Journal of Immunological Methods 422 (2015) 102–110

Contents lists available at ScienceDirect

Journal of Immunological Methods

journal homepage: www.elsevier.com/locate/jim

Efficient generation of human IgA monoclonal antibodies

Valérie Lorin, Hugo Mouquet *

Laboratory of Humoral Response to Pathogens, Department of Immunology, Institut Pasteur, Paris, 75015, France
CNRS-URA 1961, Paris, 75015, France

ARTICLE INFO

Article history:
Received 1 April 2015
Accepted 15 April 2015
Available online 22 April 2015

Keywords:
Antibodies
IgA
Transient recombinant expression system
Protein purification
Affinity/size-exclusion chromatography

ABSTRACT

Immunoglobulin A (IgA) is the most abundant antibody isotype produced in humans. IgA antibodies primarily ensure immune protection of mucosal surfaces against invading pathogens, but also circulate and are present in large quantities in blood. IgAs are heterogeneous at a molecular level, with two IgA subtypes and the capacity to form multimers by interacting with the joining (J) chain. Here, we have developed an efficient strategy to rapidly generate human IgA1 and IgA2 monoclonal antibodies in their monomeric and dimeric forms. Recombinant monomeric and dimeric IgA1/IgA2 counterparts of a prototypical IgG1 monoclonal antibody, 10-1074, targeting the HIV-1 envelope protein, were produced in large amounts after expression cloning and transient transfection of 293-F cells. 10-1074 IgAs were FPLC-purified using a novel affinity-based resin engrafted with anti-IgA chimeric Fabs, followed by a monomers/multimers separation using size exclusion-based FPLC. ELISA binding experiments confirmed that the artificial IgA class switching of 10-1074 did not alter its antigen recognition. In summary, our technical approach allows the very efficient production of various forms of purified recombinant human IgA molecules, which are precious tools in dissecting IgA B-cell responses in physiological and pathophysiological conditions, and studying the biology, function and therapeutic potential of IgAs.

1. Introduction

Humoral immunity is essential to host protection against human infections, and relies on the phenomenal diversity of antibody molecules, which sense and inactivate pathogens. At mucosal surfaces, antibodies, and more specifically IgA immunoglobulins produced locally by subepithelial plasma cells, play a key role in the protection against toxins, bacteria, viruses and protozoa (Kaetzel, 2007). There are two IgA subclasses in humans, IgA1 and IgA2, which can exist as either monomers or as multimers made of IgA monomeric units polymerized by covalent bounds with the J-chain (principally dimers). IgAs are found in diverse mucosal secretions but are also abundant in serum (2–3 mg/ml) predominantly in the monomeric and IgA1 form (Kaetzel, 2007). On the other hand, mucosal IgAs are mainly present as "secretory IgA" (SIgAs) that are IgA polymers covalently associated with the "secretory component", which corresponds to the extracellular domain of the polymeric immunoglobulin receptor (pIgR) cleaved away from the surface of epithelial cells (Kaetzel, 2007). Apart from their well-documented role in protecting epithelia from invading pathogens, SIgAs possess additional biological activities; they can act as anti-inflammatory and anti-allergenic molecules, maintain mucosal homeostasis, and regulate intestinal microbiota (Mantis et al., 2011). Surprisingly, the role of circulating serum IgAs is less clear, and should be explored more thoroughly.

In the past 15 years, the use of single cell antibody cloning techniques has enabled the rapid and efficient production of human monoclonal antibodies from different B-cell compartments (Sullivan et al., 2011; Tiller, 2011; Wilson and Andrews, 2012). The molecular and functional characterization of antibodies generated from selected single B-cell sub-populations (captured by flow cytometry or derived from screening of cultured B cells) has provided invaluable insight into the physiological and pathophysiological aspects of humoral immunity in humans (Wardemann and Nussenzweig, 2007; Meffre, 2011; Wilson and Andrews, 2012; Mouquet, 2014). Such studies have: (i) defined B-cell tolerance checkpoints at the transitions between different B-cell developmental stages (Wardemann et al., 2003; Tsuiji et al., 2006; Scheid et al., 2011); (ii) uncovered some of the tolerance mechanisms involved by studying these checkpoints in immunodeficient patients (Ng et al., 2004; Herve et al., 2005; Isnardi et al., 2008; Menard et al., 2011a); (iii) revealed defective tolerance checkpoints in autoimmune diseases (Samuels et al., 2005; Yurasov et al., 2005; Menard et al., 2011a,b); (iv) dissected antibody responses to various pathogens (Wrammert et al., 2008; Scheid et al., 2009; Muellenbeck et al., 2013); (v) identified and characterized broadly neutralizing antiviral antibodies particularly, against HIV-1 (Mouquet, 2014). In fact, probing humoral immunity using single B-cell derived monoclonal antibodies, as both effectors of and surrogates for immune responses, has not only provided invaluable insight into the mechanisms that govern adaptive B-cell responses, but has also contributed to the development of therapeutic and/or vaccine strategies to fight human diseases. However, too few studies have investigated the role IgA-mediated humoral immunity in

* Corresponding author.
E-mail address: hugo.mouquet@pasteur.fr (H. Mouquet).

http://dx.doi.org/10.1016/j.jim.2015.04.010
0022-1759/

humans (Di Niro et al., 2010, 2012; Benckert et al., 2011), and in none of those, recombinant monoclonals were produced as native IgA antibodies, which definitively restricts certain molecular and functional characterizations.

