Easy pan-detection of human IgA immunoglobulins

Cyril Planchais, Hugo Mouquet

Easy Pan-Detection of Human IgA Immunoglobulins

Cyril Planchais[1, 2], Hugo Mouquet[1, 2*]

[1]Laboratory of Humoral Immunology, Department of Immunology, Institut Pasteur, and [2]INSERM U1222, Paris, 75015, France

[*]Correspondence: hugo.mouquet@pasteur.fr (H.M.)

Keywords: Antibodies | B cells | IgA | Streptococcal IgA-binding peptide | Immunoassays

Words Count: 2, 519

Abstract

IgA antibodies are key immune effectors against invading pathogens but also possess essential immunoregulatory functions. Detecting and quantifying human IgA^+ B-cell subsets and secreted IgA molecules is needed for investigating the protective, modulatory and pathophysiologic roles of IgAs. Here, we produced a recombinant tagged trimeric form of the streptococcal IgA-binding peptide (SAP) by transient transfection-based eukaryotic expression system. The trimeric SAP (tSAP) probe had a higher production yield and apparent binding affinity to human IgA1 and IgA2 immunoglobulins when compared to the dimeric SAP molecule classically used to purify IgAs. tSAP bound both monomeric and dimeric IgAs, and allowed immunoblot detection and ELISA quantification of serum IgA antibodies in humans and non-human primates. Fluorescently labeled tSAP also permitted an accurate quantification of circulating human blood IgA-expressing memory B cells by flow-cytometric analyses. Thus, the easy-to-produce high affinity recombinant tSAP probe we developed is a versatile and valuable tool to quantify secreted and membrane-bound human but also primate IgA immunoglobulins.

1. Introduction

Immunoglobulin A (IgA) is found in animal vertebrates of the Reptilia (birds and some reptiles *i.e.*, crocodiles) and Mammalia classes (Magadan-Mompo et al., 2013; Kaetzel and Russell, 2015). In humans, IgA divides into two subclasses, IgA1 and IgA2, which distinguish in terms of sequence/structure, glycosylation state, body fluid distribution, synthesis rate/half-life, biophysical binding properties (*e.g.* bivalent interactions), and biological activities (Woof and Mestecky, 2015). Human B-cell populations expressing membrane-bound surface IgAs predominate in mucosal tissues, and IgA^+ memory B-cell subsets are also abundant in blood (Berkowska et al., 2015; Prigent et al., 2016). Secreted IgAs prevail as dimeric immunoglobulin molecules in mucosa-associated secretions but circulate mainly as antibody monomers in blood (Kaetzel, 2007). IgA antibodies play a key protective function as immune effectors against invading pathogens but also as immunosuppressors of pro-inflammatory responses, and immunomodulators of the gut microbiota (Mkaddem et al., 2014; Russell et al., 2015; Pabst and Slack, 2020). Conversely, IgA-antigen complexes can promote inflammatory processes, and IgA antibodies are pathogenic mediators in certain auto-inflammatory and autoimmune disorders (Hansen et al., 2019). Because of their paramount role in body homeostasis, protective and pathogenic humoral responses, as well as their growing interest as therapeutics (Leusen, 2015), new tools are needed to study IgA biology and functions. To this end, we recently developed a methodological strategy to efficiently clone, produce and purify recombinant monoclonal IgA antibodies from single human B cells (Lorin and Mouquet, 2015). By generating and charactering hundreds of IgA monoclonals, this approach greatly facilitated investigating IgA humoral responses and functions (Prigent et al., 2016; Lorin et al., 2017; Planchais et al., 2019).

The M22 surface protein of *Streptococcus pyogenes* and its peptide derivatives bind the Fc region of human IgA molecules, and were used to create specific IgA-binding reagents for antibody detection and purification (Johnsson et al., 1999; Sandin et al., 2002). A synthetic M22-derived 50 amino acid dimeric peptide referred as the streptococcal IgA-binding peptide (SAP) has a high-affinity for human IgAs (Sandin et al., 2002), and is

therefore currently used to fabricate commercially available affinity chromatography resin products. Taking advantage of the SAP property, here we developed of a recombinant tagged trimeric SAP protein as an easy-to-make versatile probe for detecting human IgAs in various binding assays.

