for example by flanking the RGD motif with a cysteine at both sides and oxidising the cysteines to form a disulfide bridge. The formula below shows an example of a peptide of 5-20 amino acids containing a cyclic RGD that was rendered cyclic by disulfide bridge formation of flanking cysteine residues.

In an embodiment, a peptide of 3-20 amino acids containing RGD or cyclic RGD is coupled to annexin A5 or variant thereof through chemical coupling using standard techniques such as for example coupling to an amino group or a sulfhydryl group present in the annexin polypeptide. A preferred embodiment encompasses annexin A5 or variants thereof which have RGD or cyclic RGD containing peptides of 3-20 amino

acids that are coupled to the concave side, especially the N-terminal side, of the annexin molecule.



In a particular embodiment, the annexin variant according to the invention containing the RGD motif is further modified so as to inhibit internalisation into a cell. In such a variant, one or more amino acids within the helices involved in the inter-molecular interactions between annexin molecules are replaced by different amino acids. These replacement amino acids are located at positions 16-29, 59-74, 88-102, 135-145, 156-169, 202-231, 259-266, and 305-317 in annexin A5, and these positions are underlined in Table 1 (SEQ ID No. 1). Where the annexin variant contains one or more of these modifications, the feature of the annexin not being internalised into a cell will be fulfilled. Thus, the invention also comprises an annexin variant that a) contains one or more RGD sequences and b) contains one or more of the amino acid modifications as described herein. Such annexin derivatives are described in WO 2007/069895.

Preferred modifications inhibiting internalisation are substitutions, especially substitutions of polar amino acids by non-polar amino acids. Thus, preferred amino acids for substitution include arginine (R), lysine (K), aspartate (D), glutamate (E), asparagine (N) and glutamine (Q). They may be substituted e.g. by glycine (G), alanine (A), proline (P), serine (S), threonine (T) and valine (V), especially A or G, or by a non-polar amino acid that is located in the corresponding position of another annexin (cf. Table 1). Suitable examples of substituted amino acids include E21, K25 (e.g. by G, T), R62 (e.g. G, A), D63 (e.g. G, A, P), K69, D91, K96, H97, K100, E137 (e.g. A, G, V), D138, D139, N159 (e.g. A, G, S), R160, R206, K207, Q219, D225, R226, D264, K308, K309. It is preferred in this particular embodiment, to have at least two, or even at least three, substitutions in different regions, for example R62A + E137G, or K69A + K100A + N159S etc., in order to further decrease the trimerisation of the annexin at the site of the cell.

In the embodiment described above, wherein the anxA5 variant further contains a cysteine residue at one of the concave positions 1-19, 24, 28, 46-64, 86-89, 118-135, 149-150, 157-170, 203-219, 245-248 and 280-294, said cysteine allows coupling to one or more RGD motifs as described above.

In molecules which already contain one or more RGD motifs, e.g. by suitable substitution or extension, said cysteine residue can be a suitable function for coupling to a diagnostic or therapeutic molecule, e.g. as described in WO 2006/003488. Suitable diagnostics are optical probes such as for example fluorochromes in the visible, near infrared and infrared region and nuclear probes as for example ^{99m}Tc, ^{68}Ga, ^{64}Cu, ^{111}In, ^{124}I and ^{18}F. Suitable therapeutic agents are compounds that inhibit protease activity such as for example chemical compounds or peptides that inhibit matrix metalloproteases. Other suitable therapeutics are compounds that inhibit platelet activation and blood coagulation such as for example heparin or low molecular weight derivatives thereof. Suitable therapeutic agents are also radioisotopes that have beta-emission such as for example ^{186}Re and ^{90}Y. The examples serve the purpose of indication and do not imply a limitation of possibilities.

