CD89 activation in therapy

The invention relates to the use of CD89 activating molecules, in particular Fc alpha comprising molecules, and more particularly, IgA, for inducing apoptosis in neutrophils. Anti-CD89 antibodies can alternatively be used. The CD89 activation is beneficial in the treatment of various disorders associated with increases in neutrophils, such as autoimmune disorders, inflammatory disorders, NETosis, or cystic fibrosis.

Neutrophils form an essential part of the innate immune system. They are normally found in the blood stream. During the acute phase of inflammation, e.g. as a result of a bacterial infection, neutrophils migrate towards the site of inflammation by chemotaxis, attracted by cytokines/chemokines released by activated endothelium, mast cells and macrophages at the site of infection or inflammation. Neutrophils also express and release cytokines, which amplify inflammatory reactions by other cell types. Neutrophils play a key role in the defence against invading pathogens, using phagocytosis, degranulation, thereby releasing soluble anti-microbial agents, and the generation of neutrophil extracellular traps (NETs), which comprise a web of fibres composed of chromatin and granule proteins such as serine proteases that trap and kill microbes extracellularly. NETs may also trap the pathogens and prevent their further spread. Neutrophils will often be phagocytosed themselves by macrophages.

Immunoglobulin A (IgA) is the prominent antibody class at mucosal surfaces, where it represents the key player of adaptive mucosal immunity. Diseases associated with total or partial lack of IgA, such as common variable immunodeficiency (CVID) or complete IgA deficiency, are associated with recurrent sinopulmonary and gastrointestinal infection, bronchiectasis and autoimmune diseases. One common

finding in the acute state of these immunopathological conditions is neutrophilic infiltration, which by the release of toxic mediators and catabolic enzymes causes substantial collateral tissue damage. Therefore, while neutrophils have many beneficial functions, they can also cause significant damage if not eliminated effectively when the initial stimulus has been removed.

The IgA receptor CD89 (FcAlphaR) is a transmembrane glycoprotein present on the surface of myeloid lineage cells such as neutrophils, monocytes, macrophages and eosinophils, where it mediates immunologic responses to pathogens. It interacts with IgA-opsonised targets, and triggers immunologic defence processes such as phagocytosis, antibody-dependent cell-mediated cytotoxicity, release of inflammatory mediators and triggering of respiratory burst activity.

The inventors have now surprisingly found that CD89-activation, in particular by Fc-alpha comprising molecules such as IgA or an anti-CD89 antibody, can induce apoptosis in neutrophils, and that this activity is enhanced when neutrophils have been pre-activated with, for example, microbial components such as lipopolysaccharide (LPS) or lipoteichoic acid (LTA), or cytokines such as GM-CSF or TNF-alpha. This observation is highly relevant for the treatment of diseases involving chronic neutrophilic inflammation, e.g. autoimmune inflammatory conditions, conditions involving excessive NETosis, chronic infections with pus formation or conditions of sterile neutrophil-mediated inflammation that may persist or become chronic after resolution of an infection that initially led to recruitment of neutrophils to the site of infection. Therefore, in the present invention, the activation of CD89 actually leads to an inhibition in inflammation, by activation of the apoptotic pathway in neutrophils.

One aspect of the invention is therefore a CD89-activating molecule such as an Fc-alpha-comprising molecule or anti-CD89 antibody for use in inducing apoptosis in neutrophils, preferably in pre-activated neutrophils. Preferably, the Fc-alpha-comprising molecule is immunoglobulin A (IgA). The IgA may be polyclonal, and it

may be derived from serum or plasma, preferably from human serum or plasma. The IgA may be, for example, dimeric or monomeric, or a mixture thereof. Dimeric IgA may also comprise a J-chain, and it may additionally comprise a secretory component. The use of monoclonal IgA is also contemplated. The IgA may be IgA1 or IgA2 or a mixture thereof. Preferably the IgA is human, however, primate IgA may also be used. Dimeric IgA from serum or plasma may also be combined with secretory component, preferably recombinant secretory component, even more preferably with human recombinant secretory component. Tetrameric forms of IgA can also occur occasionally.