2. Methods

2.1. Cloning and production of recombinant streptococcal IgA-binding proteins (SAPs)

Codon-optimized nucleotide fragments encoding the IgK immunoglobulin signal peptide, the SAP followed either by a single cysteine residue (dSAP) or the trimerization motif of T4 bacteriophage fibritin (tSAP), and 6xHis- and Avi-tags in C-terminal were synthetized (Invitrogen GeneArt Gene Synthesis, Thermo Fisher Scientific) and digested using Anza 5 and 11 restriction enzymes (Thermo Fisher Scientific). Digested DNA fragments were gel purified using NucleoSpin® Extract II kit (Macherey-Nagel) and cloned into pcDNA™3.1/Zeo$^{(+)}$ expression vector (Thermo Fisher Scientific). Transformed-DH10β bacteria (New England Biolabs) were screened by PCR and plasmid DNA purifications were performed from positive clones grown in 100 µg/ml ampicillin-containing Luria-Bertani broth cultures using NucleoBond® Xtra Maxi kit (Macherey-Nalgel). Freestyle™293-F suspension cells (Life Technologies) were cultured in EX-CELL® serum free medium (Sigma-Aldrich) supplemented with phenol red sodium salt (5 µg/ml, Sigma), L-glutamine (2×) and penicillin–streptomycin (0.2×; 10,000 U/ml, Life Technologies). 293-F cells were grown in suspension at 37 °C in a humidified 5% CO2 incubator on a Celltron shaker platform (Infors HT) rotating at 130 rpm (for 250 ml media in 1 l culture flasks) or 180 rpm (for 20 ml media in 50 ml bioreactors). Twenty-four hours before transfection, cell density was adjusted at 1.5 × 10^6 cells/ml, and culture grown overnight in the same conditions as mentioned above to reach ~2.5 × 10^6 cells/ml the day of transfection. Cells were harvested by centrifugation at 1250 rpm for 5 min, and resuspended in fresh FreeStyle™ 293 Expression Medium (Life technologies) without antibiotics at a density of 2.5 × 10^6 cells/ml. Plasmid DNA coding for SAPs were then added to 293-F cells at a final concentration of 2 µg/ml of culture medium.

The cultures were swirled 5 min on shaker in the culture incubator. Linear polyethylenimine (PEI) (25 kDa, Polysciences) solution (at 1 mg/ml in distilled water and 0.2 µm-filtered) was diluted 1:1 with FreeStyle™ medium and added drop by drop to the 293-F cell cultures (final concentration in medium of 9 µg/ml). The cultures were grown under agitation in the 37 °C incubator with 5% CO2. Twenty-four hours' post-transfection, cells were diluted 1:1 with EX-CELL® serum free medium, without antibiotics and supplemented with L-glutamine and phenol red sodium salt to obtain a final transfection volume of 20 ml or 250 ml. Transfected cells were cultivated for 5 days after transfection. Supernatants were then harvested, centrifuged at 4200 rpm for 30 min and 0.2 µm-filtered to remove cellular debris.

2.2. Purification and coupling of recombinant SAPs

Recombinant tagged-dSAP and -tSAP were purified by affinity chromatography by high-performance chromatography using the Ni Sepharose® Excel Resin according to manufacturer's instructions (Thermo Fisher Scientific). Beads were washed once with 50 ml of PBS, and incubated on a rolling wheel overnight at 4 °C with SAP probes-containing supernatants (500 µl for 250 ml of culture supernatant). Beads were harvested by centrifugation at 2000 rpm for 10 min without break, placed into a Poly-Prep® chromatography column (Bio-Rad) pre-equilibrated with equilibrium/wash buffer (50 mM Na2HPO4, 300 mM NaCl, 10mM Imidazole; pH 7.4), and washed 3 times with equilibrium/wash buffer. SAPs were eluted from the resin with 5 x 500 µl of elution solution (50 mM Na2HPO4, 300 mM NaCl, 150mM Imidazole - pH 7.4). For each fraction, protein concentration was measured using the NanoDrop 2000 instrument (Fisher Scientific). SAP-containing fractions were pooled, and dialyzed overnight against PBS using Slide-A-Lyzer® dialysis cassettes (10 kDa MW cut-off, Thermo Fisher Scientific). Protein purity was evaluated by in-gel protein silver-staining using Pierce® Silver Stain kit (Thermo Fisher Scientific) following protein separation of samples heated 95°C for 5 min in reducing and non-reducing conditions by SDS-PAGE using NuPAGE™ 12% Bis-Tris gels (Life Technologies, Thermo Fisher Scientific). Purified SAPs were biotinylated using BirA biotin-

protein ligase bulk reaction kit (Avidity, LLC) or directly coupled to Dylight™ 650 Dye using Dylight™ 650 NHS ester labeling kit (Thermo Fisher Scientific) according to manufacturer's instructions.

2.3. SAP-based IgA ELISA assays

For recombinant SAP-binding ELISA analyses, high-binding 96-well ELISA plates (Costar) were coated overnight with 250 ng/well of purified recombinant 10-1074 IgA1, IgA2 and dimeric IgA1 monoclonal antibodies (Lorin and Mouquet, 2015). For serum IgA detection, sera from healthy humans (Etablissement Français du Sang - EFS), mouse, rat, guinea-pig, rabbit, chicken, goat, cow (Sigma), and non-human primates (a kind gift from Drs Nicolas Huot and Michaela Muller-Trutwin, Institut Pasteur) were buffer-exchanged against PBS using Amicon® Ultra Centrifugal Filters (100 kDa MW cut-off, Merck Millipore) to partially deplete albumin, the most abundant serum protein in mammals (Moore, 1945), whose presence could interfere with protein separation by SDS-PAGE as well as with protein immobilization on ELISA plates, and as a result with the subsequent IgA detection. High-binding 96-well ELISA plates (Costar) were coated overnight with 2.5 µg/well of serum proteins. After washing with 0.05% Tween-PBS (PBST), plates were blocked for 2 h with 2% BSA, 1 µM EDTA, 0.05% Tween-PBS (blocking buffer), and then incubated for 2 h with SAP-containing supernatant, purified SAPs, and biotinylated dSAP and tSAP (560 nM), and seven consecutive 1:4 dilutions in PBS. After PBST washings, plates were revealed by addition of horseradish peroxidase (HRP)-conjugated mouse anti-6xHisTag antibodies or HRP-conjugated streptavidin (1 µg/ml in blocking solution, BD Biosciences), and HRP chromogenic substrate (ABTS solution, Euromedex). All experiments were performed in triplicate at room temperature, and using HydroSpeed™ microplate washer and Sunrise™ microplate absorbance reader (Tecan Männedorf, Switzerland).

