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Production of genetically engineered low-affinity human Immunoglobulin receptors FcγRIIa (CD32a) and FcγRIIIa (CD16a) extracellular domains in *Pichia pastoris*

Dana Ashoor[1], Sonia Bourguiba-Hachemi[2], Maryam H. A. Marzouq[3], M.Dahmani Fathallah[4]

PhD, Life Sciences Department / Health Biotechnology program, College of Graduates Studies &

College of Medicine and Medical Sciences Arabian Gulf University, Manama, Kingdom of Bahrain [1,3]

Assistant Professor, Life Sciences Department / Health Biotechnology program, College of Graduates Studies,

Arabian Gulf University, Manama, Kingdom of Bahrain [2]

Professor, King Fahd Chair for Health Biotechnology, Life Sciences Department / Health Biotechnology program,

College of Graduates Studies, Arabian Gulf University, Manama, Kingdom of Bahrain [4]

Abstract: Genetic engineering technologies allow mass production of proteins for a variety of applications mainly in the biopharmaceutical industry. FcγRIIa (CD32a) and FcγRIIIa (CD16a) are antibodies cell surface receptors that mediate important immune functions. Fcγ receptors are currently the focus in developing novel therapeutic antibodies. Herein, we report the production at a high yields of genetically engineered soluble forms of the extracellular domain of the main genetic variants of CD16a (158F/V) (1.5mg/ml, 0.75mg/ml) and CD32a (131H/R) (5.8mg/ml, 0.9mg/ml) by the yeast *Pichia pastoris*. The Proteins were produced following induction using 1% methanol. SDS-PAGE and Western blot analysis of the protein purified on Ni+ column showed bands of ~45 kDa and ~25 kDa corresponding to CD16a and CD32a respectively. The production at a high yield of good quality recombinant Fcγ receptors in yeast is an important outcome because of their importance in the development of third generation therapeutic antibodies.

Keywords: FcγRIIa (CD32a), FcγRIIIa (CD16a), recombinant protein, *Pichia pastoris*, pPICZαA.

I. INTRODUCTION

The advent of recombinant DNA technology with its myriad of genetic engineering tools was accompanied by the development of powerful prokaryotic (bacterial) and eukaryotic (mammalian cell lines and yeasts) recombinant protein expression systems along with a significant progress in fermentation process engineering [1]. Combined use of these technologies allowed industrial mass production and engineering of proteins with modified features and a large array of applications flourished in various fields, mainly in the pharmaceutical field [2].

The yeast *Pichia pastoris* expression system is of special interest to the pharmaceutical engineering community mainly because it permits the production of high levels of functionally active and secreted recombinant proteins. Also the *Pichia pastoris* expression system does not only offers the advantages of the classical *Escherichia coli* bacterial system for high-level expression, easy scale-up and an inexpensive growth medium, but also gives the advantages of eukaryotic expression systems for protein processing, folding and posttranslational modifications mainly glycosylation [3, 4].

The development of humanized yeasts by glyco-Engineering [5] is currently showing significant promise for the production of therapeutic glycoproteins for human use. Fc receptors are cell surface receptors present on monocytes, macrophages, neutrophils, natural killer (NK) cells, T and B lymphocytes.

In human, Fc receptors are divided into three receptor families [FcγRI (CD64), FcγRIIa, IIb, and IIc (CD32)] and FcγRIIIa and IIIB (CD16) that have high structural homology but different affinity and specificity to IgG subclasses. CD64 is the only high-affinity receptor that strongly binds monomeric IgG, while CD16 and CD32 are considered to be low-affinity Fc receptors [6-8]. The multiplicity of the Fc receptors is further increased by a series of polymorphisms in their extracellular domains [9].

Fc receptors play a critical role in linking IgG antibody-mediated immune responses with cellular effector functions leading to the release of inflammatory mediators, initiation of phagocytosis of immune complexes and modulation of antibody production by B cells and antibody-dependent cell-mediated cytotoxicity (ADCC)[10, 11]. ADCC has been the focus nowadays, in developing cancer therapeutic monoclonal antibodies (mAb) [12, 13], and it has been the mode of action of several approved anti-tumor medicines such as Cetuximab and Rituximab [14]. Both FcγRIIa (CD32a) and FcγRIIIa (CD16a) receptors are expressed in NK cells that make them two of the most important cell surface receptors

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mediating ADCC response [15]. Recently, several FcγR polymorphisms showed to be associated with the clinical outcome in patients treated with monoclonal antibodies [16, 17]. CD32a showed to have two alleles generating two variants differing in position 131, named low-responder (H_{131}) and high responder (R_{131}) [18]. In the case of CD16a, the difference occurs at position 158 and yields the (V_{158}) and (F_{158}) variants [19].