The Fc-alpha comprising molecule may also be Fc-alpha itself or a functional variant thereof (e.g. a tail-truncated form, lacking some of the C-terminal amino acid residues, for example the 18 C-terminal amino acid residues, see Pleass,RJ et al, J. Biol. Chem. 274, 23508-23514, 1999), but preferably it is a fusion protein of the Fc portion of IgA or functional variant thereof with another protein, for example with albumin, such as human serum albumin. However, other soluble proteins can also be used as fusion partners. In addition, single-chain Fc-alpha portions, or single chain versions of a functional variant of Fc-alpha, are contemplated, as well as dimers or even multimers of such single-chain Fc-alpha portions. When we refer to Fc-alpha in this document, functional variants of Fc-alpha, e.g. tail-truncated forms and/or other functional variants, e.g. Fc-alpha molecules with one or more amino acid substitutions, deletions or insertions that do not lead to a loss of CD89-activating activity, are also meant to be encompassed in the term “Fc-alpha”.

A functional variant is a molecule that retains at least 30% of the CD89-activating activity of the original molecule, preferably at least 40%, 50%, or 60%, more preferably at least 70%, 75%, 80%, 90%, 95%, even more preferably at least 98%, most preferably full activity. It is also contemplated that the functional variant could have enhanced CD89-activating activity.

Anti-CD89 may be an antiserum produced by immunization with CD89 or fragments thereof, purified antibodies or CD89-binding fragments thereof from an anti-CD89 antiserum, or monoclonal anti-CD89 antibodies or CD89-binding fragments thereof. Preferably, a monoclonal anti-CD89 antibody is used. Once a monoclonal antibody with the desired activity is identified, mimetics can be produced that have the same effect as the monoclonal antibody.

Another aspect of the invention is a pharmaceutical composition comprising the CD89-activating molecule, preferably the Fc-alpha-comprising molecule or anti-CD89 antibody described above, wherein at least 50% of the protein in the composition is the Fc-alpha-comprising molecule or anti-CD89 antibody. Preferably, at least 60% of the protein is the Fc-alpha comprising molecule or anti-CD89 antibody, more preferably at least 70%, 75%, 80%, 85%, 90%, or 95%, most preferably at least 98%.

In another aspect of the invention, the neutrophils that are treated with the CD89-activating molecule, preferably the Fc-alpha comprising molecule or anti-CD89 antibody, are located in a patient with an autoimmune disorder or inflammatory condition, in particular chronic neutrophilic inflammatory conditions. Examples of the autoimmune disorder or inflammatory condition are sterile neutrophilic inflammation, such as pleural empyema, or empyema in other cavities such as uterus (pyometra), infectious inflammation e.g. meningitis with pus, Cystic Fibrosis, bronchiectasis, neutrophil-induced inflammation, NETosis, arthritis, in particular rheumatoid arthritis, spondyloarthritis, ankylosing spondylitis/Morbus Bechterew or reactive arthritis (e.g. Reiter's disease). Further examples include other inflammatory conditions which are mainly mediated by neutrophils, such as inflammatory bowel disease (IBD).

In another aspect of the invention, the CD89-activating molecule, preferably the Fc-alpha comprising molecule or the anti-CD89 antibody, is administered locally to the site of increased neutrophil numbers, e.g. into the diseased joint of an arthritis

patient, in particular into the diseased joint of a rheumatoid arthritis patient, or to the site of a sterile or infectious inflammation, or to the site of chronic neutrophilic inflammation.

The neutrophils may also be located in the lung of a patient with cystic fibrosis, where NETosis, i.e. excessive NET (neutrophil extracellular trap) formation, has been correlated with impaired obstructive lung function. In this case, it is advantageous to administer the CD89-activating molecule, preferable the Fc-alpha comprising molecule, by inhalation. Preferably, it is administered in combination with DNase.

In addition, the NETosis may be associated with the pathogenesis of other autoinflammatory conditions such as preeclampsia, septic shock and autoimmune vasculitis. Another aspect of the invention is therefore the Fc-alpha comprising molecule, in particular IgA, or anti-CD89 antibody for use in the treatment of these conditions, preferably for use in the induction of apoptosis in neutrophils, preferably in activated neutrophils, located in patients suffering from these conditions.

Another aspect of the invention is a method for inducing apoptosis of neutrophils, comprising bringing the neutrophils in contact with an effective dose of a CD89-activating molecule such as an Fc-alpha comprising molecule or an anti-CD89 antibody. Preferably, the neutrophils have been or are subjected to inflammatory cytokines or microbial products or other stimuli prior or during the contact with the CD89-activating molecule.