As an alternative means for providing the annexin variant with a diagnostic or therapeutic metal species, the Annexin A5 variant of the invention may advantageously further comprise from 2 up to 10 amino acid residues at its N-terminus, allowing association with metal diagnostics or therapeutics. Such amino acids are especially histidine residues, at least two of said histidine residues being adjacent or separated by no more than one other amino acid. The metal diagnostic or therapeutic can e.g. be a radionuclide, such as gallium 67, gallium 68, indium 111, technetium 99m, rhenium 188, Copper 64 and Tin 117m. An example is an annexin variant of the invention, e.g. carrying an RGD motif in or attached to its region of amino acids 1-15, which further containing 3-6 histidine residues attached to N-terminal side of the alanine at position 1, to which one of the metals is associated. Annexin variants containing histidine residues and their metal complexes and uses are described in WO 2010/024673.

Apart from the substitutions and additions described above, the anxA5 variant of the present invention may contain further substitutions, deletions or insertions to the sequence as depicted in Table 1 (SEQ ID No 1). Such substitutions, deletions or insertions may especially be conservative substitutions as occurring when comparing the human anx5 sequence of Table 1 with that of other animal species, e.g. rat, mouse, monkey, cow or other, or with that of other annexins, such as anxA1, anxA4, anxA8, anxA9, etc., which sequences are readily accessible.

However, it is preferred that, apart from extensions at the N-terminal side or side-chains described above, the amino acid sequence of the anxA5 variant of the invention has at least 80% amino acid identity and at least 90% amino acid similarity with the sequence of SEQ ID NO. 1, more preferably at least 90% amino acid identity and at least 95% amino acid similarity, most preferably 95-99.7% amino acid identity and especially 97-99.7% amino acid similarity. It is preferred that the anxA5 variant is based on human anxA5, i.e. has the above degrees of identity with the human sequence (Table 1, SEQ ID NO. 1), and in particular so in the convex areas (the non-underlined parts of Table 1), especially an amino acid identity in the region 29-45 of at least 90% (at least 15 out of 17), more preferably at least 95% (16/17), most preferably 100% (all 17 amino acids identical).

The anxA5 variant described herein can be used in pharmaceutical compositions, methods, kits, and devices as also described herein. An embodiment of the invention relates to a method for treating a subject in need of treatment of a disease that includes administering a therapeutic composition of the variant described above which optionally contains at least one pharmaceutically acceptable excipient. Administration and dosage of a therapeutic composition can vary between patients and are well known in the medical art. The preferred dosage will depend upon the disease being treated, the therapeutic compound or mix of compounds, and the patient among other factors. Possible delivery routes of the complexes are subcutaneous, intramuscular, intra-peritoneal and intravenous administration. Those of skill in the art will know of other delivery routes. Complexes can administered as a bolus or continuously per infusion covering a longer period of time. Doses range from 0.1 μg/kg to 100 mg/kg (anxA variant over body weight) in diagnostic procedures and 1 μg/kg to 100 mg/kg in therapeutic procedures. Preferred ranges are 1 μg/kg - 1 mg/kg (diagnostic) and 5 μg/kg - 50 mg/kg (therapeutic).

The anxA5 variant as described above is suitable for use in stimulating phagocytosis of cells that expose phosphatidylserine on their surface, in particular apoptotic cells. Thus, the anxA5 variant can be used in the treatment of a disease in mammals, including humans, which comprises the augmentation of phagocytosis as a desired effect of treatment. Such diseases include diseases associated with atherosclerotic plaque and especially the advanced atherosclerotic plaque that carries an increased risk of vascular rupturing such as plaques indicated by AHA classification type II, III, IV, V and IV. The effect of the treatment will be a slow-down of plaque progression and concomitantly a reduction of risk of rupturing,

The term efferocytosis (from effero = to bury, to carry to the grave) was coined by Henson et al. for the phagocytic removal of apoptotic cells (Gardai SJ, McPhillips KA,