Over the last few years, cancer immunotherapy has benefited from newly engineered monoclonal antibodies (mAbs). These third-generation therapeutic antibodies have enhanced antitumor activity resulting from increased complement-dependent cytotoxicity (CDC) and/or antibody-dependent cellular cytotoxicity (ADCC). Indeed as a consequence of molecular engineering, and advanced *in silico* approaches it is possible to engineer a monoclonal antibody with high Fc binding affinity for the low-affinity variants of the FcγRIIIa (CD16) receptor (F_{158}) and the FcγRIIa (CD32) low-responder (H_{131}) on immune effector cells. Previous studies described the expression of recombinant soluble human FcγRs in *E. coli*, insects and mammalian cells [20-22]. However, to the best of our knowledge, expression of the extracellular domains of human Fc□Rs using yeast expression system has not been reported yet. Thus, the aim of the current study was to produce the extracellular domain of human Fc□RIIa variants (CD32a H131R) and Fc□RIIIa (CD16a F158V) as soluble recombinant proteins in *Pichia pastoris* KM71H using the expression vector pPICZαA and the enzyme-controlled glucose delivery EnBase® technology.

II. MATERIALS AND METHODS

A. Strains and plasmids

Table 1 describes the strains and expression vector used in our study. *Escherichia coli* Top 10, used for the plasmids propagation, was grown in LB broth (MO BIO, USA) at 37°C under agitation (250 rpm). *Pichia pastoris* KM71H (Invitrogen) was grown in YPD medium (Sigma, USA) at 30°C under agitation (250 rpm).

Table1: Strains and Plasmid used in this study.

Strains or plasmid	Description	Source
Esherichia coli Top10F'	rec A, end A	Invitrogen
Pichia pastoris KM71H	Mut^s, Arg+ (arg4 aox1::ARG4)	Invitrogen
pPICZaA	Zeo^r, carrying the secretion signal sequence from the S. cerevisiae α-factor and functional sites for integration at the 5′ AOX1 locu	Invitrogen

rec A: recombinant deficient;
end A: endonuclease A deficient;
Mut^s : Methanol utilization slow;
arg4: arginosuccinate lyase gene;
Zeo^r: Zeocin resistance

B. Construction of recombinant plasmids

Synthetic genes encoding the extracellular domains of CD16a (NM_000569.6) and CD32a (NM_001136219.1) were generated satisfying the codon usage of P. pastoris for wild-type codons, digested with EcoRI and NotI, and then ligated into similarly digested pPICZaA vector to create the recombinant expression vector. The synthesized genes were cloned downstream of the *Saccharomyces cerevisiae*-□ factor signal sequence and in frame with a c-myc epitope and an additional 6X Histidine (His) tag on the 3`-end (GeneCust, Dudelange, Luxembourg). Competent E. Coli Top 10 was used to clone and propagate the expression vector. The integrity of the cloned plasmids was validated by restriction enzyme digestion, and by Dye termination sequencing method [23] (GenoScreen, Lille, France) using universal AOX1 primers: 5´AOX1: 5´-GACTGGTTCCAATTGACAAGC-3´ and 3´AOX1: 5´-GCAAATGGCATTCTGACATCC-3´ (GeneCust). Approximately 1µg of recombinant plasmid was linearized with BstX I (Promega). Then, linearized vector was transformed into competent yeast cells by electroporation using a Gene Pulser instrument (Bio-Rad) under the following setting: cuvette gap, 2.0 mm; charging voltage, 1500 V; resistance, 200Ω; capacitance, 25µF. Transformants were selected on YPD plates supplemented with 500, 1000, and 2000 µg/ml Zeocin. After 72h incubation at 30°C, single clones were picked and spotted on YPD plates containing 100µg/ml Zeocin. Efficient integration of the synthetic gene in the transformed P. pastoris was confirmed by colony PCR method using AOX1 primers. PCR-positive colonies exhibiting the highest expression levels were stored at 4°C on YPD plates supplemented with 100µg/ml zeocin.