The effective dose of an Fc-alpha comprising molecule may be above the concentration of IgA in plasma, preferably about three times the IgA plasma concentration, more preferably about 10 times, 15 times or 20 times, the IgA plasma concentration, or even higher.

The CD89-activating molecule, preferably the Fc-alpha comprising molecule or the anti-CD89 antibody, is preferably comprised in a pharmaceutical composition, comprising one or more pharmaceutically acceptable excipients. The composition may additionally comprise a stabilizer.

The route of administration can be intravenous, subcutaneous, inhaled, intranasal, topical (i.e. skin or mucosal surface including gut or eye, administered as eyedrops), oral, but preferably the administration occurs locally, i.e. to the site of excessive neutrophil infiltration or activity, e.g. into the arthritic joint, by inhalation for cystic fibrosis, by injection to the site of a sterile infection etc. The dosage form may be a tablet, capsule, cream, suppository, but preferably it is a sterile solution. The product may be provided in lyophilized form and reconstituted into liquid form prior to use, or it may be provided as a stable liquid formulation.

Another aspect of the invention is the CD89-activating molecule, such as the Fc-alpha comprising molecule or anti-CD89 antibody, used in combination with other therapeutic agents, such as anti-inflammatory compounds, e.g. NSAIDs, antibodies that can modulate the immune response such as anti-CD20 (e.g. Rituxan), anti-TNFalpha (e.g. Remicade, Humira), antibiotics, anti-viral compounds such as ganciclovir, anti-fungal compounds such as Voriconazol, or anti-protozoan compounds.

The Fc-alpha-comprising molecule may be polyclonal IgA, e.g. isolated from serum or plasma, preferably from human serum or plasma. More preferably, it is purified from a pool of human plasma. Even more preferably, it is purified as a by-product of human plasma fractionation and/or subsequent purification of plasma proteins, most preferably from a side fraction that is produced during the manufacture of IgG preparations (e.g. IVIg or SCIg) from pooled human plasma, and/or from a precipitate obtained during the manufacture of IgG preparations from plasma.

Preferably, the IgA dimers are isolated (or enriched) from human pooled plasma. More preferably, these dimers are further combined with secretory component, e.g. secretory component produced recombinantly. The resulting secretory IgA is likely to be more stable in environments with high level of proteases, e.g. synovial fluid of an arthritic joint or the inflamed lung or gut. As most neutrophil granule proteins are proteases, this may be a particularly preferred way to carry out the present invention.

Alternatively, the IgA may be monoclonal. Monoclonal IgA may be produced by hybridoma cell lines, or by engineered cell lines, comprising the cDNA for the light chain and the heavy chain in an expression plasmid with an appropriate promoter and optionally further regulatory elements. The skilled person is well aware of methods to produce hybridomas, e.g. by cell fusion or immortalization of IgA-producing B-lymphocytes. Methods to isolate the cDNAs encoding the antibody heavy chain and light chain, and clone these cDNAs into appropriate expression vectors are also well known in the art. The expression vectors can then be transfected into cell lines, for example mammalian cell lines such as CHO cells or HEK293 cells, and cell lines can be selected that stably express the desired antibody. Suitable techniques are found, for example, in Current Protocols in Molecular Biology, Ausubel FM et al. (eds.) John Wiley & Sons, Inc.; http://www.currentprotocols.com/WileyCDA/. Functional variants of IgA, e.g. IgA with one or more amino acid substitutions, deletions or insertions that do not lead to a loss of CD89-activating activity, are also meant to be encompassed when the term “IgA” is used throughout this document.

The Fc-alpha comprising molecule may also be an engineered fusion protein. For example, the cDNA encoding the Fc-alpha portion of the IgA heavy chain is fused in frame to the cDNA encoding another protein, preferably a soluble protein, for example human serum albumin. Examples of suitable Fc-alpha cDNA sequences are shown in SEQ ID NOs: 1 and 2.