Frasch SC, Janssen WJ, Starefeldt A, Murphy-Ullrich JE, et al., *Cell* 2005; 123: 321–334). Chronic obstructive pulmonary disease (COPD) is associated with defective efferocytosis in humans and animal models (S. Hodge et al,, Am. J. Respiratory Cell and Molecular Biology, vol.37, pp 748-755, 2007; Vandivier et al., *Chest*, June 2006 vol. 129, No. 6, 1673-1682). Apoptotic cells can undergo secondary necrosis and thereby perpetuate inflammation. Low dose administrations of the macrolide antibiotic Azithromycin to patients with COPD have been used to improve phagocytic ability of Alveolar Macrophages as a novel approach to supplement existing therapies (Hodge et al., *American Journal of Respiratory and Critical Care Medicine*, Vol. 178, pp 139-148, 2008). The anxA5 variant will therefore be useful in the treatment of COPD by stimulating the removal of apoptotic cells.

The anxA5 variant according to the invention can be used in a method for stimulating phagocytosis of cells that expose phosphatidylserine on their surface, which method comprises administering to a subject in need thereof the anxA5 variant, in a pharmaceutically acceptable composition. Such composition may be administered through intravenous infusion of a solution or through inhalation of a pharmaceutical aerosol delivery formulation.

The anxA5 variants of the invention are particularly useful for treating conditions in which phagocytosis is defective and inflammatory responses play a central role. Hence, the invention relates to methods of treatment of chronic inflammatory diseases such as arthritis, atherosclerosis, chronic obstructive pulmonary diseases (COPD), including chronic bronchitis and emphysema, and chronic autoimmune diseases such as systemic lupus erythematodes (SLE), inflammatory bowel diseases, including Crohn's disease, ulcerative colitis and celiac disease, psoriasis, rheumatoid arthritis, multiple sclerosis, myasthenia gravis, Sjögren's syndrome, etc.

Description of the figures

Figure 1A shows a histogram of the flow cytometric analysis of MCF-7 cells incubated with anxA5-FITC; figure 1B shows the histogram of MCF-7 cells incubated with RGD-anxA5-FITC produced according to Example 1. Incubation of MCF-7 cells with RGD-anxA5-FITC resulted in increased binding of RGD-anxA5-F to MCF-7 cells.

Figure 2 shows that AnxA5 inhibits and RGD-anxA5 stimulates phagocytosis of apoptotic Jurkat cells by differentiated THP-1 cells.

Examples:

AnxA5 variants are made that carry a phagocytosis inducing signal in the N-terminal tail. The variants can further contain a cysteine residue in the N-terminal tail either as part of an extension of the tail, or within the tail by replacement of glutamine at position 2.

Example 1: Production of Annexin A5 variant having an RGD motif

The human annexin A5 cDNA was prepared from a white blood cell cDNA library from a healthy volunteer with standard techniques known in the art. The cDNA sequence encoded the amino acid sequence presented in Table 1. Primers were designed to mutate annexin A5 by PCR techniques such that the resulting cDNA encoded the amino acid sequence of Table 1 with the exception of the substitution of T (Thr), encoded by ACN (N = T, C, A or G), at position 7 by D (Asp), encoded by GAY (Y = T or C). The variant is denoted as A5 T7D, and its sequence is represented in SEQ ID No.2.

The annexin A5-T7D cDNA was cloned into a bacterial expression vector with standard techniques known in the art. *E. coli* transformed with the resulting bacterial expression vectors were grown in a fermentor. The annexin A5-T7D variant produced by the bacteria was isolated and purified from *E. coli* lysates with standard chromatography techniques known to persons skilled in the art.

The purified annexin A5-T7D variant appeared as a homogenous band with an apparent molecular weight of around 34kDa on SDS-PAGE and exhibited full calcium-dependent phosphatidylserine binding activity as measured by plasmon surface resonance technique using the BiaCore system.