C. Protein expression and purification

The expression of the recombinant CD16a and CD32a proteins was performed using the enzyme-controlled glucose delivery EnBase® strategy. Protein production was methanol-induced in EnPresso® Yeast Defined Tablet Medium from BioSilta (Oulu, Finland) [24, 25]. To prepare a pre-culture, 1 ml of YPD broth was inoculated with a single colony from YPD/zeocin plates. The pre-culture was incubated at 30°C under agitation (250 rpm) for 6h. Then, 1:100 of the pre-culture volume was inoculated into 50 ml EnPresso® Yeast Defined Tablet Medium, which was supplemented with 1.5U/L glucose-releasing biocatalyst (EnZ1'm). The culture flask was incubated overnight on an orbital shaker at 250 rpm and 30°C. Afterwards, methanol (inducing agents) was added at a concentration of 5ml/L and EnZ1'm at a concentration of 9 U/L. The incubation was continued at 28°C for another 8 hr, and then More methanol (10 ml/L) and EnZ 1'm (9U/L) were added and the incubation was continued overnight. Next morning, methanol (5 ml/L) was added and the culture was harvested after 5 hr incubation. The pellet was discarded, and the supernatant was stored at -20°C.

The recombinant CD16a and CD32a His-tagged proteins were purified from the supernatant using 5 ml HiTrap Chelating HP columns (GE Healthcare, UK). The columns were supplied free of metal ions, and they were charged

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with Nickle ions (Ni^{2+}) as a metal salt solution (0.1 M Ni_2Cl). Different imidazole concentrations in both the binding buffer and elution buffer were tested for the best elution (0 and 20mM imidazole respectively for CD16a and 40 and 500mM respectively for CD32a). The fractions containing the purified CD16a and CD32a proteins were desalted and concentrated with 30K Amicon Ultra-0.5 ml centrifugal filter units (Millipore, Germany). Microvolume protein concentration was determined by a direct A280 measurement using Thermo Scientific NanoDrop 2000c Spectrophotometer (Wilmington, USA).

D. SDS-PAGE and Western blot analysis

Purified recombinant proteins were separated by SDS-PAGE using 15% (v/v) polyacrylamide gels according to Laemmli's protocol [26]. The recombinant proteins were detected by Coomassie Brilliant Blue R-250 staining and transferred to a Hybond-P polyvinylidene difluoride membrane (PVDF, Amersham, GE Healthcare). The membrane was blocked with 0.1% bovine serum albumin (BSA) in Tris-buffered saline, pH 7.4 (TBS) containing 0.05% Tween 20 for 1 hour at room temperature. Western blotting analysis was performed with horseradish peroxidase (HRP)-conjugated anti-*His* antibody (1/5000) (Invitrogen). Target proteins were visualized using an enhanced chemiluminescence (ECL) detection system (Amersham, GE Healthcare).

III. RESULTS

A. Cloning of synthetic FcγRIIa (CD32a) and FcγRIIIa (CD16a) genes.

The DNA sequences encoding the extracellular domains CD16a (Met 18 – Gln 208; Fig.1A) and CD32a (Ala 36 – Ile 218; Fig.1B) were fused to the secretion signal sequence of the α-factor from *S.cerevisiae* in the pPICZ□A expression cassette between EcoRI and NotI restriction sites (Fig. 1C). The cloning of synthetic genes resulted in the recombinant plasmids pPICZ□A CD16a and pPICZ□A CD32a.