2.4. Infrared fluorescent immunoblotting

Purified SAP proteins (125 to 500 ng /well) heated at 95°C for 5 min in reducing and non-reducing were separated by SDS-PAGE using NuPAGE™ 12% Bis-Tris gels (Life Technologies, Thermo Fisher Scientific), and then electrotransferred onto nitrocellulose membranes. Membranes were then saturated overnight at 4°C in PBS-0.05% Tween 20 (PBST)-5% dry milk. After washing 3 times with PBST, membranes were incubated with mouse anti-6xHis antibody (1µg/ml; BD biosciences) in PBST-5% dry milk for 2 h. Membranes were then washed with PBST and incubated for 1 h with 1:10,000-diluted IRDye 800CW-conjugated goat anti-mouse IgG (LI-COR Biosciences) in PBST-5% dry milk.

For SAP-based IgA detection, total serum proteins (20 µg/well) heat-treated for 5 min at 95°C were separated by SDS-PAGE using NuPAGE™ 4-12% Bis-Tris gels (Invitrogen, Thermo Fisher Scientific) in non-reducing conditions, and electrotransferred onto nitrocellulose membranes. Saturated membranes were PBST-washed 3 times, and incubated for 2 h with biotinylated-tSAP (1 µg/ml) in PBST-5% BSA. After washings, membranes were incubated for 1 h with 1:10,000-diluted Alexa fluor™ 790-conjugated streptavidin (Thermo Fisher Scientific) in PBST-5% BSA. Finally, membranes were PBST-washed and scanned with the Odyssey Infrared Imaging system (LI-COR Biosciences).

2.5. IgA flow cytometric staining by SAP probes

Peripheral blood mononuclear cells from healthy donors (Etablissement Français du Sang - EFS) were purified by Ficoll density gradient centrifugation according to the manufacturer's instructions (GE Healthcare), and then washed twice with 1% BSA-PBS (FACS buffer). Two millions cells in 200 µl of FACS buffer *per* tube were incubated for 30 min at 4°C with LIVE/DEAD fixable dead cell stain kit (Molecular Probes, Thermo Fisher Scientific) to exclude dead cells. After washing with FACS buffer, cells in 200 µl of FACS buffer were incubated for 30 min at 4°C with Dylight™ 650-conjugated SAP probes (ranging from 14 to 520 nM), and with a cocktail of mouse anti-human antibodies: CD19-A700 (HIB19, BD Biosciences, San Jose, CA), IgG BV786 (G18-145, BD Biosciences) and IgA FITC (IS11-8E10, Miltenyi Biotec, Bergisch Gladbach, Germany). Finally, cells were washed with FACS

buffer and resuspended in 400 µl 1% paraformaldehyde (PFA)-PBS solution. Following a lymphocyte and single cell gating, live cells were gated on $CD19^+$ B cells. FACS analyses were performed using a FACS Aria II cytometer (BD Biosciences) and FlowJo software (v10.3, FlowJo LLC, Ashland, OR).

3. Results

3.1. Design and production of recombinant streptococcal IgA-binding proteins

Dimeric SAP display higher binding affinity to human IgAs than its monomeric counterpart (Sandin et al., 2002). Thus, we designed two multimeric SAP gene constructs for mammalian cell-based expression of recombinant proteins, each containing a N-terminal leader peptide for secretion, and C-terminal 6xHis- and Avi-tags for purification and biotynilation, respectively (**Fig. 1A**). Dimeric SAP (dSAP) corresponding to the original di-sulfide bonded peptide M dimer (Sandin et al., 2002), and a trimeric form of the SAP (tSAP) for which we replaced the C-terminal cysteine residue in dSAP by the trimerization motif of T4 bacteriophage fibritin (**Fig. 1A**) were produced. To this end, FreeStyle™ 293-F cells were transiently transfected with purified dSAP and tSAP vector DNA, and cultivated for five days. The concentration of produced recombinant SAPs in harvested supernatants measured by sandwich ELISA showed a ~ 20-fold higher production yield for tSAP compared to dSAP (44.4 µg/ml *vs* 2.3 µg/ml in average, respectively) (**Fig. 1B**). Accordingly, purification of histidine-tagged SAPs by high-performance immobilized metal ion affinity chromatography (IMAC) directly from supernatants allowed recovering higher amounts of protein products for tSAP compared to dSAP (*per* 20 ml culture, 686 µg *vs* 117 µg in average, respectively). The purity of SAPs in phosphate buffered saline (PBS) solution was confirmed by visualizing specific protein bands on silver-stained SDS-PAGE gels, and by infrared immunoblotting using anti-HisTag antibody detection (**Fig. 1C**). As expected, purified dSAPs were well detected as dimers both on the stained gel and immunoblot under non-reducing conditions (**Fig. 1C**). To compare the reactivity of dSAP and tSAP against human IgA molecules, we performed ELISA experiments using as antigens purified IgA monoclonal antibodies (Lorin

and Mouquet, 2015). ELISA binding analyses showed although both SAPs recognize human monomeric IgA1 and IgA2 as well as dimeric IgA1 (dIgA1) antibodies, tSAP displayed a higher relative affinity to human IgAs (30-, 13- and 9-fold greater than dSAP for IgA1, IgA2 and dIgA1, respectively) (**Fig. 1D**).