The Fc-alpha comprising molecule may also be a single chain Fc-alpha protein, where two Fc-alpha cDNAs are linked by a nucleic acid encoding a linker peptide, similar to the way single-chain Fv fragments are engineered. (Wörn A, Plückthun A. J. Mol. Biol, 2001;305:989-1010). Two or more of such single-chain Fc-alpha proteins may also be connected to form a dimer or multimer of Fc-alpha units. Such molecules can be produced by routine techniques, for example as described in Current Protocols in Molecular Biology, Ausubel FM et al. (eds.) John Wiley & Sons, Inc.; http://www.currentprotocols.com/WileyCDA/. For example, the Fc-alpha portion of the IgA heavy chain can be amplified from an IgA producing cell by RT-PCR, using primers designed to contain appropriate restriction sites. The resulting cDNA can then be digested with the corresponding restriction enzyme(s) and cloned into a suitably prepared expression vector. Synthetic oligonucleotides encoding linker peptides can be inserted into the appropriate positions, for example by digesting the cDNA with appropriate restriction enzymes and ligating the oligonucleotides with appropriately designed ends into the vector. The complete expression plasmid can then be produced in large amounts, e.g. in E. coli, using standard techniques. After purification, it can be transfected into mammalian cells, and cell lines stably expressing the desired proteins can be produced. The proteins can then be purified. Standard techniques are available to carry out these procedures.

Chimeric Fc-alpha portions, comprising, for example, one constant domain from the heavy chain of IgA1 and one constant domain from the heavy chain of IgA2 are also contemplated; the skilled person is well aware of the immunoglobulin domains and can identify the domain borders and combine immunoglobulin domains as desired. Combinations of Fc-alpha portions with J-chain are also included; additionally the resulting molecule may also be combined with secretory component. As mentioned above, functional fragments of Fc-alpha and functional variants can also be used; fusion proteins, dimers, multimers, combinations of such functional variants with J-chain, or with J-chain and secretory component, are also included.

The preferred secretory component contains at least a segment of the amino acid residues 1 to residue about 606 of the human polymeric immunoglobulin receptor (pIgR) amino acid sequence or variants thereof, e.g. analogous portions from a different mammalian species. However, the human sequence is preferred. The protein sequence of the human pIgR can be found under SwissProt accession number P01833 (see also Krajci et al, 1989, Biochem. Biophys. Res. Comm 158, 783-789).

Alternatively, the CD89 activating protein may be anti-CD89. The skilled person will be well aware of methods to produce anti-CD89. CD89, or fragments thereof, may be used as an antigen to produce a specific antiserum. Antibodies may be isolated from such an antiserum. Monoclonal antibodies may also be produced, and the methods of producing such monoclonal antibodies are well known to the skilled person. Preferably, the monoclonal anti-CD89 antibody is a human antibody, e.g. produced from immortalized human B-lymphocytes or in an animal where the animal's own immunoglobulin genes have been at least partially replaced by the corresponding human genes. The techniques used for the production of antibodies are well known, and are described in detail in various laboratory manuals, e.g. Ed Harlow & David Lane: Antibodies, A Laboratory Manual, or Sambrook et al: Molecular Cloning, A Laboratory Manual (both from CSHL).

The invention is exemplified in the following examples. The examples are intended as an illustration of a way to perform the invention and are not supposed to be limiting the invention.

Examples: Induction of apoptosis in neutrophils

Materials and Methods

Antibodies: Anti–CD89 (Clone A59) (both unlabeled and phycoerythrin [PE]–conjugated), and PE-conjugated immunoglobulin G1 (IgG1) isotype control were from BD Biosciences (Basel, Switzerland). Goat anti–mouse IgG (GaM) and unlabelled control IgG1 were obtained from Jackson ImmunoResearch Laboratories (distributed by Milan Analytica, La Roche, Switzerland). Anti-Fas agonistic mAb (CH11) was obtained from LabForce AG (Nunningen, Switzerland). Pooled serum IgA was obtained from CSL Behring AG (Wankdorf, Bern), and was prepared as follows: IgA was purified from human plasma and side-fraction of the IVIg/SCIg manufacturing process by affinity chromatography using CaptureSelect Human IgA resin from BAC (Naarden, Netherlands). Briefly, cryo-depleted human pool plasma or side-fraction was loaded onto an equilibrated CaptureSelect Human IgA column under near physiological conditions (pH, conductivity), without exceeding the IgA-binding capacity of the column. After loading the column was washed with phosphate buffered saline and IgA was eluted with a glycin buffer at pH 3. The eluates were pH-corrected and concentrated up to 50 mg/ml protein. Monomeric IgA can be purified from plasma IgA obtained as described above by preparative size-exclusion chromatography, for example using Superdex 200 gel resin.**Cells:** Blood neutrophils were isolated from healthy individuals as well as patients suffering from rheumatoid arthritis (RA) associated with acute joint inflammation. Briefly, peripheral blood mononuclear cells (PBMCs) were separated by centrifugation on Ficoll-Hypaque (Seromed-Fakola AG, Basel, Switzerland). The lower phase, mainly granulocytes and erythrocytes, was treated with erythrocyte lysis solution (155 mM NH_4Cl, 10 mM $KHCO_3$, and 0.1 mM EDTA [ethylenediaminetetraacetic acid], pH 7.3). The resulting cell populations contained mostly neutrophils. Joint fluid neutrophils from patients with RA were isolated using the same protocol. Cell purity was assessed by staining with Diff-Quik (Baxter, Düdingen, Switzerland) and light microscopy analysis. The purity of the resulting populations was at least 95% for neutrophils.