A

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gcagctcccccaaaggctgtgctgaaacttgagcccccgtggatcaacgtgctccaggag  60
 A  A  P  P  K  A  V  L  K  L  E  P  P  W  I  N  V  L  Q  E
gactctgtgactctgacatgccaggggctcgcagcccctgagagcgactccattcagtgg  120
 D  S  V  T  L  T  C  Q  G  A  R  S  P  E  S  D  S  I  Q  W
ttccacaatgggaatctcattcccacccacacgcagcccagctacaggttcaaggccaac  180
 F  H  N  G  N  L  I  P  T  H  T  Q  P  S  Y  R  F  K  A  N
aacaatgacagcggggagtacacgtgccagactggccagaccagcctcagcgaccctgtg  240
 N  N  D  S  G  E  Y  T  C  Q  T  G  Q  T  S  L  S  D  P  V
catctgactgtgctttccgaatggctggtgctccagacccctcacctggagttccaggag  300
 H  L  T  V  L  S  E  W  L  V  L  Q  T  P  H  L  E  F  Q  E
ggagaaaccatcatgctgaggtgccacagctggaaggacaagcctctggtcaaggtcaca  360
 G  E  T  I  M  L  R  C  H  S  W  K  D  K  P  L  V  K  V  T
ttcttccagaatggaaaatcccagaaatttctccatttggatcccaccttctccatccca  420
 F  F  Q  N  G  K  S  Q  K  F  S  H  L  D  P  T  F  S  I  P
caagcaaaccacagtcacagtggtgattaccactgcacaggaaacataggctacacgctg  480
 Q  A  N  H  S  H  S  G  D  Y  H  C  T  G  N  I  G  Y  T  L
ttctcatccaagcctgtgaccatcactgtccaagtgcccagcatgggcagctcttcacca  540
 F  S  S  K  P  V  T  I  T  V  Q  V  P  S  M  G  S  S  S  P
atggggatc
 M  G  I
```

B

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atgcggactgaagatctcccaaaggctgtggtgttcctggagcctcaatggtacagggtg  60
 M  R  T  E  D  L  P  K  A  V  V  F  L  E  P  Q  W  Y  R  V
ctcgagaaggacagtgtgactctgaagtgccaggggagcctactcccctgaggacaattcc  120
 L  E  K  D  S  V  T  L  K  C  Q  G  A  Y  S  P  E  D  N  S
acacagtggtttcacaatgagagcctcatctcaagccaggcctcgagctacttcattgac  180
 T  Q  W  F  H  N  E  S  L  I  S  S  Q  A  S  S  Y  F  I  D
gctgccacagtcgacgacagtggagagtacaggtgccagacaaacctctccaccctcagt  240
 A  A  T  V  D  D  S  G  E  Y  R  C  Q  T  N  L  S  T  L  S
gacccggtgcagctagaagtccatatcggctggctgttgctccaggcccctcggtgggtg  300
 D  P  V  Q  L  E  V  H  I  G  W  L  L  L  Q  A  P  R  W  V
ttcaaggaggaagaccctattcacctgaggtgtcacagctggaagaacactgctctgcat  360
 F  K  E  E  D  P  I  H  L  R  C  H  S  W  K  N  T  A  L  H
aaggtcacatatttacagaatggcaaaggcaggaagtattttcatcataattctgacttc  420
 K  V  T  Y  L  Q  N  G  K  G  R  K  Y  F  H  H  N  S  D  F
tacattccaaaagccacactcaaagacagcggctcctacttctgcaggggggctttttggg  480
 Y  I  P  K  A  T  L  K  D  S  G  S  Y  F  C  R  G  L  F  G
agtaaaaatgtgtcttcagagactgtgaacatcaccatcactcaaggtttggcagtgtca  540
 S  K  N  V  S  S  E  T  V  N  I  T  I  T  Q  G  L  A  V  S
accatctcatcattctttccacctgggtaccaa
 T  I  S  S  F  F  P  P  G  Y  Q
```

C

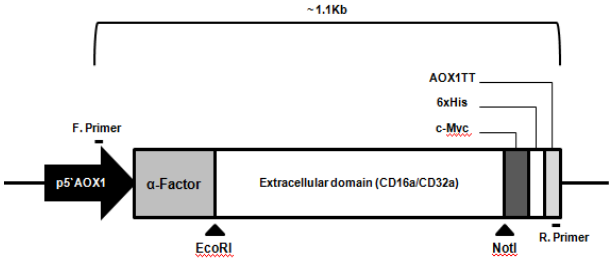

Fig. 1: Recombinant pPICZ□A FcγRIIa (CD32a) and pPICZ□A FcγRIIIa (CD16a) vectors construction. **A:** Nucleotide and amino acid (Ala 36–Ile 218) sequences of the extracellular domain of human CD32a variants. The variant allele position is underlined (**H**131R). **B:** Nucleotide and amino acid (Met 18–Gln 208) sequences of the extracellular domain of human CD16a variants. The variant allele position is underlined (**F**158V). **C:** Schematic drawing of the FcγRs expression cassette of the pPICZ□A used to electroporate yeast *P.pastoris*. p5'AOX1: AOX1 promoter induced by methanol. α-factor: α-factor signal from *S.cerevisiae*. c-Myc and 6x His tags at the C-terminal of the produced protein. AOX1TT: Aox transcription termination region. Forward (F.Primer) and reverse (R.Primer) AOX1 primers are represented by black bars.

