COMPOSITIONS COMPRISING SECRETORY-LIKE IMMUNOGLOBULINS

Compositions

The invention relates to methods for preparing compositions comprising secretory-like immunoglobulin, in particular secretory-like IgA and/or secretory-like IgM, and compositions obtainable by the methods.

IgA is the second most abundant Ig class after IgG in human plasma where it is found at 0.88-4.10 g/L. There are two subclasses of IgA, IgA1 and IgA2 (Fig. 1) (Zuercher AW et al. Plasma derived immunoglobulins. Principles of Immunopharmacology. 3rd ed. Birkhäuser, 2011: p. 271-301) which differ in their disulfide bonds linking heavy and light chains as well as in their antigenic diversity due to significant differences in the hinge region of the molecule. The glycosylation pattern of the two subclasses is different: the heavy chain of both subclasses is N-glycosylated; in contrast, O-glycosylation is found on IgA1 but not IgA2, due to the truncated hinge region of IgA2.

Human IgA is found in two major forms: either circulating in blood/ plasma, or secreted to mucosal surfaces. In plasma, IgA is predominantly present as monomers (80-90%) and is produced by bone marrow plasma cells; the major subclass in plasma is IgA1.

The term polymeric IgA (pIgA) describes dimeric, occasionally tetrameric, IgA covalently joined at their "tail-pieces" by the Joining (J) chain (Fig. 1).

IgM molecules make up 10% of the total serum Ig content. They are confined predominantly to the intravascular pool and are part of the primary, antigen-specific, humoral immune response; phylogenetically and ontogenetically they are the earliest antibody (Ab) molecules. IgM exists predominantly as a pentamer joined by

the J chain and arranged into a planar structure; occasionally, IgM can also be found in a hexameric form lacking the J chain (Zuercher AW et al. Plasma derived immunoglobulins. Principles of Immunopharmacology. 3rd ed. Birkhäuser, 2011: p. 271-301).

Mucosal surfaces of the digestive, respiratory and urogenital tracts, as well as the ducts of exocrine glands are lined by layers of epithelial cells that form a tight barrier separating the body's internal compartments from the outside environment. In humans, these vast surfaces cover 400 m^2, an area that is permanently exposed to exogenous pathogens (Corthésy, B. (2010) Future Microbiol. 5:817-829). The combination of innate and inducible cellular and molecular mechanisms ensures protection against colonization and entry/invasion by microbes. In healthy individuals, secretory IgA (SIgA) is the most abundant antibody (Ab) fulfilling the function of immune exclusion on the luminal side of mucosal surfaces (Macpherson, A.J. et al. (2008) Mucosal Immunol. 1:11-22), whereas secretory IgM Abs take over in IgA-deficient patients. SIgA is synthesised by mucosal plasma cells of the intestinal lamina propria, the upper respiratory tract or the uro-genital tract. SIgA consists of a pIgA dimer, and a highly glycosylated secretory component (SC) of approximately 75 kDa (Fig. 1); similarly pentameric, J chain-containing IgM associated with SC constitutes SIgM. The SC represents the extracellular part of the polymeric Ig receptor (pIgR). pIgR is needed for the trans-epithelial transport of pIgA or pentameric IgM from the site of production to the mucosal surface where the pIgR-pIgA / pIgR-IgM complex is converted to SIgA / SIgM by enzymatic cleavage (Zuercher AW et al. Plasma derived immunoglobulins. Principles of Immunopharmacology. 3rd ed. Birkhäuser, 2011: p. 1-31). Association with SC protects IgA or IgM from proteolytic degradation. SIgA is the predominant Ig in seromucous secretions such as saliva, trachea-bronchial secretions, colostrum, milk, tear fluid, intestinal secretions and uro-genital secretions. It is the most prominent Ig produced at mucosal linings (and thus in the human body); approx. 3-5 g of SIgA is secreted daily into the intestinal lumen. SIgA is thus essential for

immune exclusion and to maintain epithelial integrity. SIgM is present at lower levels but fulfills the same immune exclusion functions as SIgA.

For a few pathogens such as Poliovirus, *Salmonella,* or influenza, protection against mucosal infection can be induced by active mucosal immunization with licensed vaccines. However, for the majority of mucosal pathogens no active mucosal vaccines are available. Alternatively, protective levels of Abs might directly be delivered to mucosal surfaces by passive immunization. In nature this occurs physiologically in many mammalian species by transfer of maternal antibodies to their offspring via milk (Brandtzaeg, P. (2003) Vaccine 21:3382-3388). Human and animal studies using passive mucosal immunization have demonstrated that pIgA and SIgA antibody molecules administered by oral, intranasal, intrauterine or lung instillation can prevent, diminish, or cure bacterial and viral infections (Corthésy, B. (2003) Curr. Pharm. Biotechnol. 4:51-67). However, the secretory form of IgA naturally found at mucosal surfaces was rarely used, and large scale production of SIgA is not possible to date. Construction of SIgA with biotechnological methods is challenging but such molecules could have important clinical applications (Corthésy, B. (2002) Trends Biotechnol. 20:65-71). The same also applies to secretory component-containing IgM.

Summary of the invention

The inventors have surprisingly found that it is possible to combine plasma-derived J chain-containing immunoglobulin, in particular IgA and/or IgM, with secretory component without the need to first purify the J chain-containing immunoglobulin. This invention opens the door to large-scale production of secretory-like IgA and/or IgM which can be used in medicine, for example for the prevention and treatment of infections on mucosal surfaces in subjects, in particular in human subjects.

One aspect of the invention is a method for producing a composition comprising secretory-like immunoglobulin, in particular IgA and/or IgM, in vitro, comprising the steps of

(a) obtaining a blood-derived protein composition comprising J chain-containing immunoglobulin, in particular IgA and/or IgM, in a non-purified form; and

(b) admixing the composition of step (a) with secretory component.

Preferably, the secretory-like immunoglobulin is secretory-like IgA. Preferably, the composition of step (a) contains at least 5% J chain-containing IgA, more preferably at least 10%, more preferably at least 20%, even more preferably at least 30%, most preferably it will contain at least 50% J chain-containing IgA. Preferably, the composition of step (a) is derived from human blood, e.g. human plasma or fractions thereof enriched for IgA or even J chain-containing IgA, but no purification of J chain-containing IgA is required.

Preferably, the secretory-like immunoglobulin is secretory-like IgM. Preferably, the composition of step (a) contains at least 5% J chain-containing IgM, more preferably at least 10%, more preferably at least 20%, even more preferably at least 30%, most preferably it will contain at least 50% J chain-containing IgM. Preferably, the composition of step (a) is derived from human blood, e.g. human plasma or fractions thereof enriched for IgM or even J chain-containing IgM, but no purification of J chain-containing IgM is required.

The secretory component used in step (b) is preferably recombinant secretory component, more preferably human recombinant secretory component, preferably produced by a mammalian cell line. However, secretory component from a natural source can also be used, such as secretory component purified from milk, saliva, mucus or similar sources.

The molar ratio between secretory component and J chain within IgA dimers/polymers or IgM pentamers in the composition of step (a) ranges between 1:10 and 10:1, preferably between 1:5 and 5:1, more preferably between 1:2 and 2:1.

In another aspect of the invention, the molar ratio between secretory component and J chain within the composition of step a) ranges between 1:10 and 10:1, preferably between 1:5 and 5:1, more preferably between 1:2 and 2:1.

Another aspect of the invention is a composition comprising secretory-like IgA and/or secretory-like IgM or combinations thereof obtainable by a method of the invention. The composition may further comprise a pharmaceutically acceptable carrier or excipient.

A further aspect of the invention is the composition described above for medical use.

Detailed description of the invention

As already mentioned above, the inventors have surprisingly found that it is possible to combine plasma-derived J chain-containing immunoglobulins, in particular J chain-containing IgA and/or J chain-containing IgM with secretory component without the need to first purify the J chain-containing immunoglobulin. This invention opens the door to large-scale production of secretory-like IgA and/or secretory-like IgM which can be used in medicine, for example for the prevention and treatment of infections on mucosal surfaces in subjects, in particular in human subjects.

One aspect of the invention is a method for producing a composition comprising secretory-like immunoglobulin, in particular secretory-like IgA or secretory-like IgM, in vitro, comprising the steps of

(a) Obtaining a blood-derived protein composition comprising J chain-containing IgA or J chain-containing IgM in a non-purified form,

(b) admixing or combining the composition of step (a) with secretory component.

Preferably, the composition of step (a) contains at least 5% (w/w) J chain-containing IgA, more preferably at least 10%, more preferably at least 20%, even more preferably at least 30%, more preferably at least 50%, most preferably it will contain at least 70% J chain-containing IgA. Preferably, the composition of step (a) is derived from human blood, e.g. human plasma or fractions thereof enriched for IgA or even J chain-containing IgA, but no specific purification steps for J chain-containing IgA dimers or polymers is required. Therefore, the J chain-containing IgA will be in a composition also comprising other proteins such as monomeric IgA, IgM, or IgG. For example, the composition may comprise more than 10% monomeric IgA, and/or more than 10% IgG, and/or more than 10% IgM. While an enrichment for J chain-containing dimeric IgA may happen as part of the processing of human plasma fractions for the purification of plasma proteins such as IgG, albumin, alpha-1 antitrypsin, and coagulation factors, no purification step specifically designed to separate J chain-containing dimeric IgA or polymers from other proteins, like affinity chromatography or size exclusion and selection of the fraction of the relevant molecular mass, is necessary to obtain the composition of step (a).

In another preferred aspect of the invention, the composition of step (a) contains at least 5% (w/w) J chain-containing IgM, more preferably at least 10%, more preferably at least 20%, even more preferably at least 30%, more preferably at least 50%, most preferably it will contain at least 70% J chain-containing IgM. Preferably, the composition of step (a) is derived from human blood, e.g. human plasma or fractions thereof enriched for IgM or even J chain-containing IgM, but no specific purification steps for J chain-containing IgM is required. Therefore, the J chain-containing IgM will be in a composition also comprising other proteins such

as IgA or IgG. For example, the composition may comprise more than 10% IgA, and/or more than 10% IgG. While an enrichment for J chain-containing IgM may happen as part of the processing of human plasma fractions for the purification of plasma proteins such as IgG, albumin, alpha-1 antitrypsin, and coagulation factors, no purification step specifically designed to separate J chain-containing IgM from other proteins, like affinity chromatography or size exclusion and selection of the fraction of the relevant molecular mass, is necessary to obtain the composition of step (a).

The term "secretory component" as used herein refers to a protein that specifically binds to J-chain-containing immunoglobulin, and is related to or derivable from or identical to an extracellular portion of the polymeric immunoglobulin receptor (pIgR), preferably a mammalian pIgR, more preferably a primate pIGR, most preferably a human pIgR. Preferably, the secretory component confers increased stability to the J-chain containing immunoglobulin. The secretory component comprised in the composition may be recombinant secretory component, preferably secretory component produced in a mammalian cell line. Secretory component in its traditional, narrow meaning (referred to as "natural secretory component" herein) is the extracellular portion of the polymeric immunoglobulin receptor (pIgR), which usually gets associated during secretion with dimeric or polymeric IgA or pentameric IgM comprising a J chain. J chain-containing IgA/IgM binds to the polymeric immunoglobulin receptor at the basolateral surface of epithelial cells and is taken up into the cell by transcytosis. This receptor complex then transits through the cellular compartments before being transported to the luminal surface of the epithelial cells. The transcytosed IgA/IgM-pIgR complex is then released through proteolysis, and part of the polymeric immunoglobulin receptor (pIgR), referred to as the natural secretory component, stays associated with the J chain-containing IgA/IgM, releasing secretory IgA/IgM. However, there is evidence that reverse transcytosis of IgA, i.e. from the luminal surface to the basolateral surface, can also take place.

The human pIgR is cloned and sequenced, its sequence is available as SwissProt entry P01833, and shown in Seq ID NO: 1. Human pIgR is a glycoprotein with 764 amino acid residues, containing a signal peptide (residues 1 to 18), an extracellular part (residues 19 to 638), a transmembrane region (residues 639 to 661), and a cytoplasmic region (residues 662 to 764). Residues 19 to 603 are thought to associate with J chain-containing IgA as described above, and this part of this glycoprotein is usually referred to as the secretory component (referred to as "natural secretory component" herein).

The secretory component used in the composition of the invention can comprise any extracellular pIgR sequence that is capable of associating with J chain-containing IgA. For example, secretory component may comprise extracellular domains of pIgR from mammalian sources, e.g. from primates, cattle, horses, cats, dogs, rabbits, guinea pigs, rats or mice, or variants thereof. Functional hybrids of the extracellular domains from several mammalian species or variants thereof are also contemplated for use in the invention, e.g. prepared by fusing the immunoglobulin-like domains from different species into a secretory component-like protein. A functional secretory component may also be formed by fusing a selection of immunoglobulin-like domains normally present, e.g. rabbit secretory component is functional being composed of only domains 1, 4 and 5. Preferably, however, the human secretory component or functional variants thereof is used.

Therefore the secretory component used in the composition of the invention preferably comprises residues 19 to 603 of SEQ ID NO: 1 or functional variants thereof. Functional variants may include deletions, insertions, and/or substitutions, preferably substitutions are conservative substitutions, e.g. a basic amino acid residue is substituted for another basic amino acid, a hydrophobic amino acid is substituted for another hydrophobic amino acid, etc. The variant secretory component is at least 50% identical in sequence to residues 19 to 603 of SEQ ID NO: 1, preferably at least 55%, 60%, 65%, 70%, 75%, 80%, more preferably at least 85% or even 90%, even more preferably at least 92%, 94%, 95%, 97%, 98%,

or even 99% identical to residues 19 to 603 of SEQ ID NO: 1. Preferably, the secretory component comprises the extracellular portion of the pIgR, more preferably the extracellular portion of the human pIgR, most preferably the secretory component comprises or even consists of residues 19 to 603 of SEQ ID NO: 1.

The skilled person is well aware how to produce the secretory component by recombinant techniques. An example of expression of human secretory component in CHO cells has been described by Phalipon et al (Phalipon A et al (2002) Immunity 17:107-115), but the invention is not limited to secretory component produced by this system. For example, the desired cDNA sequence can be produced synthetically or cloned via RT-PCR, using RNA isolated from cells or tissue expressing pIgR as template. The cDNA can then be inserted into a mammalian expression vector such as pcDNA3 – many alternative expression vectors are available. The recombinant expression vector will then be introduced into a suitable host cell line, such as CHO, Cos, HEK293, or BHK. Other cell lines are available and can also be used. Methods for introducing such vectors into a cell line include lipofection, electroporation and other techniques well known to the skilled person. Usually cells harboring the expression vector and expressing the protein of interest are then selected and cloned. Viral expression systems can also be used, for example, vaccinia virus can be used to express proteins at high levels in mammalian cells, baculovirus expression systems can be used to express proteins at high levels in insect cells. Yeast or bacterial expression systems can also be envisaged, and such expression systems are known to the skilled person. Likewise, plant expression systems can also be envisaged, and such systems are known to the skilled person.

The secretory component or variant thereof used in the composition of the invention may also comprise a tag, such as a hexa-Histidine tag, which can aid in the purification of the resulting protein. If such a tag is attached via a cleavable linker, the tag may be cleaved off prior to use in the invention. Similarly, the secretory

component may be produced as a fusion protein. Again, a cleavable linker may be used so that the fusion partner may be cleaved off the secretory component prior to use in the invention.

The skilled person can then purify the expressed protein with standard methods. Recombinant secretory component may be purified to high purity with a suitable method, for example size-exclusion and/or ion exchange chromatography. Preferably the final preparation of recombinant secretory component will be essentially free of contaminants, particularly host cell proteins. However, secretory component can also specifically associate with J-chain containing immunoglobulin in unpurified form, thus purification prior to association with the J-chain-containing immunoglobulin is not essential.

The secretory component may also be obtained from a natural source, preferably from milk, saliva or mucus. Preferably the secretory component is of human origin, but secretory component from other species can also be used in the invention.

The molar ratio between secretory component and J chain within IgA dimers/polymers or IgM pentamers in the composition of step (a) ranges between 1:10 and 10:1, preferably between 1:5 and 5:1, more preferably between 1:2 and 2:1.

The molar ratio between secretory component and J chain within the composition of step (a) is between 1:10 and 10:1, preferably between 1:5 and 5:1, more preferably between 1:2 and 2:1.

The amount of secretory component used in step (b) may be at least 1 part (by weight) of secretory component to 50 parts (by weight) of protein in the composition of step (a), preferably at least 1 part to 40, 30, 20, 15, 10, most preferably at least 1 part of secretory component to 5 parts of protein in the composition of step (a).

Another aspect of the invention is a composition comprising secretory-like IgA obtainable by a method of the invention. Yet another aspect of the invention is a composition comprising secretory-like IgM obtainable by a method of the invention. Yet a further aspect is a composition comprising secretory-like IgA and secretory-like IgM obtainable by a method of the invention, for example in a molar ratio of between 10:1 and 1:10, preferably between 5:1 and 1:5, more preferably between 2:1 and 1:2. In another aspect of the invention the combined content of IgA and IgM in the composition exceeds 50%, preferably 60%, more preferably 70%, even more preferably 80%, even more preferably 90%, most preferably it is 100%.

A further aspect of the invention is the composition described above for medical use. For example, the compositions of the invention can be used advantageously to treat necrotizing enterocolitis, and generally infections at mucosal surfaces.

The composition may further comprise one or more pharmaceutically acceptable carrier or excipient, and/or a stabilizer. The composition may be formulated in liquid form, as a syrup, a lotion, an ointment, a powder which may be reconstituted with a liquid prior to administration, a capsule, a pill, a gel, a cream, a jelly, a controlled release formulation, or any other formulation suitable for the intended medical use. For example, for the treatment of GI diseases, the composition may be formulated with a protective coating that dissolves in the desired area of the GI tract to release the composition. The composition may be taken orally, administered topically, enterally, by inhalation, or any other suitable route for the intended use. For oral application acid pump inhibitors may be co-administered.