In order to study IgA biology and IgA B-cell responses in physiological and pathophysiological conditions, we designed an expression cloning-based methodological approach to rapidly produce recombinant human IgA monoclonal antibodies. Expression vectors for human IgA1 and IgA2 compatible with the single B-cell antibody cloning method (Tiller et al., 2008) were created, and used to clone the immunoglobulin variable domain genes of a prototypic antigen-specific antibody, 10-1074, a neutralizing HIV-1 IgG1. Efficient production of purified monomeric and dimeric 10-1074 IgA molecules was made possible by combining a transient transfection-based eukaryotic expression system with fast protein liquid chromatography (FPLC) purification. Human IgA1 and IgA2 were FPLC-purified using a new affinity resin engrafted with anti-IgA chimeric Fabs. Finally, IgA monomers and dimers were separated by FPLC-based size exclusion chromatography.

2. Materials and methods

2.1. Expression vectors and cloning of human IgA monoclonal antibodies

Total RNAs were previously extracted from human peripheral blood mononuclear cells (PBMCs) of a healthy donor (Scheid et al., 2011), using TRIzol® reagent (Life Technologies) following manufacturer's instructions. cDNAs were obtained by reverse transcription of RNAs primed with random hexamers (Roche) using SuperScript® III reverse transcriptase (Life technologies) according to the manufacturer's protocol. Primers used to amplify the Fc part of IgA1 and IgA2, and J chain gene products were as follows: *huIgA.Fc sense*, 5′-GCATCCCCGACCAG CCCCAA-3′; *huIgA.Fc antisense*, 5′-GTAGCAGGTGCCGTCCACCT-3′; *huJc sense*, 5′-GGATCCATGAAGAACCATTTGCTT-3′; *huJc antisense*, 5′-GAAT TCTTAGTCAGGATAGCAGGC-3′. Specific gene products were amplified by PCR using 5 μl of cDNAs as template, 250 nM each dNTP, 200 nM each primer and 2.5 U of HotStarTaq DNA polymerase (Qiagen) in a total reaction volume of 50 μl. PCR conditions comprised one cycle of 94 °C for 5 min, 35 cycles of 94 °C for 30 s, 50 °C (*J chain*) or 57 °C (*IgA Fc*) for 30 s and 72 °C for 50 s (*J chain*) or 2 min (*IgA Fc*), and a final elongation step of 72 °C for 10 min. After electrophoretic separation, PCR products visualized by UV on ethidium bromide-stained agarose gel were purified using NucleoSpin® Extract II kit (Macherey-Nagel). Purified J chain DNA product was directly cloned into pcDNA™3.1/Zeo$^{(+)}$ expression vector (Life technologies) using Bam*HI* and Eco*RI* restrictions sites. Purified IgA Fc DNA products were cloned into pCR® 2.1-TOPO® vector using TOPO TA Cloning® Kit (Life technologies) to allow the selection of either IgA1 or IgA2 Fc DNA inserts. Following bacterial colony screening by PCR and sequencing, plasmid DNA of selected clones were prepared from transformed-DH10β bacteria (New England Biolabs) using NucleoSpin®Plasmid kit (Macherey-Nagel), and used as templates to introduce Sal*I* and Hind*III* restriction sites by PCR using the same conditions as described above with the following primers: Sal*I*-*huIgA.Fc*, 5′-CGTACGGTCGACCACCAGCCCCAAGGTC-3′; Hind*III*-*huIgA.Fc*, 5′-CCAAGCTTTCAGTAGCAGGTGCCGTCCA-3′. Purified PCR products were then cloned into a human Igγ$_1$-expression vector described previously (Tiller et al., 2008) using Sal*I* and Hind*III* restriction sites. This cloning strategy allows for the complete substitution of the DNA fragment coding for the human Fc part of IgG1 by either IgA1 or IgA2, and an easy subsequent cloning of V_H domain DNA segment using Age*I* and Sal*I* restriction sites as previously described for recombinant IgG1 (Tiller et al., 2008). However, it also introduces a Proline to Threonine substitution in position 3 of IgH constant domain 1 (C_H1). All final constructs were isolated from transformed-DH10β bacteria using plasmid PureLink™ plasmid maxiprep kit (Life technologies), sequenced and compared to the original product sequences. For the cloning of anti-HIV antibody 10-1074 (Mouquet et al., 2012), 10-1074 V_H domain gene inserted into the human Igγ$_1$-expressing vector was extracted using Age*I* and Sal*I* enzymatic digestion (New England Biolabs), and cloned after purification into human Igα1- and Igα2-expressing vectors using 0.5 μl of T4 DNA ligase (New England Biolabs). Plasmids were prepared from transformed-DH10β bacteria using plasmid PureLink™ plasmid maxiprep kit (Life Technologies), sequenced and compared to the original product sequences.