Single and double digest with EcoRI and NotI confirmed the integrity of the expression vectors (Fig. 1D). The digestions yielded 587 bp, and 563 bp bands visualized on agarose gel electrophoresis representing the extracellular domain of CD16a (F158V) and CD32a (H131R) respectively.

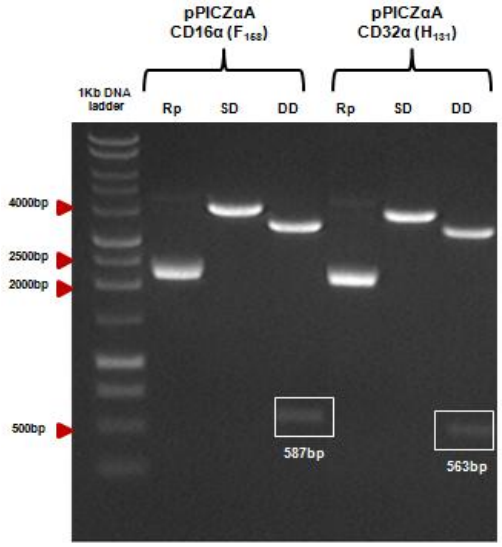

Fig. 2: Recombinant pPICZ□A CD16a (variant F) and pPICZ□A CD32a (variant H) plasmids restriction map. Rp: recombinant plasmid. SD: single digest with *Eco*RI. DD: double digest with *Eco*RI and *Not*I.

B. Recombinant human FcγRIIa (CD32a) and FcγRIIIa (CD16a) proteins expression and purification

The *P.pastori*s system was used to produce the recombinant human FcγRIIa (CD32a H131R) and FcγRIIIa (CD16a F158V) as soluble proteins. The yeast system was used to produce the extracellular domains of

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human FcγRIIa (CD32a) and FcγRIIIa (CD16a) as soluble recombinant proteins. P. pastoris strain KM71H was transformed with the synthetic genes cloned into the expression vector pPICZαA under the tightly regulated methanol-inducible alcohol-oxidase-1 (AOX1) promoter, along with the open reading frame of the α-factor signal sequence, to drive the secretion of the induced protein into the culture medium. Successful integration of the expression cassette into the yeast was confirmed by PCR and DNA sequencing (data not shown). Selected transformant was grown in methanol-induced in EnPresso® Yeast Defined Medium. Recombinant FcγRIIa (CD32a) and FcγRIIIa (CD16a) proteins were expressed as fusion proteins carrying a c-myc epitope and a His-tag on their C-terminal. Expressed proteins were purified using HiTrap Chelating HP columns. The purified FcγRIIa (CD32a) and FcγRIIIa (CD16a) proteins were detected by SDS-PAGE. The gel was stained with Coomassie blue (Fig.3A). As expected, major bands with an estimated molecular mass of ~25 and ~45 kDa, representing the two variants of the extracellular domains of CD32a and CD16 respectively, were detected. The identity of the recombinant proteins was confirmed by western blot analysis under reducing conditions (Fig. 3B). Here again, the protein bands of approximately 45 and 25 kDa were detected by anti-His antibody validating the successful production of fusions CD16a (F and V variants) and CD32a (R and H variants) proteins by the recombinant *P.pastoris* yeast.

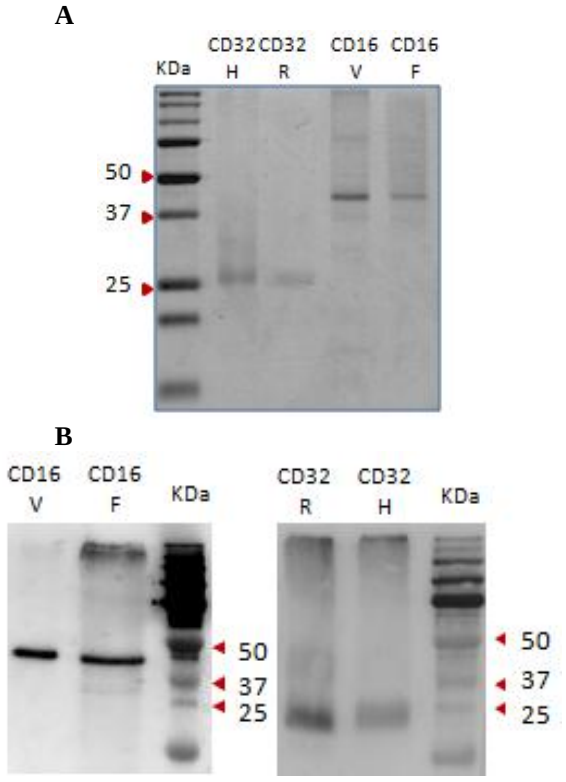

Fig. 3: Expression and purification of the CD16a and CD32a fusion proteins variants (CD16V, CD16 F, CD32 R and CD32 H). **A**: Analysis of the expressed and purified proteins by SDS-PAGE. The gel was stained with Coomassie blue. **B**: Analysis of the purified fusion proteins by western blotting using anti-His antibody (1/5000). Molecular weights markers are shown by red arrowhead.

The purification process yielded a concentration of 1538µg/ml, 754 µg/ml for CD16a (F158) and (V158) respectively and 5859µg/ml, 910µg/ml for CD32 (H131) and (R131) respectively.

IV. DISCUSSION

The studies of proteins structure-function, as well as their use for industrial purpose as drugs or reagents require a large amount of high-quality protein. Until few decades ago, the only sources for proteins were biological samples and protein production and purification were cumbersome and costly. The quantities obtained allowed only analytical studies. Genetic engineering and recombinant DNA techniques have introduced a revolution in life sciences by allowing the production of the quasi indefinite amount of protein. Heterologous gene expression across species facilitated the development of powerful recombinant protein expression systems that use microorganisms such as bacteria, yeast and algae's. These technological developments boosted protein structure-function research and spurred the development of the biotech-industry mainly the biopharmaceutical industry.

In this study, we achieved for the first time the expression in the yeast *P.pastoris* of 2 types of human Immunoglobulin G cell surface receptors or Fc receptors namely CD16a and CD32. In total, we have produced four recombinant proteins, i.e. two genetic variants of the CD16a, CD16a/F158 and CD16a/V158 respectively and two genetic variants of the CD32, CD32/H131 and CD32/R131. The secreted recombinant human proteins CD16a and CD32a were expected to have a molecular size between 25 and 23 kDa respectively, but the results showed a protein with a molecular size of ~45 kDa for CD16a variants and ~25 kDa for CD32a variants. These results can be explained by posttranslational modification (glycosylation). The data indicate less posttranslational modification of CD32a than that of CD16a. Recombinant Fc receptors with a glycosylation pattern similar to that of native proteins would be achieved if a humanized yeast clone is used for protein expression. Fc receptors were previously expressed in mammalian cells, however he high production yields we achieved using the methylotropic *P.pastoris* that varied from 1.5gr/l up to 12gr/l cannot be foreseen even with the most advanced mammalian cells expression systems.

The high yields obtained with the different Fc receptors variants we produced are probably associated with the use of cDNA sequences that are adjusted to the *P.pastoris* codon bias [27]. To improve the expression of heterologous gene, codon optimization by using the host frequently used codons is now a proven efficient approach. Commonly, it is achieved by replacing all codons with the host preferred codons [28]. Moreover, the use of the enzyme-controlled glucose delivery EnBase and the EnPresso Yeast Defined culture medium in a fed-batch-like culture provides cells with constant intracellular physiological conditions, avoiding excess metabolism in

the initial cultivation phase [24, 25]. All the produced recombinant Fc receptors were detected by specific anti-His antibody in addition to the direct binding to IgG Fc region observed by Surface Plasmon Resonance, Biacore analysis (data not shown). These data show that the Fc receptors recombinant extracellular domains produced in *P.pastoris* are properly folded and thus suitable for structure-function studies and that the glycosylation pattern may not significantly contribute to the interaction with the IgG Fc moiety.

The importance of the Fc receptors that are normally expressed at the surface of effector immune cells, such as the NK (natural killer) cells, lies in their role in bringing these cells close to their target via their binding to the Fc region of antibodies that already bind to the target. When the target is a tumour cell, the quality of the binding between an anti-cancer antibody Fc region and the Fc receptors determines the efficiency of the immune clearance of the target through ADCC. Characterization of the molecular basis of the interaction between the Fc receptors and the antibody Fc region is critical to the design of therapeutic antibody with improved biological activities. Therefore, the availability of high structural quality recombinant Fc receptors is of paramount importance for validating novel antibodies particularly for cancer therapy.

V. CONCLUSION

In conclusion, this data is the first to report the expression of extracellular domains of human CD16a and CD32a receptors using *P.pastoris* expression system. We have generated recombinant *P.pastoris* clones that produce properly folded, secreted forms of the extracellular domain of CD16a (158F/V) and CD32a (131H/R) at high yields.

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REFERENCES

[1] Graumann, K.; Premstaller, A., Manufacturing of recombinant therapeutic proteins in microbial systems. Biotechnol J 2006, 1, (2), 164-86.
[2] Schmidt, F. R., Recombinant expression systems in the pharmaceutical industry. Appl Microbiol Biotechnol 2004, 65, (4), 363-72.
[3] Benabdesselem, C.; Barbouche, M. R.; Jarboui, M. A.; Dellagi, K.; Ho, J. L.; Fathallah, D. M., High level expression of recombinant Mycobacterium tuberculosis culture filtrate protein CFP32 in Pichia pastoris. Molecular biotechnology 2007, 35, (1), 41-9.
[4] Daly, R.; Hearn, M. T., Expression of heterologous proteins in Pichia pastoris: a useful experimental tool in protein engineering and production. Journal of molecular recognition : JMR 2005, 18, (2), 119-38.
[5] Hamilton, S. R.; Gerngross, T. U., Glycosylation engineering in yeast: the advent of fully humanized yeast. Current opinion in biotechnology 2007, 18, (5), 387-92.
[6] Ravetch, J. V.; Kinet, J. P., Fc receptors. Annu Rev Immunol 1991, 9, 457-92.
[7] Daeron, M., Fc receptor biology. Annu Rev Immunol 1997, 15, 203-34.
[8] Daeron, M., Structural bases of Fc gamma R functions. Int Rev Immunol 1997, 16, (1-2), 1-27.
[9] van Sorge, N. M.; van der Pol, W. L.; van de Winkel, J. G., FcgammaR polymorphisms: Implications for function, disease susceptibility and immunotherapy. Tissue Antigens 2003, 61, (3), 189-202.
[10] Seidel, U. J.; Schlegel, P.; Lang, P., Natural killer cell mediated antibody-dependent cellular cytotoxicity in tumor immunotherapy with therapeutic antibodies. Frontiers in immunology 2013, 4, 76.
[11] Raghavan, M.; Bjorkman, P. J., Fc receptors and their interactions with immunoglobulins. Annu Rev Cell Dev Biol 1996, 12, 181-220.
[12] Dillman, R. O., Monoclonal antibodies for treating cancer. Ann Intern Med 1989, 111, (7), 592-603.
[13] Oldham, R. K.; Dillman, R. O., Monoclonal antibodies in cancer therapy: 25 years of progress. J Clin Oncol 2008, 26, (11), 1774-7.
[14] McLaughlin, P.; Grillo-Lopez, A. J.; Link, B. K.; Levy, R.; Czuczman, M. S.; Williams, M. E.; Heyman, M. R.; Bence-Bruckler, I.; White, C. A.; Cabanillas, F.; Jain, V.; Ho, A. D.; Lister, J.; Wey, K.; Shen, D.; Dallaire, B. K., Rituximab chimeric anti-CD20 monoclonal antibody therapy for relapsed indolent lymphoma: half of patients respond to a four-dose treatment program. J Clin Oncol 1998, 16, (8), 2825-33.
[15] Metes, D.; Ernst, L. K.; Chambers, W. H.; Sulica, A.; Herberman, R. B.; Morel, P. A., Expression of functional CD32 molecules on human NK cells is determined by an allelic polymorphism of the FcgammaRIIC gene. Blood 1998, 91, (7), 2369-80.
[16] 16. Kim, D. H.; Jung, H. D.; Kim, J. G.; Lee, J. J.; Yang, D. H.; