Reinhart et al. BMC Proceedings 2011, 5(Suppl 8):P56
http://www.biomedcentral.com/1753-6561/5/S8/P56

BMC Proceedings

MEETING ABSTRACT Open Access

3D6 and 4B3: Recombinant expression of two anti-gp41 antibodies as dimeric and secretory IgA

David Reinhart[1,2*], Robert Weik[2], Renate Kunert[2]

From 22nd European Society for Animal Cell Technology (ESACT) Meeting on Cell Based Technologies
Vienna, Austria. 15-18 May 2011

Background

Sexually transmitted diseases are predominantly acquired via mucosal membranes of the rectal or genital tract during sexual intercourse. This major port of virus entry is naturally defended by the humoral immune response, with immunoglobulin A (IgA) as the primary antibody class to elicit mucosal immunity. Dimeric IgA (dIgA) reaches the luminal side of mucosal tissues by transcytosis through epithelial cells lining the mucosa. In a first step, dIgAs specifically bind to the basolaterally expressed polymeric immunoglobulin receptor (pIgR) on epithelial cells. For release of IgAs on the luminal side the extracellular portion, termed secretory component (SC), remains attached to the antibody to form secretory IgA (sIgA) [1,2].

One example of a sexually transmitted disease is the human immunodeficiency virus (HIV) which annually infects several million individuals on a global scale and potentially leads to the acquired immunodeficiency syndrome (AIDS). Although current therapies can reduce disease progression in infected individuals, no cure is yet available or within reach in near future. As a consequence increased attention is now being paid to develop drugs that could prevent virus acquisition.

3D6 and 4B3 are two monoclonal antibodies (mAb) which have originally been isolated as IgG1 isotype from seroconverted HIV-1 patients and bind to the principal immunodominant domain of gp41. In the course of this project both mAbs were isotype switched to IgA1. Recombinant CHO cell lines were established for the production of 3D6 and 4B3 as dimeric as well as secretory IgA. While dIgA were expressed by a single cell line, sIgA are produced by a biochemical association of dIgA with SC. Both dIgA and sIgA variants were characterized and the contribution of the heavily glycosylated SC on IgA stability will be investigated.

Antibody expression

MAbs 3D6 and 4B3 (IgG1) were developed at the Institute of Applied Microbiology [3,4]. Isotype switching was performed by substitution of the original heavy chain constant region with that for IgA1 (GenBank Accession Number 184743). For their recombinant expression as dIgA three plasmids were generated containing the coding region of either heavy chain, light chain or joining (J) chain. The latter one should increase dimerization of IgA monomers. Recombinant CHO clones were selected whereas high producers were isolated by applying the dihydrofolate reductase (dhfr) system.

Assembly of 3D6 and 4B3 as sIgA is intended by an *in vitro* biochemical association of dimeric IgA with human secretory component (hSC). Thus, CHO cell lines which solely express hSC were established by co-transfection of two plasmids containing the coding region for hSC and dhfr, respectiviely.

Cultivation temperature greatly influences productivity

Mammalian cells are commonly cultivated at 37°C. In order to investigate the effect on mAb secretion, clones expressing 3D6-dIgA and 4B3-dIgA were propagated at sub-physiological temperatures. It was observed that temperature markedly impacts final IgA quantities in culture supernatants. Product titers of clones expressing 3D6-dIgA were almost 3-fold increased when incubated at 33 °C as compared to 37 °C. Furthermore, cultivation of 4B3-dIgA-expressing clones at 33 °C, rather than at 37 °C, resulted in end titers which were approximately 13-fold increased.

* Correspondence: David.Reinhart@boku.ac.at
[1]Department of Biotechnology, Institute of Applied Microbiology, University of Natural Resources and Life Sciences, Vienna, Austria
Full list of author information is available at the end of the article

BioMed Central
© 2011 Reinhart et al; licensee BioMed Central Ltd. This is an open access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/2.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Table 1 Purification scheme developed for isolation of 3D6 and 4B3 dIgA.

STEP	EQUIPMENT	PROCEDURE	RECOVERY	
			3D6 dIgA	4B3 dIgA
Ultra/Diafiltration	Kvick Start UDF Cassette, Millipore, 30 kD	Harvested cell culture supernatant containing dIgA is concentrated and buffer exchanged against PBS, pH 7.4	>95 %	>95 %
Lectin Affinity Chromatography	Immobilized Jacalin, Thermo Scientific	UDF retentate is immobilized onto a lectin affinity resin. After a washing step, bound product is eluted by applying 1.5 M D-galactose in PBS, pH 7.4	98.7 %	106.1 %
Ultra/Diafiltration	Kvick Start UDF Cassette, Millipore, 30 kD	The eluate is buffer exchanged against 20 mM Tris, 10 mM NaCl, pH 8.5	>95 %	>95 %
Anion Exchange Chromatography	DEAE Sepharose FF, GE Healthcare	The retentate is applied onto the anion exchanger. Post washing with 100mM NaCl product is eluted from the resin using 20 mM Tris, 200 mM NaCl, pH 8.5	88.4 %	86.7 %
Hydrophobic Interaction Chromatography	Phenyl-Sepharose 6FF low sub, GE Healthcare	$(NH_4)_2SO_4$ is added to 0.75 M for 4B3-dIgA or 1.25 M for 3D6-dIgA to precipitate host cell proteins but not mAb itself. After a washing step 3D6-dIgA is eluted with 0.4 M $(NH_4)_2SO_4$, which allows to isolate dimeric IgA from other IgA isoforms. 4B3-dIgA desorbs at 0 M $(NH_4)_2SO_4$	44.1 %	59.1 %