Example 2: Effect of Annexin A5 variant having an RGD motif

In order to demonstrate that the T7D substitution results in a functional RGD motif on anxA5 MCF-7 cells (figure 1) and THP-1 cells were incubated with fluorescein labeled anxA5 or fluorescein labeled T7D anxA5. MCF-7 cells and THP-1 cells express $\alpha_v\beta_3$ *and* $\alpha_v\beta_5$. Preliminary data confirm that incorporation of RGD (Arginine-Glycine-Aspartic-acid) into the N-terminal tail of anxA5 increases the binding to $\alpha_v\beta_3$ *and* $\alpha_v\beta_5$ expressing cells, indicating that RGD is exposed for interaction with integrin-receptor (see figure 1).

Further, the effect of anxA5 or T7D anxA5 on phagocytosis of apoptotic cells was studied by addition of anxA5 or T7D anxA5 to a mixture of THP-1 cells (phagocytes) and anti-Fas stimulated Jurkat cells (apoptotic cells). AnxA5 inhibits phagocytosis of apoptotic cells likely by shielding the expressed PS on the apoptotic surface. By introducing RGD into the N-terminal tail of anxA5 inhibition is reversed into

potentiating of phagocytosis (see figure 2). Therefore the overall conclusion of the preliminary data is that RGD in the N-terminal tail of anxA5 stimulates phagocytosis of apoptotic cells likely through activation of integrin receptors of the phagocyte.

Figure 2 shows that AnxA5 inhibits and RGD-anxA5 stimulates phagocytosis of apoptotic Jurkat cells by differentiated THP-1 cells.

Example 3: Production of Annexin A5 variant having an RGD motif and a Cys residue at the N-terminus

Human annexin A5 cDNA was mutated in the manner as described in example 1, with the further substitution of Q (Gln), encoded by GAR (R = A or G), at position 2 by C (Cys), encoded by TGY (Y = T or C). The variant is denoted as A5 Q2C T7D.

The cysteine of this variant can be used for coupling diagnostics of therapeutics The variant can be further mutated by substitution of Cys-315 by e.g. Ser or Ala.

Claims

1. An Annexin A5 variant having at least 80% amino acid identity with the full length sequence of SEQ ID NO. 1, comprising at least one (1) and not more than twenty (20) RGD sequences within the Annexin, wherein at least one of the RGD sequences substitutes sequences of three amino acids within the range 1-19, 46-64, 86-89, 118-135, 157-170, 203-219, 245-248 and 280-294 of SEQ ID No. 1 or is located on an amino acid sequence extension at the N-terminus of the Annexin A5, wherein said Annexin A5 variant has the ability to selectively bind to a cell with phosphatidylserine exposed on its surface.

2. An annexin A5 variant according to claim 1, wherein said at least one RGD sequence is flanked by a neutral amino acid selected from A, G, I, L, M, P, S, T and V.

3. An Annexin A5 variant according to claim 1 or 2, which comprises more than one RGD sequence, each pair of RGD sequences being separated by 1-3 amino acids selected from A, G, I, L, M, P, S, T and V.

4. An Annexin A5 variant according to any one of claims 1-3, wherein at least one RGD sequence substitutes a sequence of three amino acids within the range 1-15.

5. An Annexin A5 variant according to claim 4, wherein at least one RGD sequence substitutes a sequence of three amino acids within the range 1-10 of SEQ ID No. 1.

6. An Annexin A5 variant according to any one of claims 1-5, wherein at least one RGD sequence is located on an amino acid sequence extension at the N-terminus of the Annexin A5.

7. An Annexin A5 variant according to any one of claims 1-6, wherein at least one RGD sequence is part of a cyclic sequence containing 3-20 amino acids.

8. An Annexin A5 variant according to any one of claims 1-7, wherein at least one amino acid selected from D, E, K, R, N, Q at positions 16-29, 59-74, 88-102, 135-145, 156-169, 202-231, 259-266, and 305-317, is substituted by an amino acid selected from G, A, P, S, T and V.