The proteins in the composition may be concentrated, e.g. using dia/ultrafiltration or other standard methods, prior to being formulated. In addition, the composition may be lyophilized, and then reconstituted with a suitable solution prior to use.

Definitions

The term "secretory-like IgA" or "secretory-like plasma IgA" is intended to encompass J chain-containing (plasma) IgA combined with a protein that is secretory component or a functional variant thereof, which serves to provide some protection from proteolytic digestion. Typically, the J chain-containing IgA will comprise two or four, or even more IgA monomers. Typically, the J chain-containing IgA will be mixed with a secretory component or variant thereof in vitro, i.e. the association between secretory component and J chain-containing IgA takes place in vitro rather than during transcytosis.

The term "secretory-like IgM" is intended to encompass J chain-containing (plasma) IgM combined with secretory component or a functional variant thereof in vitro. Preferably, the J chain-containing IgM will be pentameric IgM.

"Specific purification steps for J chain-containing IgA dimers or polymers" relate to purification steps that would be included in a purification process specifically designed to separate J chain-containing IgA dimers or polymers from other proteins, such as monomeric IgA, other immunoglobulins, and other plasma proteins. Such specific purification steps may, for example, include affinity chromatography with a ligand specifically binding to J chain, or size exclusion chromatography selecting the fractions containing proteins of a molecular weight corresponding to J chain-containing IgA dimers. While the process for preparing the composition from plasma may comprise methods that lead to an enrichment of IgA or even J chain-containing IgA, such as ion exchange chromatography, the J chain-containing IgA is not specifically purified. Thus "J chain-containing IgA in a non-purified form" refers to a composition containing less than 80% J chain-containing IgA, typically less than 70%, 60%, or 50% J chain-containing IgA, it may even contain less than 40%, 30%, 25%, 20%, 15% or even 10% J chain containing IgA.

"Specific purification steps for J chain-containing IgM" relate to purification steps that would be included in a purification process specifically designed to separate J chain-containing IgM from other proteins, such as other immunoglobulins, and other plasma proteins. Such specific purification steps may, for example, include affinity chromatography with a ligand specifically binding to IgM or to J chain, or size exclusion chromatography selecting the fractions containing proteins of a molecular weight corresponding to J chain-containing IgM pentamers. While the process for preparing the composition from plasma may comprise methods that lead to an enrichment of J chain-containing IgM, such as ion exchange chromatography, the J chain-containing IgM is not specifically purified. Thus "J chain-containing IgM in a non-purified form" refers to a composition containing less than 80% J chain-containing IgM, typically less than 70%, 60%, or 50% J chain-containing IgM, it may even contain less than 40%, 30%, 25%, 20%, 15% or even 10% J chain containing IgM.

The term "secretory component" as used herein refers to a protein that specifically binds to J-chain-containing immunoglobulin, and is related to or derivable from or identical to an extracellular portion of the polymeric immunoglobulin receptor (pIgR), preferably a mammalian pIgR, more preferably a primate pIGR, most preferably a human pIgR. Preferably, the secretory component confers increased stability to the J-chain containing immunoglobulin. As detailed above, the most preferred secretory component is human secretory component, e.g. corresponding to residues 19 to 603 of SEQ ID NO: 1. However, amino acid deletions, insertions, substitutions may be included, as long as they lead to a functional protein, i.e. one that is still capable of associating with J chain-containing IgA and preferably conferring protection from proteolytic digestion to it. Homologues from other mammalian species are also included, as are chimeric proteins comprising parts from different species.

The term "% [percent]" when used to describe the content of immunoglobulin in a composition/preparation means weight per weight protein.

List of Figures

The invention will now be illustrated by the following, non-limiting examples, with reference to the following figures and sequence listing:

Figure 1 shows a diagram of the structure of monomeric, dimeric and J chain-containing secretory IgA.

Figure 2 shows Western blots of different IgA preparations, developed with different antibodies:

- Fig 2A shows a Western blot of different IgA preparations, developed with anti-α chain, anti-J chain or anti-secretory component antibody.
- Fig 2B shows a Western blot of different secretory-like and secretory IgA preparations, developed with anti-secretory component antibody.
- Fig 2C shows a Western blot of different size exclusion chromatography fractions of secretory-like IgAF5, developed with antibodies to secretory component, α chain and J chain.
- Fig 2D shows a chromatogram of a size exclusion chromatography run of secretory-like IgAF5.

Figure 3 shows dot blots, using immobilized secretory component:

- Fig 3A shows a flow diagram of how the assay was set up
- Fig 3B shows SC capturing J chain-containing IgA
- Fig 3C shows SC capturing J chain-containing IgM

Figure 4 shows Western blots of time course experiments of different IgA preparations (A) or IgM preparations (B) incubated with intestinal washes. The blots were developed using anti-heavy chain antibodies.

Figure 5 shows the protection against infection with Shigella by different IgA preparations:

- Fig 5A shows the decrease in transepithelial resistance by Shigella on polarized Caco-2 monolayers, and the protection from such decrease in TER by anti-Shigella SIgA (see Phalipon A et al (1995) J. Exp. Med. 182: 769-778), IgAF5 and SIgAF5.

- Fig 5B shows the reduction of bound and internalized bacteria by anti-Shigella SIgA, IgAF5 and SIgAF5.

Figure 6 shows images of *Shigella* in immune complexes obtained after incubation with anti-*Shigella* SIgA (SIgAC5) used as a positive control, and plasma-derived polymeric IgAF5, SIgAF5, monomeric IgAF5 and IgG.

Figure 7 shows secretion of cytokine TNF-α and chemokines CXCL8 and CCL3 by polarized Caco-2 epithelial cell monolayers exposed to *Shigella* alone or in complex with various Abs.

Figure 8 shows the protection against infection with Shigella by pentameric IgM and SIgM preparations.

- Fig 8 shows the decrease in transepithelial resistance by *Shigella* on polarized Caco-2 monolayers, and the protection from such decrease in TER by anti-Shigella SIgA (see Phalipon A et al (1995) J. Exp. Med. 182: 769-778), IgM and SIgM.