Purification of recombinant dIgA

The two established mAb-expressing cell lines secrete dIgA at different quantities: The best clone producing 3D6-dIgA achieved a mean specific productivity of 59.1 ±14 pg/cell*day (pcd). Conversely, the highest 4B3-dIgA secreting clone reaches a mean specific productivity of 0.6±0.4 pcd. Hence, an increased amount of host cell proteins is present in cell culture supernatants of 4B3-dIgA as compared to 3D6-dIgA. Therefore, we elaborated a purification protocol which allows the recovery of both dimeric IgAs at a greatly enhanced purity. The purification procedure is shown in table 1.

Formation of secretory IgA

Secretory IgA can be assembled *in vitro* due to the natural affinity of dimeric IgA for secretory component and *vice versa* (5). Initially, supernatants from recombinant cell lines expressing hSC, 3D6-dIgA or 4B3-dIgA were buffer exchanged against PBS. Subsequently, sIgA formation was performed by incubation of hSC with dIgA at 25°C for 3 hours at different molar ratios. However, applying a molar ratio of 1:1 is commonly described as optimal [5]. The association of 3D6-dIgA or 4B3-dIgA with hSC was successfully verified by SDS-PAGE following Western blotting (data not shown). Conjugation of the immunoblot was performed with a mouse anti-human secretory component antiserum (Sigma) and detected via an anti-mouse IgG1 antiserum (Sigma).

Conclusions

The anti-HIV-1 mAbs 3D6 and 4B3 were isotype switched and could successfully be expressed as dIgA in stably transfected CHO cells. Furthermore, continuous product secretion was obtained for at least 20 passages in spinner flasks.

Cultivation of the recombinant cell lines at different temperatures revealed that their product expression can markedly be influenced: 3D6-dIgA expression was nearly triplicated by shifting the temperature from 37 °C to 33 °C. Applying the same conditions, 4B3-dIgA product secretion was nearly 13-fold increased.

A purification protocol was developed which allows the recovery of the dIgAs 3D6 and 4B3 in highly pure fractions. Furthermore, the elaborated scheme enables the isolation of dimeric 3D6 from its various high molecular weight isotypes.

Secretory IgA of both 3D6 and 4B3 can successfully be produced by mixing dimeric IgA with human secretory component.

Acknowledgement
Funding from the European Community's Seventh Framework Programme (FP7/2002-2013) under grant agreement N°201038, EuroNeut-41.

Author details
[1]Department of Biotechnology, Institute of Applied Microbiology, University of Natural Resources and Life Sciences, Vienna, Austria. [2]Polymun Scientific Immunbiologische Forschung GmbH, Vienna, Austria.

Published: 22 November 2011

References

1. Snoeck V: The IgA system: a comparison of structure and function in different species. *Vet Res* 2006, 37(3):455-67.
2. Bonner A, Perrier C, Corthésy B, Perkins SJ: Solution structure of human secretory component and implications for biological function. *J Biol Chem* 2007, 282(23):16969-80, Epub 2007 Apr 11.
3. Buchacher A, Predl R, Strutzenberger K, Steinfellner W, Trkola A, Purtscher M, Gruber G, Tauer C, Steindl F, Jungbauer A, *et al*: Generation of human monoclonal antibodies against HIV-1 proteins; electrofusion and Epstein-Barr virus transformation for peripheral blood lymphocyte immortalization. *AIDS Res Hum Retroviruses* 1994, 10(4):359-69.
4. Kunert R, Rüker F, Katinger H: Molecular characterization of five neutralizing anti-HIV type 1 antibodies: identification of nonconventional D segments in the human monoclonal antibodies 2G12 and 2F5. *AIDS Res Hum Retroviruses* 1998, 14(13):1115-28.
5. Crottet P, Corthésy B: Secretory component delays the conversion of secretory IgA into antigen-binding competent F(ab')2: a possible implication for mucosal defense. *J Immunol* 1998, 161(10):5445-53.

doi:10.1186/1753-6561-5-S8-P56
Cite this article as: Reinhart *et al.*: 3D6 and 4B3: Recombinant expression of two anti-gp41 antibodies as dimeric and secretory IgA. *BMC Proceedings* 2011 5(Suppl 8):P56.