Immunofluorescence: CD89 surface expression was analyzed by flow cytometry following incubation of the cells with saturating concentrations of PE-conjugated anti–CD89 and isotype-matched PE-conjugated control mAbs.

Cell cultures: Neutrophils were cultured at $1x10^6$/mL in the presence or absence of cytokines or LPS and/or antibodies for the indicated times using complete culture medium (RPMI 1640 containing 10% fetal calf serum [FCS] and 200 IU/mL penicillin/ 100 µg/mL streptomycin, all from Life Technologies, Basel, Switzerland). If not indicated otherwise, cells were stimulated with 17.5 µg/mL anti-CD89 mAb or with 10 mg/ml plasma IgA. GM-CSF (Novartis Pharma GmbH, Nürnberg, Germany) was used at 25 ng/mL, anti-Fas mAb (CH11) at 1 µg/mL. LPS (Sigma Aldrich, Buchs, Switzerland) was used at 100 ng/ml. Goat anti-mouse was used at 30 µg/ml , G-CSF (Aventis Pharma, Zurich, Switzerland) at 25 ng/mL, IFN-γ (R&D Systems, Wiesbaden-Nordenstadt, Germany) at 85 ng/mL, IFN-α (PBL Biomedical Laboratories, distributed by Alexis) at 500 U/mL, TNF-α (R&D Systems, Wiesbaden-Nordenstadt, Germany) at 25 ng/mL, and LTA (Gift from T. Hartung University of Konstanz) at 10 µg/mL. The caspase inhibitor qVD-OPh (quinoline-Val-Asp-difluorophenoxymethylketone) was used at 20 µM.

Determination of cell death and apoptosis: Cell death was assessed by uptake of 1 µM ethidium bromide and flow cytometric analysis (FACSCalibur, Becton Dickinson). To determine the form of cell death, morphologic analysis and annexin V analysis were performed. For morphologic analysis, the cells were cultured for 15 hours, and stained with Giemsa-May-Grünwald (Diff-Quik). An Axiovert 35 microscope equipped with a 630 /1.4 oil objective lens was used (Carl Zeiss, Heidelberg, Germany). Images were processed with Adobe Photoshop 5.0 software (Adobe, San Jose, CA). To determine whether cell death was apoptosis, redistribution of phosphatidylserine (PS) on the neutrophil's membrane was measured by Annexin V staining and flow cytometry (FACSCalibur, Becton Dickinson), using a commercially available apoptosis detection kit according to the manufacturer's protocol (BD Biosciences), in the presence of propidium iodide (PI) (von Gunten S et al., Blood 2005; 106:1423-1431.). To detect DNA fragmentation,

a typical hallmark of apoptosis, the cells were permeabilized by a hypotonic fluorochrome solution (50 µg/ml Propidium iodide, 0.1% Sodium citrate, 0.1% Triton X-100), leading to a leakage of DNA multimers (182 bp) out of the cell. The remaining, reduced amount of DNA was then stained by the DNA intercalating agent propidium iodide and analyzed by flow cytometry (Nicoletti I et al., J Immunol. Methods 1991;139:271-279).

Statistical analysis: Statistical analysis was performed by using the Student *t* test. If mean levels are presented, the standard error of the mean (SEM) and the number (n) of independent experiments are indicated in each case. A probability value of less than 0.05 was considered statistically significant.