3.2. Sensitive detection of human and non-human primate IgAs by SAP trimers

The M22 protein of *S. pyogenes* has been shown to interact with two loops in the Cα2-Cα3 interdomain of the human IgA Fc region (Pleass et al., 2001). Interestingly, the ^{257}LLG^{259} loop motif in Cα2 is well conserved across several mammal species known to possess circulating IgAs (Kaetzel and Russell, 2015), whereas the ^{440}PLAF^{443} loop motif in Cα3 is only found in primate and bovine IgAs (**Fig. 2A**). We thus evaluated the capacity of purified recombinant SAPs to bind serum IgAs from these various mammals using AviTag-biotinylated dSAP and tSAP as ELISA probes. Binding analyses showed that SAPs react with immobilized IgA-containing serum proteins from human and macaque but not from goat, cow, chicken, rabbit, guinea pig, rat and mouse (**Fig. 2A**). In agreement, SAP probes were also able to bind serum IgAs by ELISA from other non-human primate species: African green monkey, Mandrill, Cercopithecus cephus, Golden-bellied and Black crested mangabeys, Allen's swamp and Hamlyn's monkeys (**Fig. 2B**). Consistent with the data obtained for human IgAs, tSAP exhibited a higher relative binding affinity than dSAP against non-human primate IgAs (**Figs. 2A** and **2B**). To confirm the recognition of IgAs in total serum proteins by SAPs, we next performed an infrared immunoblotting using biotinylated tSAP as a detection probe. As expected, tSAPs revealed immunoreactive protein bands with molecular weights corresponding to IgAs in sera from the human subject and non-human primates but not from the other tested mammals (**Fig. 2C**).

3.3. Efficient flow cytometric capture of IgA-expressing B cells by SAP probes

Human B-cell subsets expressing IgA B-cell receptors are abundant in the blood and mucosa-associated lymphoid tissues. To investigate whether SAP probes also recognize

membrane-bound IgAs on B cells, human peripheral blood mononuclear cells from healthy individuals (n=3) were stained with a concentration range of Dylight^{TM}650-conjugated (DyL^{650})-SAP probes, as well as FITC- and Alexa 786-conjugated anti-human IgA and IgG antibodies, respectively (**Fig. 3**). Flow cytometric analysis showed that fluorescent SAP probes bind to blood IgA^{+} B cells in a dose dependent manner (**Fig. 3B** and **3C**). All SAP^{+}CD19^{+} cells were IgA^{+} and IgG^{-} indicating that SAP probes bind specifically IgA-expressing B cells, without competition with fluorescently labeled anti-human IgA antibodies ordinarily used in flow cytometry (**Fig. 3B**). Optimal binding of human IgA^{+} B cells was achieved by staining with DyL^{650}-tSAP at a final concentration of 520 nM (mean 97.5%, CV 11%; **Fig. 3C**).

4. Concluding remarks

tSAP is a novel, easy to produce, high-affinity IgA-binding reagent, which possesses superior activity compared to the originally-described SAP molecule. Recombinant SAP trimers can be utilized for a sensitive detection and quantification of human but also non-human primate IgAs in various immunoassays. tSAP is a small non-glycosylated protein secreted in the culture cell supernatant, which may thus also be easily produced using prokaryote-based expression systems. Moreover, considering its very high production yield and binding affinity, coupling tSAPs to polysaccharide beads would permit a low-cost and efficient purification of IgAs by affinity chromatography. Finally, due to its binding capacity to non-human primate IgA antibodies, the tSAP probe constitutes a very useful tool to investigate IgA-mediated immunity in infected or vaccinated monkeys (Amos et al., 2015; Nelson et al., 2016; Ackerman et al., 2018; Eudailey et al., 2018).

Acknowledgements

We are grateful to Drs Nicolas Huot and Michaela Muller-Trutwin (HIV, inflammation and persistence Unit, Institut Pasteur) for providing the sera from non-human primates. We also thank Valérie Lorin (Humoral Immunology lab, Institut Pasteur) for providing purified 10-1074 IgA monoclonal antibodies. H.M. received core grants from the Institut Pasteur, the INSERM and the *Milieu Intérieur* Program (ANR-10-LABX-69-01). This work was funded by the European Research Council (ERC) – Seventh Frame-work Program (ERC-2013-StG 337146). C.P. was supported by the ERC-2013-StG 337146 program and by a one-year fellowship from the Agence Nationale de Recherches sur le Sida et les Hépatites Virales (ANRS).

Author Contributions

C.P. performed and analyzed experiments, and wrote the manuscript. H.M. conceived and supervised the study, designed experiments, and wrote the manuscript.

Declaration of Interests

The authors declare no competing interests.