9. An Annexin A5 variant according to any one of claims 1-8, which further contains a cysteine residue at one of the positions 1-19, 24, 28, 46-64, 86-89, 118-135, 149-150, 157-170, 203-219, 245-248 and 280-294, said cysteine residue optionally being coupled to a diagnostic or therapeutic molecule.

10. An Annexin A5 variant according to any one of claims 1-9, which further comprises from 2 up to 10 histidine residues at its N-terminus, at least two of said histidine residues being adjacent or separated by no more than one other amino acid, and optionally being bound to a radionuclide.

11. An Annexin A5 variant according to any one of claims 1-10, for use in stimulating phagocytosis of cells that expose phosphatidylserine on their surface.

12. An Annexin A5 variant according to any one of claims 1-11, for use in the treatment of a disease in mammals, which disease comprises the augmentation of phagocytosis as a desired effect of treatment.

13. An Annexin A5 variant according to claim 12, wherein mammals are humans.

14. An Annexin A5 variant according to claim 12, for use in the treatment of a chronic inflammatory disease.

15. An Annexin A5 variant according to claim 14, for use in the treatment of a chronic autoimmune disease.

16. An Annexin A5 variant according to any one of claims 12-14, for use in the treatment of atherosclerotic plaque.

17. An Annexin A5 variant according to any one of claims 12-14, for use in the treatment of chronic obstructive pulmonary disease.

18. An Annexin A5 variant according to any one of claims 12-14, for use in the treatment of systemic lupus erythematodes.

19. An Annexin A5 variant according to any one of claims 12-14, for use in the treatment of rheumatoid arthritis.

20. A composition comprising an Annexin A5 variant according to any one of claims 1-11 and a pharmaceutically acceptable excipient, carrier, and/or diluent.