2.2. Hybridoma culture and mouse IgG purification

Hybridoma CH-EB6 cells (ATCC® HB-200™) were cultivated in Dulbecco's modified Eagle's medium (DMEM) containing 4.5 g/l D-glucose and 4 mM L-glutamine (Life Technologies), supplemented with 10% fetal bovine serum (HyClone™ Thermo Scientific), Antibiotic/Antimycotic (1X) and 1% sodium pyruvate (Life Technologies). HB-200 cell cultures were passaged every 2–3 days to maintain a cell density between 1.0×10^5 and 1.0×10^6 cells/ml. Supernatants were collected and stored at 4 °C after cell debris removal by centrifugation at 4200 rpm for 30 min (4 °C) and 0.2 μm filtration. Mouse IgG1 anti-human IgA antibodies were purified by affinity chromatography using protein G sepharose high performance beads (GE Healthcare) according to the manufacturer's instructions. The concentration of each elution fraction was measured using the NanoDrop 2000 instrument (Fisher Scientific). The fractions of interest were pooled, and dialyzed overnight against PBS using Slide-A-Lyzer® dialysis cassettes with a 10 kD molecular weight cutoff (Thermo Scientific).

2.3. Generation of a human IgA-specific chimeric Fab

Total RNAs extraction was performed on 5×10^6 of HB-200 hybridoma cells by TRIzol® reagent (Life Technologies) following the manufacturer's instructions. The quality of isolated total RNAs was checked on an ethidium bromide-stained agarose gel after electrophoretic separation, and their concentration was determined using NanoDrop 2000 instrument (Thermo Scientific). Total RNAs were reverse transcribed in a 50 μl reaction mix containing 5 μg RNAs primed with random hexamers (Roche) using SuperScript® III reverse transcriptase (Life technologies) according to the manufacturer's protocol. Reverse transcription conditions were comprised of successive cycles as follow: 42 °C for 10 min, 25 °C for 10 min, 50 °C for 60 min and 94 °C for 5 min. cDNAs were stored at −20 °C.

Murine IgH and Igκ variable domain genes were amplified by semi-nested (IgH) and nested (Igκ) PCRs using 5 μl of cDNAs as template as previously described (Tiller et al., 2009). PCR products were separated by electrophoresis on an ethidium bromide-stained gel (2% agarose), purified using NucleoSpin® Gel/PCR Clean-up (Macherey-Nagel), and sequenced (Eurofins genomics) using the following primers: Cγ1-231 (5′-GCCCTTGAACTTCTCATTG-3′) and 5′ mVkappa/3′ BsiWI P-mJK mix (Tiller et al., 2009), for IgH and Igκ, respectively. PCR sequences were then compared to germline Ig reference sequences and analyzed using IgBLAST (http://www.ncbi.nlm.nih.gov/igblast) and IMGT® (http://www.imgt.org).

To allow the cloning of IgH and Igκ DNA fragments into expression vectors, specific PCR amplifications were performed using 5 μl of first PCR products as template, and single gene-specific V and J gene primers including restriction sites as previously described (Tiller et al., 2009): 5′ AgeIP-mVH1S132 (5′-CTGCAACCGGTGTACATTCCGAGGTGCAGCTGCAGG AGTCTGG-3′) and 3′SalIP-mJH02 (Tiller et al., 2009) for IgH, and 5′ AgeIP-mVK10-96 (5′-CTGCAACCGGTGTACATTCCGATATTGTGATGACAC AGACTACATCC-3′) and 3′BsiWI P-mJK01 (Tiller et al., 2009) for Igκ. Purified digested PCR products were cloned into human Igγ$_1$*Fab*- or Igκ-expressing vectors (Wardemann et al., 2003; Mouquet et al., 2012) as previously described (Tiller et al., 2008). Vectors containing HB-200 Igγ$_1$*Fab* and Igκ genes were isolated from transformed-DH10β bacteria (New England Biolabs) using plasmid DNA purification kits (NucleoSpin®Plasmid, Macherey-Nagel), sequenced and compared to the original PCR-product