References

Ackerman, M.E., Das, J., Pittala, S., Broge, T., Linde, C., Suscovich, T.J., Brown, E.P., Bradley, T., Natarajan, H., Lin, S., Sassic, J.K., O'Keefe, S., Mehta, N., Goodman, D., Sips, M., Weiner, J.A., Tomaras, G.D., Haynes, B.F., Lauffenburger, D.A., Bailey-Kellogg, C., Roederer, M. and Alter, G., 2018, Route of immunization defines multiple mechanisms of vaccine-mediated protection against SIV. Nature medicine 24, 1590-1598.

Amos, J.D., Himes, J.E., Armand, L., Gurley, T.C., Martinez, D.R., Colvin, L., Beck, K., Overman, R.G., Liao, H.X., Moody, M.A. and Permar, S.R., 2015, Rapid Development of gp120-Focused Neutralizing B Cell Responses during Acute Simian Immunodeficiency Virus Infection of African Green Monkeys. Journal of virology 89, 9485-98.

Berkowska, M.A., Schickel, J.N., Grosserichter-Wagener, C., de Ridder, D., Ng, Y.S., van Dongen, J.J., Meffre, E. and van Zelm, M.C., 2015, Circulating Human CD27-IgA+ Memory B Cells Recognize Bacteria with Polyreactive Igs. Journal of immunology 195, 1417-26.

Eudailey, J.A., Dennis, M.L., Parker, M.E., Phillips, B.L., Huffman, T.N., Bay, C.P., Hudgens, M.G., Wiseman, R.W., Pollara, J.J., Fouda, G.G., Ferrari, G., Pickup, D.J., Kozlowski, P.A., Van Rompay, K.K.A., De Paris, K. and Permar, S.R., 2018, Maternal HIV-1 Env Vaccination for Systemic and Breast Milk Immunity To Prevent Oral SHIV Acquisition in Infant Macaques. mSphere 3.

Hansen, I.S., Baeten, D.L.P. and den Dunnen, J., 2019, The inflammatory function of human IgA. Cellular and molecular life sciences : CMLS 76, 1041-1055.

Herr, A.B., Ballister, E.R. and Bjorkman, P.J., 2003, Insights into IgA-mediated immune responses from the crystal structures of human FcalphaRI and its complex with IgA1-Fc. Nature 423, 614-20.

Johnsson, E., Areschoug, T., Mestecky, J. and Lindahl, G., 1999, An IgA-binding peptide derived from a streptococcal surface protein. The Journal of biological chemistry 274, 14521-4.

Kaetzel, C.S. 2007. Mucosal immune defense : immunoglobulin A. Springer, New York.

Kaetzel, C.S. and Russell, M.W. 2015 Chapter 18 - Phylogeny and Comparative Physiology of Mucosal Immunoglobulins. In: J. Mestecky, W. Strober, M.W. Russell, B.L. Kelsall, H. Cheroutre and B.N. Lambrecht (Eds.) Mucosal Immunology (Fourth Edition). Academic Press, Boston, p. 325-347.

Leusen, J.H., 2015, IgA as therapeutic antibody. Molecular immunology 68, 35-9.

Lorin, V., Malbec, M., Eden, C., Bruel, T., Porrot, F., Seaman, M.S., Schwartz, O. and Mouquet, H., 2017, Broadly neutralizing antibodies suppress post-transcytosis HIV-1 infectivity. Mucosal immunology 10, 814-826.

Lorin, V. and Mouquet, H., 2015, Efficient generation of human IgA monoclonal antibodies. J Immunol Methods 422, 102-10.

Magadan-Mompo, S., Sanchez-Espinel, C. and Gambon-Deza, F., 2013, IgH loci of American alligator and saltwater crocodile shed light on IgA evolution. Immunogenetics 65, 531-41.

Mkaddem, S.B., Christou, I., Rossato, E., Berthelot, L., Lehuen, A. and Monteiro, R.C., 2014, IgA, IgA receptors, and their anti-inflammatory properties. Current topics in microbiology and immunology 382, 221-35.

Moore, D.H., 1945, Species difference in serum protein patterns. The Journal of biological chemistry 161, 21-32.

Nelson, C.S., Pollara, J., Kunz, E.L., Jeffries, T.L., Jr., Duffy, R., Beck, C., Stamper, L., Wang, M., Shen, X., Pickup, D.J., Staats, H.F., Hudgens, M.G., Kepler, T.B., Montefiori, D.C., Moody, M.A., Tomaras, G.D., Liao, H.X., Haynes, B.F., Ferrari, G., Fouda, G.G.A. and Permar, S.R., 2016, Combined HIV-1 Envelope Systemic and Mucosal Immunization of Lactating Rhesus Monkeys Induces a Robust