Fig 1a

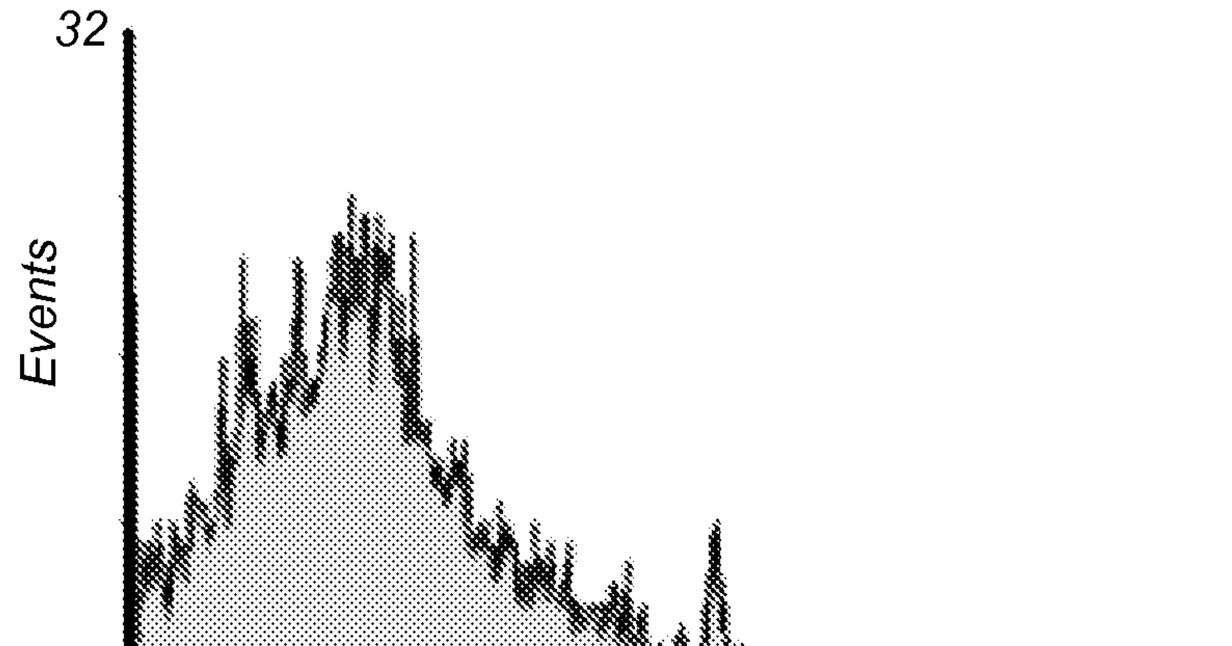


Fig 1b

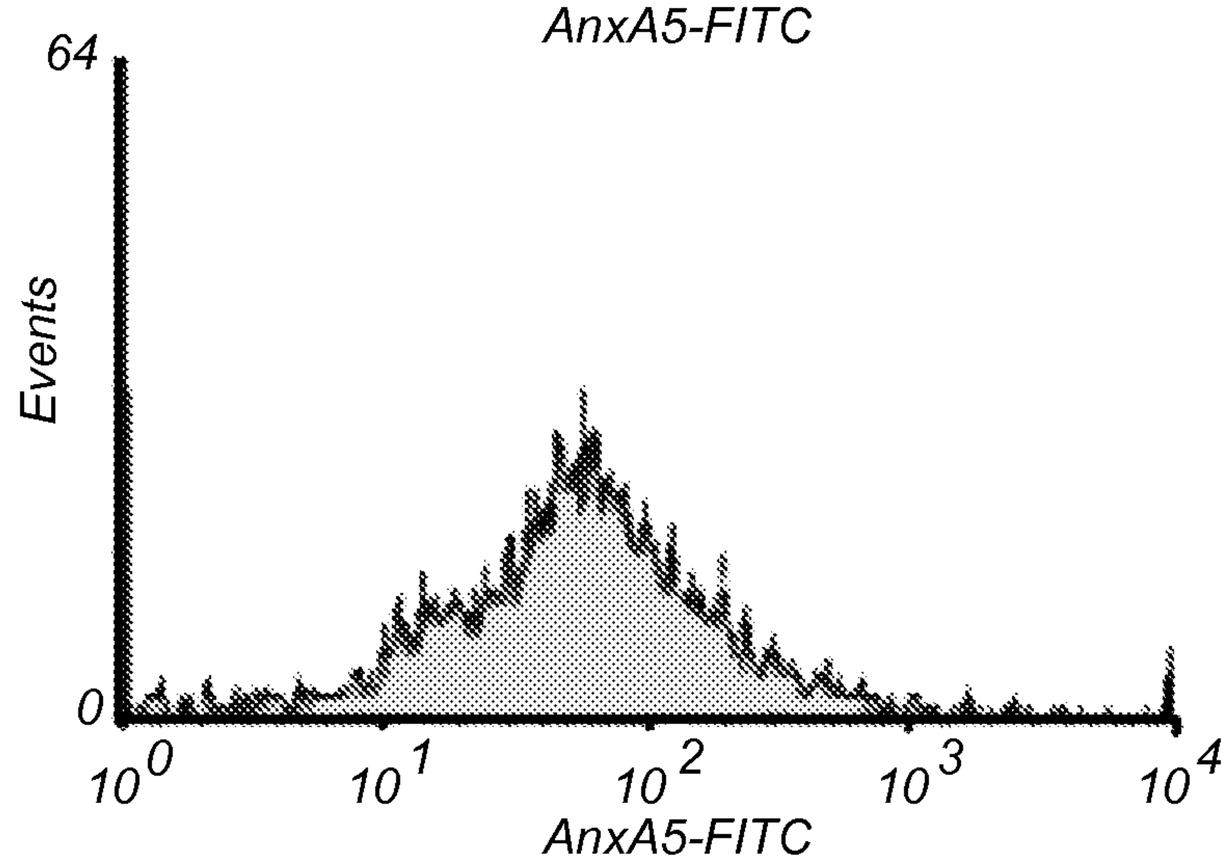


Fig 2