Example 1: CD89 expression on neutrophils upon stimulation with LPS or GM-CSF

Neutrophils were isolated from blood of healthy volunteers as described above. The cells were incubated in the presence or absence of inflammatory stimulus (100 ng/ml LPS or 10 ng/ml GM-CSF) for 8 hours. The cells were then incubated with phycoerythrin (PE)-labeled anti-CD89 or PE-labeled isotype-matched control antibody. CD89 expression was then assessed by flow cytometry.

Neutrophils expressed CD89 in the absence of inflammatory stimulus (Fig. 1A), and CD89 expression was increased when the cells were incubated in the presence of an inflammatory stimulus such as LPS (Fig 1A, left panel) or GM-CSF (Fig 1A, right panel). The profiles of the cells stained with the control antibody are also shown.

Figure 1B shows the time course of CD89 upregulation with LPS and GM-CSF. Significant upregulation of expression was observed after 4 hours.

Example 2: CD89 ligation stimulates neutrophil cell death

Neutrophils were isolated as described above. The cells were then incubated with anti-CD89 monoclonal antibody, anti-Fas antibody, anti-Fas + anti-CD89, each in the presence and absence of goat-anti mouse antibody (GaM). Cell death was assessed by ethidum bromide uptake and flow cytometric analysis. Ligation of CD89 by an anti-CD89 monoclonal antibody (mAb) resulted in significantly

increased neutrophil cell death (Fig. 2A), similar to anti-Fas induced cell death. CD89-mediated death was further significantly enhanced by crosslinking. Results of 22-hour cultures are shown (n = 5). (B) A concentration effect curve of anti-CD89 mAb in 22-hour neutrophil cultures indicated maximal death effects at 17.5 μg/ml (n = 5) (Fig. 2B).

IgA isolated from plasma or monoclonal IgA, and other Fc-alpha-comprising molecules will show similar effects, in particular if cross-linking of CD89 is achieved.

Example 3: Serum IgA and CD89 induce neutrophil cell death

Blood neutrophils were isolated from healthy volunteers as described above. The cells were incubated with medium in the presence or absence of plasma IgA at 10 mg/ml (Fig. 3A) or anti-CD89 mAb at 17.5 μg/ml (Fig. 3B) in the presence of various inflammatory stimuli. IgA clearly increased cell death in activated neutrophils (Fig. 3A). Similar results were obtained with anti-CD89 monoclonal antibody, shown in Figure 3B. Total plasma IgA (containing monomers and dimers) and a monomeric subfraction of plasma IgA were equally effective in inducing neutrophil cell death (Fig. 3C).

Example 4: Neutrophils from ankylosing spondyloarthritis patients express enhanced levels of CD89

Neutrophils were isolated from blood of a healthy volunteer and of an ankylosing spondyloarthritis patient. They were then incubated with PE-labeled anti-CD89 mAb or PE-labeled control antibody and CD89 expression was analyzed by flow cytometry. The neutrophils from the ankylosing spondylarthritis patient showed significantly increased surface expression of CD89 (Fig. 4, solid line) compared to the healthy donor (Fig. 4, dotted line). Staining with isotype-matched control mAb of the same samples of neutrophils is also shown.

Example 5: Neutrophils isolated from patients are more susceptible to anti-CD89- and IgA-mediated cell death

Neutrophils were isolated from blood of healthy volunteers for control purposes, and from joint/synovial fluid of arthritis patients (patients suffering from rheumatoid arthritis, ankylosing spodyolarthritis, psoriatric arthritis and "unknown" arthritis), and were cultured for 24 hours in presence or absence of anti-CD89 antibody or medium; thereafter neutrophil cell death was analyzed as described above. Anti-CD89 led to a high rate of death in synovial neutrophils from arthritis patients. The death rate was significantly higher than that of neutrophils from blood of healthy volunteers as shown in Figure 5A. The same effect was observed with plasma IgA at 10mg/ml (Fig 5B).

Example 6: Pan-caspase inhibitor qVD-OPh inhibits IgA-mediated cell death in neutrophils

Neutrophils were isolated from blood from healthy volunteers as described above. The cells were then cultured for 24 hours in the presence or absence of inflammatory stimulus (LPS, GM-CSF), and cell death in the absence or presence of IgA and/or pan-caspase inhibitor qVD-OPh (20 μM) was assessed. The results are shown in Figure 6. The pan caspase-inhibitor qVD-OPh blocked the plasma IgA-mediated neutrophil cell death both in the presence and absence of LPS and GM-CSF. As a control apoptosis induction with an anti-Fas mAb and its inhibition with qVD-OPh is shown. These findings indicate that IgA-mediated neutrophil cell death is caspase-dependent programmed cell death, i.e.apoptosis.

Example 7: Phosphatidyl serine redistribution upon pooled serum IgA binding to neutrophils, as shown by Annexin V staining

Neutrophils were isolated from blood of healthy volunteers as described above, and incubated in medium, medium with GM-CSF or LPS, in the presence or absence of plasma IgA or anti-CD89 mAb. For control neutrophils were incubated with an apoptosis-inducing anti-Fas mAb. After 6 hours, the phosphatidyl serine distribution was assessed by Annexin V staining and flow cytometry. After 10 hours the DNA fragmentation was assessed by propidium iodine staining and flow cytometry.

Pooled serum IgA ligation in presence of GM-CSF or LPS resulted in strong phosphatidyl-serine redistribution (Fig. 7A) and DNA fragmentation (Fig. 7B), both indicative for the induction of neutrophil apoptosis. Comparable results were obtained using anti-CD89 mAb instead of plasma IgA (Fig 7A, 7B). These scatter plots show one representative experiment out of 5. Percentages of cells in each quadrant are indicated. In Figure 7B, the relative number of apoptotic cells (percentage) is indicated in each histogram.

Example 8: Morphology of CD89-mediated neutrophil death

Neutrophils were isolated from blood from healthy volunteers as described above. They were cultured for 15 hours in the presence of medium alone, GM-CSF, or LPS, in the presence and absence of anti-CD89. They were stained with Giemsa-May-Grünwald (Diff-Quik), and examined by microscopy. CD89-mediated neutrophil death showed the typical morphological features of classic apoptosis (reduced cell volume, fragmented nuclei). IgA-mediated neutrophil death is expected to show the same morphology.

Claims

1. A CD89-activating molecule for use in inducing apoptosis in neutrophils.

2. The CD89-activating molecule of claim 1, wherein it is a molecule comprising Fc-alpha or a functional equivalent thereof.

3. The molecule of claim 2, wherein it is immunoglobulin A (IgA).

4. The IgA of claim 3, wherein the IgA is derived from serum or plasma.

5. The IgA of claim 3 or claim 4, wherein the IgA is dimeric.

6. The IgA of claim 3 or claim 4, wherein the IgA is monomeric.

7. The IgA of claim 5, wherein the IgA dimer comprises a J-chain.

8. The IgA of claim 7, wherein the IgA dimer comprises a secretory component.

9. The IgA of claim 3 or claim 4, wherein the IgA comprises monomeric and dimeric IgA.

10. The IgA of any of claims 3 to 9, which is polyclonal.

11. The IgA of any of claims 3 or 5 to 9, which is monoclonal.

12. The IgA of any of claims 3 to 11, wherein the IgA is IgA1 or IgA2 or a mixture thereof.

13. The CD89-activating molecule of claim 1, wherein it is an anti-CD89 antibody, preferably a monoclonal antibody.

14. A pharmaceutical composition comprising the CD89-activating molecule of any of claims 1 to 13, wherein at least 50% of the protein in the composition is the CD89-activating molecule.

15. The CD89-activating molecule of any of claims 1 to 13, wherein the neutrophils are comprised in a patient with an autoimmune disorder or inflammatory condition.

16. The CD89-activating molecule of claim 15, wherein the autoimmune disorder or inflammatory condition is selected from sterile neutrophilic inflammation, infectious inflammation, neutrophil-induced inflammation, inflammatory bowel disease and NETosis.

17. The CD89-activating molecule of claim 15, wherein the autoimmune disorder is arthritis, in particular rheumatoid arthritis, spondyloarthritis, ankylosing spondylitis/Morbus Bechterew, or reactive arthritis.

18. The CD89-activating molecule according to any of claims 1 to 13, wherein the CD89-activating molecule is administered locally.

19. The CD89-activating molecule according to any of claims 1 to 13, wherein the neutrophils are in a patient with cystic fibrosis.

20. Method for inducing apoptosis of neutrophils, comprising bringing the neutrophils in contact with an effective dose of the CD89-activating molecule of any of claims 1 to 13.

21. The method of claim 20, wherein the neutrophils have been pre-activated by an inflammatory stimulus such as inflammatory cytokines or microbial components (e.g. LPS).