Immunoglobulin A Isotype B Cell Response in Breast Milk. Journal of virology 90, 4951-4965.
Pabst, O. and Slack, E., 2020, IgA and the intestinal microbiota: the importance of being specific. Mucosal immunology 13, 12-21.
Planchais, C., Kok, A., Kanyavuz, A., Lorin, V., Bruel, T., Guivel-Benhassine, F., Rollenske, T., Prigent, J., Hieu, T., Prazuck, T., Lefrou, L., Wardemann, H., Schwartz, O., Dimitrov, J.D., Hocqueloux, L. and Mouquet, H., 2019, HIV-1 Envelope Recognition by Polyreactive and Cross-Reactive Intestinal B Cells. Cell reports 27, 572-585 e7.
Pleass, R.J., Areschoug, T., Lindahl, G. and Woof, J.M., 2001, Streptococcal IgA-binding proteins bind in the Calpha 2-Calpha 3 interdomain region and inhibit binding of IgA to human CD89. The Journal of biological chemistry 276, 8197-204.
Prigent, J., Lorin, V., Kok, A., Hieu, T., Bourgeau, S. and Mouquet, H., 2016, Scarcity of autoreactive human blood IgA(+) memory B cells. European journal of immunology 46, 2340-2351.
Russell, M.W., Kilian, M., Mantis, N.J. and Corthésy, B. 2015 Chapter 21 - Biological Activities of IgA. In: J. Mestecky, W. Strober, M.W. Russell, B.L. Kelsall, H. Cheroutre and B.N. Lambrecht (Eds.) Mucosal Immunology (Fourth Edition). Academic Press, Boston, p. 429-454.
Sandin, C., Linse, S., Areschoug, T., Woof, J.M., Reinholdt, J. and Lindahl, G., 2002, Isolation and detection of human IgA using a streptococcal IgA-binding peptide. Journal of immunology 169, 1357-64.
Woof, J.M. and Mestecky, J. 2015 Chapter 17 - Mucosal Immunoglobulins. In: J. Mestecky, W. Strober, M.W. Russell, B.L. Kelsall, H. Cheroutre and B.N. Lambrecht (Eds.) Mucosal Immunology (Fourth Edition). Academic Press, Boston, p. 287-324.