SEQ ID NO: 1 shows the protein sequence of human pIgR.

Examples

Example 1: Western blot of IgA preparations from plasma mixed with recombinant secretory component

Materials and Methods

1.1 IgA preparation from plasma by affinity chromatography and/or by sequential elution of MPHQ column

Human plasma IgA was purified by affinity chromatography using CaptureSelect Human IgA resin (Bioaffinity Company BAC, Naarden, Netherlands) according to the resin manufacturer's protocol using 3 different sources of plasma IgA as starting material, namely cryo-depleted plasma, re-solubilised cold ethanol fractionation paste, or a strip fraction from an anion-exchange (AIEX) chromatography column obtained by sanitizing said column, according to the commercially applied IgG purification process of CSL Behring AG (Berne, Switzerland). Briefly, cryo-depleted pool plasma, re-solubilised paste or AIEX strip fraction was diluted in phosphate buffered saline (PBS) to an IgA concentration of approximately 1 mg/mL and then loaded onto a PBS-equilibrated CaptureSelect Human IgA column, without exceeding the IgA binding capacity of the column. After loading the column was washed with PBS, and IgA was eluted with glycin buffer at pH 3. The eluate was adjusted with 0.5M Tris (pH 8) to pH 4.5 and concentrated up to 16 mG/mL protein in PBS. SIgA from human milk was purified by the same method.

From the AIEX chromatography step of the IVIg manufacture process of CSL Behring AG (Berne, Switzerland), fraction F4 was obtained after a post-wash of the Macro-Prep High Q (Bio-Rad, Hercule, CA) column with 10 mM phosphate / 30 mM acetate at pH 6.5 by elution with 55 mM tartrate / 5 mM acetate at pH 7.6. Then, fraction F5 was eluted with 50 mM phosphate / 25 mM citrate at pH 5.0. F4 and F5 were brought to approximately 1 mG/mL in PBS by ultra-/diafiltration, and then

depleted of IgG by affinity chromatography using IgSelect resin (GE Healthcare, Glattbrugg, Switzerland). IgAF4 was directly harvested in the flowthrough of the IgSelect chromatography of F4 load. To obtain IgAF5, the IgSelect flowthrough of F5 load was depleted of IgM by affinity chromatography using CaptureSelect Human IgM resin (Bioaffinity Company BAC). IgAF4 and IgAF5 were brought to final concentrations by ultra-/diafiltration.

1.2 IgM preparation from plasma by affinity chromatography

Human plasma IgM was purified by affinity chromatography using CaptureSelect Human IgM resin (Bioaffinity Company BAC, Naarden, Netherlands) according to the resin manufacturer's protocol using the same 3 different sources as starting material as described in section 1.1 for IgA, namely cryo-depleted plasma, re-solubilised cold ethanol fractionation paste, or a strip fraction from an anion-exchange (AIEX) chromatography column obtained by sanitizing said column, according to the commercially applied IgG purification process of CSL Behring AG (Berne, Switzerland). Briefly, cryo-depleted pool plasma, re-solubilised paste or AIEX strip fraction was diluted in phosphate buffered saline (PBS) to an IgM concentration of approximately 1 mg/mL and then loaded onto a PBS-equilibrated CaptureSelect Human IgM column, without exceeding the IgM binding capacity of the column. After loading the column was washed with PBS, and IgM was eluted with glycin buffer at pH 3. The eluate was adjusted with 0.5M Tris (pH 8) to pH 4.5 and concentrated up to 10 mG/mL protein in PBS.

1.3. Western blots

SDS-PAGE and electrotransfer onto nitrocellulose (NC) membranes were carried out using the Mini-Cell system from Invitrogen (Carlsbad, CA), according to the manufacturer's protocols. Briefly, samples were denatured in sample buffer under reducing or non-reducing conditions, respectively, and electrophoretically separated on pre-cast gradient gels, NuPAGE Novex Bis-Tris 4-12% 1.0 mm 10 well, using

NuPAGE MOPS Electrophoresis Buffer (Invitrogen). Wet transfer onto NC membranes (0.2 µm) was performed with the XCell II Blot Module (Invitrogen) and NuPAGE Transfer Buffer. The membranes were then blocked for 30 min in PBS-0.5% Tween 20 solution (PBS-T) containing 4% Rapilait skim milk powder (Migros, Switzerland). For immunoblotting polyclonal rabbit antibodies were used: 1) rabbit anti-human alpha chain (Dako, horseradish peroxidase (HRP)-conjugated: 1/5'000 dilution); 2) rabbit anti-human J chain (BioGenex, Fremont, CA; 1/300 dilution), followed by secondary anti-rabbit HRP-conjugated antiserum (Sigma; 1/10'000 dilution); 3) rabbit anti-human SC (Dako; 1/5000 dilution), followed by secondary anti-rabbit HRP-conjugated antiserum (Sigma; 1/10'000 dilution). All incubations were performed in PBS-T containing 4% milk powder at ambient temperature for 1-2 hours. After final washing with PBS-T, immunodetection on membranes was revealed by chemiluminescence and digitally recorded in an ImageQuant LAS 4000 system (GE Healthcare Lifesciences).

1.4 Association of plasma derived IgA with recombinant secretory component

Secretory-like IgA was obtained by combining in vitro 100 mg of IgAF5 with 4 mg of recombinant human secretory component (recSC). Association was performed in PBS for 30 min at room temperature as previously described in (Crottet, P., and Corthésy, B. (1998) J. Immunol. 161:5445-5453).

1.5 Size exclusion chromatography (SEC) fractionation

IgAF5 comprising secretory-like IgA (associated *in vitro* with recSC) was injected at 200 µG/ 20µL into an Agilent Technologies 1050 HPLC system for size exclusion chromatography at a flowrate of 1.5 mL/min over a TSKgel G3000SWXL 7.8 mm ID x 30 cm column (Tosoh Bioscience). Fractions of 0.75 mL were collected between 8.0 and 13.5 min retention time in intervals of 30 sec.

Results

The results are shown in Figure 2. Figure 2A demonstrates a comparison of IgA purified by affinity chromatography from plasma, from re-solubilised paste and from AIEX strip fraction or by sequential elution to obtain IgAF5 as described in 1.1 with SIgA from human milk. Secretory component was found in SIgA from milk but not in any of the IgA fractions purified from human plasma. All preparations contained the same amount of IgA heavy chain/alpha-chain. As expected, SIgA from milk contained the highest amount of J chain as essentially all IgA molecules are expected to be present as J chain-containing dimers. The amount of J chain and thus J chain-containing IgA dimers in IgA purified from plasma was low. This is expected as only a small portion of plasma IgA is present in dimeric form. A similar content of J chain was observed in IgA purified from re-solubilised paste. Surprisingly an increased fraction of IgA was present as J chain-containing dimers in the column strip fraction as evidenced by the increased amount of J chain. Surprisingly, this was further increased in IgAF5. This accumulation occurred without application of a specific process step for enrichment.

Figure 2B shows that no secretory-component containing IgA was present in IgAF5. After association with recSC free recSC (75kDa) and dimeric IgA associated with recSC were found. Indeed the secretory-like plasma IgA appeared similar to SIgA from milk. It was estimated that in the preparation of IgAF5 used in the shown experiment the content of J chain-containing IgAF5 was about 20%. Indeed, the signal strength observed in lane 2 was comparable to the signal of 1:5-diluted human milk SIgA.

Figure 2C shows the content of SC, IgA alpha chain and J chain in fractions obtained by size-exclusion chromatography of secretory-like IgAF5. recSC was observed in early fractions corresponding to high molecular weight forms of IgA – likely polymeric and dimeric forms. Appearance of SC coincided with appearance of J chain, indicating that indeed the SC-containing fraction of IgAF5 was the dimeric,

J chain-containing fraction. In addition IgA alpha-chain was detected in fractions of smaller molecular weight, likely comprising the monomeric fraction of IgAF5; these fractions were devoid of SC and J chain. These data demonstrate that recSC admixed with plasma-derived IgA containing monomeric and dimeric forms of IgA specifically associated with the dimeric, J chain-containing forms of IgA.

Figure 2D shows the chromatogram of the SEC run during which fractions were collected between retention time 8.0 min and 13.5 min. Peaks representing IgA polymers, dimers and monomers are indicated.

Example 2: Dot blot re-association assay (DORA)

A dot-blot re-association assay was used to show the association of immobilised secretory component with plasma-derived IgA or IgM *in vitro*. Briefly, as shown in Figure 3A, secretory component was dotted onto blotting membranes; non-specific binding sites were blocked. Thereafter plasma-derived IgA (Figure 3B) or IgM (Figure 3C) obtained by affinity chromatography from plasma (lane 4), from re-solubilised paste (lane 5) or from AIEX strip fraction (lane 6) obtained as described in 1.1 and 1.2 were applied to the membrane. After washing off unbound IgA or IgM, bound IgA/IgM was detected as described briefly below.

DORA was carried out essentially as described (Rindisbacher, L. et al (1995) J. Biol. Chem. 270:14220-14228), with the following modifications: Blotting membranes consisted of polyvinylidone fluoride (PVDF) polymer, blocking solution was phosphate-buffered saline-0.05% Tween-20 (PBS-T) containing 1% bovine serum albumin (BSA), crude preparations enriched in IgA were used for overlay incubation in 200 µl of PBS-T containing 0.1 % BSA, and detection antibodies were directly coupled to HRP.

The results for IgA are shown in Figure 3B. Immobilised secretory component was capable of capturing plasma-derived IgA. Similar to what is shown in Figure 2C this demonstrates that recSC associated with plasma-derived IgA dimers.

The results for IgM are shown in Figure 3C. Immobilised secretory component was capable of capturing plasma-derived IgM. This demonstrates that recSC associated with plasma-derived IgM.

Example 3: Digestion of secretory-like IgA and secretory-like IgM with intestinal washes

In order to prove a functional advantage of association of purified secretory component with J chain-containing IgA and IgM, respectively, IgA and IgM were prepared as described in paragraph 1.1 and 1.2. Secretory-like IgA was obtained by enriching J chain-containing IgA using size-exclusion chromatography and combining *in vitro* 10 μg thereof with 2.5 μg of recombinant human secretory component (hSCrec). Secretory-like IgM was obtained by enriching pentameric IgM using size-exclusion chromatography and combining *in vitro* 25 μg thereof with 2.5 μg of recombinant human secretory component (hSCrec). Association was performed in PBS for 30 min at room temperature as previously described (Crottet, P., and Corthésy, B. (1998) J. Immunol. 161:5445-5453). Integrity and proper assembly of the molecules into possibly covalent complexes were examined by SDS-PAGE under non-reducing and reducing conditions, followed by Western blotting and immunodetection with antiserum specific for hSC as indicated above.

Collection of intestinal washes from BALB/c mice (4-6 weeks old) was done according to the published procedure (Crottet, P., and Corthésy, B. (1998) J. Immunol. 161:5445-5453). For in vitro digestion, 120 ng of purified J chain-containing IgA and reconstituted secretory-like IgA were mixed (or not) with 1 or 2 μl of intestinal washes in a final volume of 20 μl of PBS and incubated at 37°C for various periods of time as indicated in Figure 4 (T= time in hours). For in vitro

digestion of IgM, 250 ng purified IgM and secretory-like IgM were mixed with 4 µl of intestinal washes. Reactions were stopped by the addition of 2 µl of Complete™ protease inhibitor mixture (Roche Applied Science, Rotkreuz, Switzerland), and kept frozen until analysis by Western blot detecting the reduced form of heavy chain of the antibody.

The results are shown in Figure 4A and B. IgA from re-solubilized paste and from column strip obtained as described in 1.1. and IgM from column strip as described in 1.2. were either used as such or both after association with recSC to form secretory-like IgA (Fig. 4A) or secretory-like IgM (Fig. 4B). For IgA, after 4 hours of digestion with intestinal enzymes the signal of non-associated IgA started to decrease, indicative of proteolytic digestion; the effect was stronger after 6 hours and after overnight digestion, no intact IgA alpha-chain was detected by Western blot. In contrast, secretory-like IgA was much less sensitive to digestion by intestinal proteases and even after overnight exposure a significant portion of IgA alpha-chain within secretory-like IgA remained intact.

For IgM (Fig. 4B) a comparison of IgM and freshly associated secretory-like IgM (SIgM) with the same preparations after 24h and 48h of digestion is shown. Appearance of degraded mu-chain fragments occurred more rapidly and more extensively for IgM compared to SIgM, confirming for IgM – similar as for IgA – that association with recSC provided improved structural stability.

Overall, this demonstrates that the specific association with recSC provided improved structural stability, and will make the digestion-prone plasma IgA molecules fit for mucosal application, e.g. via the oral route.

Example 4: *Shigella flexneri*

The human colonic adenocarcinoma epithelial Caco-2 cell line (American Type Tissue Collection) was seeded on polyester Snapwell filters (diameter, 12 mm; pore size, 0.4 µm; Corning Costar) as described (Crottet, S., Corthésy-Theulaz, I., Spertini, F., and Corthésy, B. (2002) J. Biol. Chem. 277:33978-33986). The integrity of the polarized Caco-2 cell monolayer was checked by measuring the transepithelial electrical resistance (TER) using a Millicell-ERS device (Millipore). TER values of well-differentiated monolayers ranged between 450–550 $\Omega \times cm^2$.

2×10^7 bacteria (Phalipon A. et al (1995) J. Exp. Med. 182:769-778) were mixed with 100 µg of IgA,125 µg of secretory-like IgA, 275 µg IgM or 300 µg SIgM in a final volume of 500 µl of plain DMEM (P-DMEM: DMEM complemented with 10 mM HEPES, 20 µg/ml transferrin, 2 mM glutamine, 1% non-essential amino acids, 1 mM sodium pyruvate) and incubated for 1 h at RT under gentle agitation. The mixtures were resuspended in P-DMEM to infect polarized Caco-2 cell monolayers.

1 h before the use of polarized Caco-2 cell monolayers, C-DMEM was replaced by P-DMEM in both the apical and basolateral compartments. Apical medium was then replaced by 500 µl of bacterial suspensions (2×10^7 bacteria) as such or in combination with the antibody. TER values were measured at selected time-points from the beginning of the infection onward.

To quantify bacteria that had adhered and infected the cells, Caco-2 cells in filters were washed three times with PBS, cells were incubated in 500 µl of cold lysis buffer [10 mM Tris-HCl (pH 7), 0.2 % Nonidet P-40, 50 mM NaCl, 2 mM EDTA (pH 8)] for 5 min on ice and lysed by up-and-down pipetting. Serial dilutions (10^{-2}-10^{-6}) of cell lysates were applied onto LB agar plates and after 24 h of incubation at 37 °C, colony-forming units (CFU) were determined by eye counting of duplicate plates.

To examine the integrity of Caco-2 cell monolayers, Snapwells were washed with PBS, prior to fixation overnight with 5 ml of 4% paraformaldehyde at 4°C. After washing with PBS, filters were permeabilized and non-specific binding sites were blocked using PBS containing 5% FCS and 0.2% Triton X-100 (PBS-Tr) for 30 min at RT. All antibodies were diluted in PBS-Tr. Filters were incubated with rabbit anti-human ZO-1 (1/200, Invitrogen) for 2 h at RT, washed in PBS, followed by goat anti-rabbit IgG conjugated with Alexa Fluor® 647 (1/100, Invitrogen) for 90 min at RT. To visualize cells, filters were finally incubated with 100 ng/ml of 4',6-diamidino-2-phenylindole (DAPI) in PBS (Invitrogen) for 30 min. Filters were cut out of their holders, and mounted in Vectashield solution for observation using a Zeiss LSM 710 Meta confocal microscope (Carl Zeiss, Germany) equipped with either a 10x or a 40x objective. Images were processed using the Zeiss ZEN 2009 light software.

To examine *Shigella*-IgA complexes, bacteria constitutively expressing green fluorescent protein were used and the formation of immune complexes was verified after incubation with biotinylated mouse anti-human IgA1/IgA2 (1/10, BD) for 30 min at RT under gentle agitation, followed by cyanine 5-conjugated Streptavidin (1/400, GE HealthCare) for 30 min at RT under gentle agitation. Three washes with PBS were performed between each step and all antibodies were diluted in PBS/5% FCS. Labeled immune complexes were laid onto glass slides (Thermo Scientific), mounted and immediately visualized using a Zeiss LSM 710 Meta confocal microscope (Carl Zeiss, Germany) equipped with a 63x objective. Images were processed with the Zeiss ZEN 2009 light software.

Human CXCL8 (IL-8), TNF-α, and CCL3 (MIP-3α) in the basolateral compartment of polarized Caco-2 cell monolayers were quantitated by ELISA with commercial kits (BD Biosciences and R&D Systems, respectively).

Results

Exposure of the polarized intestinal epithelial cell monolayer to *Shigella* led to disruption of the integrity of the monolayer, evidenced by a decrease of TER (Figure 5A), massive invasion, evidenced by elevated bacterial counts found in association with the epithelial cells (Figure 5B), and by a visibly compromised cell monolayer as observed by laser scanning confocal microscopy. Addition of secretory-like IgA delayed and partially inhibited the destruction of the monolayer, indicated by a significant inhibition of the reduction of TER (Figure 5A), by a reduction in the number of epithelial cell-bound bacteria (Figure 5B), and by a more preserved cell monolayer integrity in analysis by confocal microscopy.

Association of *Shigella*-specific monoclonal SIgA SIgAC5, polymeric IgA and secretory-like IgA resulted in the formation of immune aggregates (Figure 6) of multiple bacteria, in contrast to monomeric IgA and IgG that coated the bacterium only (Figure 6). It is likely that bacterial aggregation by the specific mAb and plasma-derived pIgA and secretory-like IgA contributed to the reduction of bacterial adhesion to Caco-2 cells observed in Figure 5B. The same three antibody preparations markedly reduced the production of pro-inflammatory cytokines/chemokines by Caco-2 cells, while monomeric IgAF4 and IgG had no (TNF- α and CCL3) or weak (CXCL8) effects (Figure 7). This indicates that neutralization of *Shigella* by secretory and polymeric IgA decreases the responsiveness of Caco-2 cells, ultimately contributing to the overall anti-inflammatory properties of IgA.

Protection of the polarized Caco-2 cell monolayer from infection with *Shigella* was similarly achieved with IgM and secretory-like IgM, to a level at least similar to that recovered when using specific SIgAC5 (Figure 8). Maintenance of TER for at least 12h30 indicated that the IgM isotype possesses neutralizing properties protecting the Caco-2 monolayer from damages induced by exposure to *Shigella*.

Example 5: Prevention of recurrence of *Clostridium difficile* infection (CDI)

The composition of the invention is used in a mouse model of *Clostridium difficile* infection.

C57BL/6 mice are treated with a mixture of oral antibiotics (kanamycin, gentamicin, colistin, metronidazole, and vancomycin) for 3 days as previously described (Chen X, et al. Gastroenterology 2008 Dec;135(6):1984-92). Two days later, they are given parenteral clindamycin phosphate (10 mg/kg s.c.) [Day -1]. One day later [Day 0] they are challenged by gavage with 0.5 x 10^5 cfu of toxinogenic *C. difficile* strain 10465. A moderate to fulminant colitis develops 1 to 5 days after the administration of *C. difficile*. Untreated, this progresses rapidly into severe and fatal colitis in the majority of animals. To treat primary infection animals receive vancomycin, for 5 days after *C. difficile* challenge, and the animals are monitored for mortality, as well as the presence or absence of severe CDI with diarrhea. Animals judged to be in a moribund state are euthanized by a single injection of sodium pentobarbital. To study recurrence of CDI animals surviving primary *C. difficile* challenge are maintained under observation until day 28. Animals are weighed 3 times weekly from day 7 to 28. After cessation of vancomycin treatment animals receive IgA or secretory-like IgA (400 mg/kg body weight via the oral route) for 5 days starting the day after the last dose of vancomycin.

Result

Animals treated with vancomycin survive the primary infection with *C. difficile*. However, a significant proportion of animals - up to 70% - succumb to recurrence of *C. difficile* infection within 3-4 days after termination of vancomycin treatment. In contrast, recurrence of infection is prevented if animals are treated with secretory-like IgA via the oral route. Plasma IgA alone is not effective (or at least not as effective) as secretory-like IgA in preventing recurrence of *C. difficile* infection.

Alternatively, the composition is used in a model of oral mucositis similar as described in Watkins et al (Oral Dis 2010, 16:655-660).

Appropriately formulated IgA preparations (or vehicle solution for control) are given prophylactically (e.g. starting at day -3) three times daily to Syrian Golden Hamsters for the entire duration of the study up to day 28. In a model of acute radiation-induced mucositis, on day 0 one everted buccal cheek pouch is irradiated (40 Gy), the other cheek pouch is left untreated for control. Alternatively, in a model of fractionated radiation-induced mucositis, a cumulative dose of 60 Gy is applied, partitioned into eight fractions of 7.5 Gy as described in (Watkins, Oral Dis 2010, 16:655-660). In yet another model of combined cisplatin and acute radiation-induced mucositis, disease is induced by a combination of cisplatin (5 mg/kg) and 35 Gy radiation on day 0. Clinical evaluation of oral mucositis and monitoring of body weight is done daily, starting on Day 6 until the end of the study, typically on Day 28. The scoring system is described in (Watkins Oral Dis, 2010 16:655-660). In addition, tissue and plasma samples are collected and appropriately processed throughout the study for histological analyses, determination of inflammatory markers in plasma and for gene expression studies of various tissues.

Results

Untreated/vehicle treated animal develop oral mucositis, disease peaks around day 16-18, spontaneous healing, evidenced by a regression of the mucositis, starts around day 18-20. Animals treated with IgA and in particular with Secretory-like IgA have significantly lower mucositis scores compared to control animals and lose less weight, paralleled by less severe histological findings and reduced levels of inflammatory markers (including but not limited to inflammatory cytokines and chemokines). Reduction of inflammation and promotion of wound-healing is confirmed at the level of mRNA expression by gene-expression analysis techniques.

Claims

1. Method for producing a composition comprising secretory-like immunoglobulin in vitro, comprising the steps of
(a) Obtaining a blood-derived protein composition comprising J chain-containing immunoglobulin in a non-purified form,
(b) admixing the composition of step (a) with secretory component.

2. The method of claim 1, wherein the secretory-like immunoglobulin is secretory-like IgA and/or secretory-like IgM.

3. The method of claim 1 or claim 2, wherein the composition of step (a) contains at least 5% J chain-linked IgA.

4. The method of claim 3, wherein the composition of step (a) contains at least 10% J chain-linked IgA.

5. The method of claim 3, wherein the composition of step (a) contains at least 20% J chain-linked IgA.

6. The method of claim 3, wherein the composition of step (a) contains at least 30% J chain-linked IgA.

7. The method of claim 3, wherein the composition of step (a) contains at least 50% J chain-linked IgA.

8. The method of any previous claim, wherein the composition of step (a) is derived from human blood.

9. The method of any previous claim, wherein the secretory component in step (b) is recombinant secretory component.

10. The method of any previous claim, wherein the secretory component is human secretory component.

11. The method of any previous claim, wherein the secretory component is produced in a mammalian cell line.

12. The method of any previous claim, wherein the secretory component is the extracellular portion of the polymeric immunoglobulin receptor pIgR.

13. The method of any previous claim, wherein in step (b) the molar ratio between added secretory component and J chain within IgA dimers/polymers ranges between 1:10 and 10:1.

14. The method of claim 13, wherein the ratio ranges between about 1:5 and 5:1.

15. The method of claim 14, wherein the ratio ranges between about 1:2 and 2:1.

16. A composition comprising secretory-like IgA and/or secretory-like IgM or a combination thereof obtainable by a method of any of claims 1 to 15.

17. The composition of claim 16, further comprising a pharmaceutically acceptable carrier or excipient.

18. The composition of claim 16 or claim 17 for medical use.