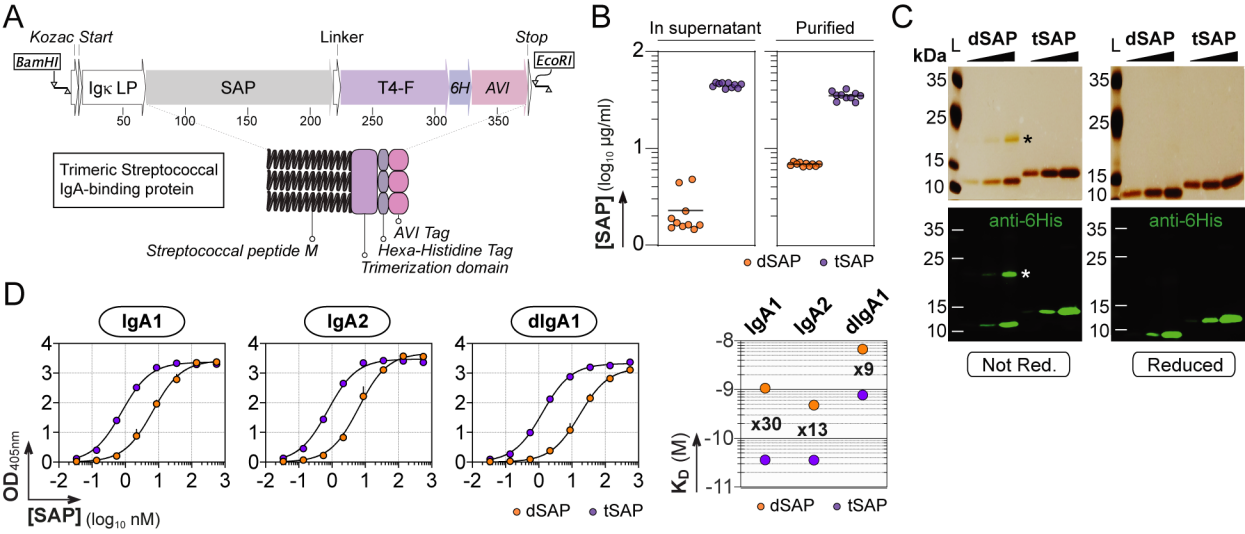
Figure legends

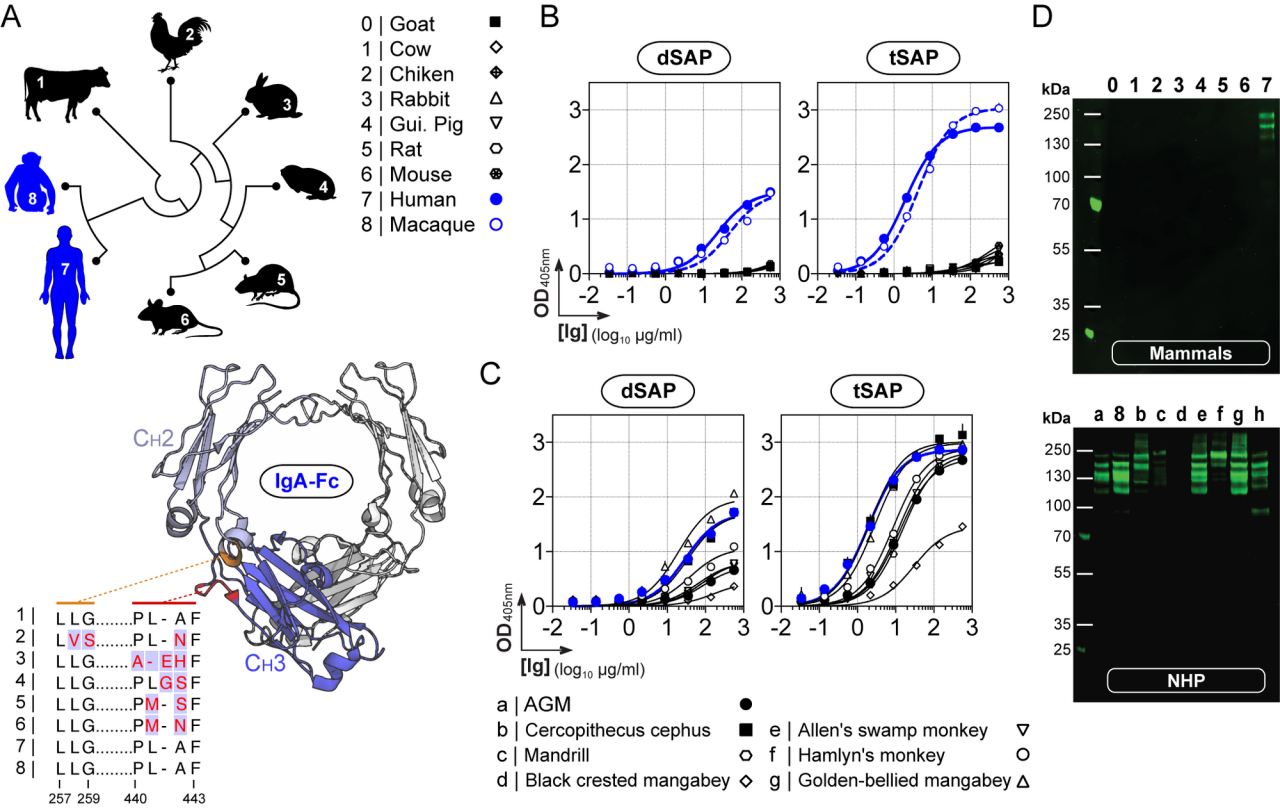
Figure 1. Production of recombinant streptococcal IgA-binding proteins. (**A**) Schematic diagram shows the expression-cloning construct for producing trimeric streptococcal IgA-binding proteins. (**B**) Dot plots comparing the production yield of the dimeric and trimeric streptococcal IgA-binding protein (dSAP and tSAP, respectively), determined by ELISA in cell culture supernatant and after affinity purification. Values of each dot correspond to the means of intra-assay duplicate measurements. (**C**) Silver-stained SDS-PAGE gels (top) and infrared immuoblots (bottom) show purified dSAPs and tSAPs (ranging from 125 to 500 ng) in non-reducing (not red.) and reducing conditions. Representative stained gel and infrared immunoblot images (from two independent experiments) are shown. Asterisks indicate SAP dimers under non-reducing conditions. (**D**) ELISA graphs comparing the dSAP and tSAP binding to purified monomeric and dimeric IgA1, and monomeric IgA2 monoclonal antibodies (left). Means ± SD from intra-assay triplicate measurements are shown. Dot plots comparing the relative binding affinity of recombinant SAPs against monomeric and dimeric IgAs (right). Fold differences of K_D values between dSAP and tSAP against IgA antibodies are indicated on the graph.

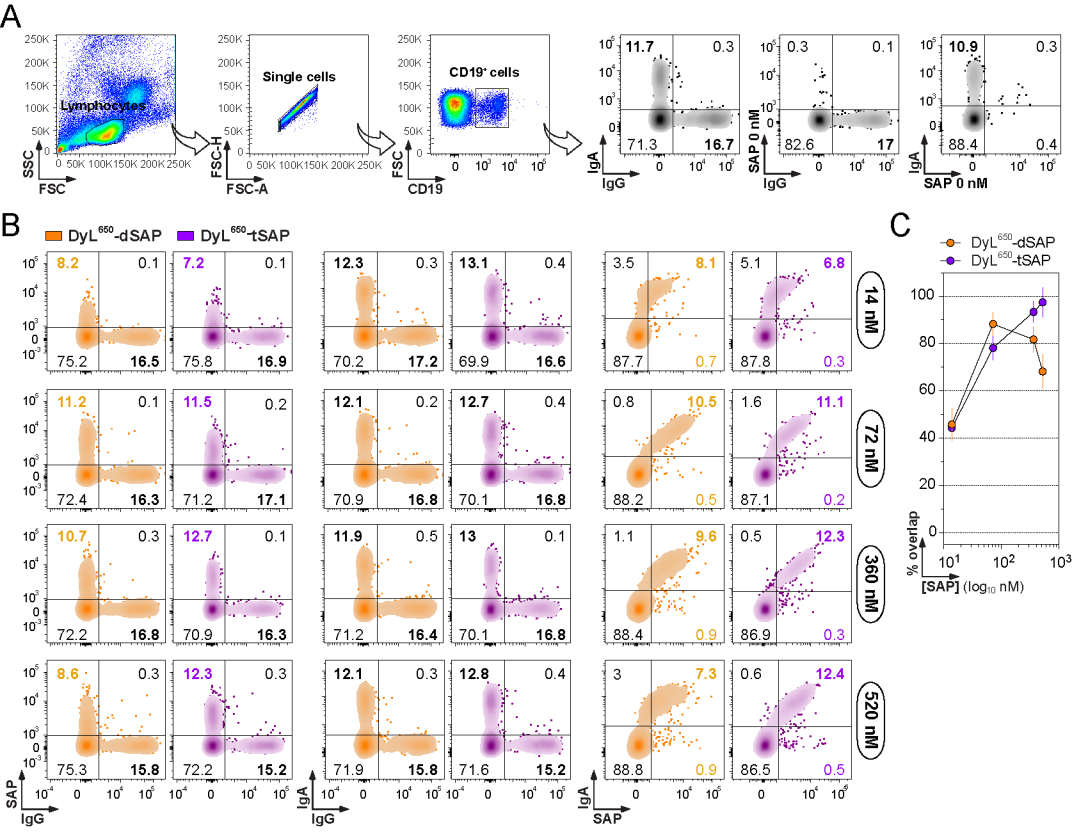
Figure 2. SAP binding capacity to mammalian serum IgA antibodies. (**A**) Circular cladogram built from the amino acid alignment of immunoglobulin α1 chains from various mammal species (top). Gui. Pig, Guinea pig. Ribbon diagrams showing the crystal structure of the human IgA1 Fc (1OW0) (Herr et al., 2003) (bottom). Amino acid stretches predicted to interact with *S. pyogenes* M22 protein are depicted in orange and red in the Cα2 and Cα3 domain, respectively. Amino acid alignments the ^{257}LLG^{259} and ^{440}PLAF^{443} loop motifs from the selected mammal species are shown. (**B**) ELISA graph comparing the reactivity of dSAP and tSAP against serum IgA antibodies from selected mammals as shown in (A). Means ± SD from intra-assay triplicate measurements in one representative experiment (from two independent experiments) are shown. (**C**) ELISA graph showing the binding of dSAP and tSAP to serum IgAs from selected non human-primate species. Human serum IgAs were

used as positive control (blue lines). Means ± SD from intra-assay triplicate measurements are shown. (**D**) Infrared immunoblotting showing the reactivity of biotinylated tSAPs against serum IgA antibodies from selected mammal (left) and primate (right) species.

Figure 3. SAP-based flow cytometric detection of human IgA-expressing B cells. (**A**) Representative cytograms showing the flow cytometric gating strategy to determine frequencies of circulating blood IgA^+ B cells from healthy individuals (n=3) by fluorescent SAP probes' staining. (**B**) Titration of fluorescently labeled dSAP and tSAP (concentrations varying from 14 to 520 nM) to quantify human IgA^+ B cells. Data from one representative healthy subject are shown. Commercially available anti-IgA antibody was used as positive control. (**C**) Graphs comparing the frequency of circulating blood IgA^+ B cells revealed using commercial anti-IgA antibodies simultaneously bound by the SAP probes at different concentrations. Mean values obtained from duplicate measurements in 3 healthy individuals are shown. Error bars indicate SEM.


A
Kozac Start
BamHI
Igκ LP
SAP
T4-F
6H
AVI
EcoRI
Linker
Stop
50
100
150
200
250
300
350
Trimeric Streptococcal IgA-binding protein
Streptococcal peptide M
AVI Tag
Hexa-Histidine Tag
Trimerization domain
B
In supernatant
Purified
[SAP] (log10 µg/ml)
dSAP
tSAP
C
kDa
L
dSAP
tSAP
35
25
15
10
anti-6His
Not Red.
Reduced
D
IgA1
IgA2
dIgA1
OD405nm
[SAP] (log10 nM)
dSAP
tSAP
KD (M)
x9
x30
x13


A
0 | Goat
1 | Cow
2 | Chiken
3 | Rabbit
4 | Gui. Pig
5 | Rat
6 | Mouse
7 | Human
8 | Macaque
B
dSAP
tSAP
OD405nm
[Ig] (log10 µg/ml)
C
dSAP
tSAP
OD405nm
[Ig] (log10 µg/ml)
CH2
IgA-Fc
CH3
1 | LLG.....PL-AF
2 | LVS.....PL-NF
3 | LLG.....A-EHF
4 | LLG.....PLGSF
5 | LLG.....PM-SF
6 | LLG.....PM-NF
7 | LLG.....PL-AF
8 | LLG.....PL-AF
257 259 440 443
a | AGM
b | Cercopithecus cephus
c | Mandrill
d | Black crested mangabey
e | Allen's swamp monkey
f | Hamlyn's monkey
g | Golden-bellied mangabey
D
kDa
0 1 2 3 4 5 6 7
250
130
100
70
55
35
25
Mammals
kDa
a 8 b c d e f g h
250
130
100
70
55
35
25
NHP


A
Lymphocytes
SSC
FSC
Single cells
FSC-H
FSC-A
CD19+ cells
FSC
CD19
11.7
0.3
71.3
16.7
IgA
IgG
0.3
0.1
82.6
17
SAP 0 nM
IgG
10.9
0.3
88.4
0.4
IgA
SAP 0 nM
B
DyL650-dSAP
DyL650-tSAP
14 nM
72 nM
360 nM
520 nM
SAP
IgG
IgA
IgG
IgA
SAP
C
DyL650-dSAP
DyL650-tSAP
% overlap
[SAP] (log10 nM)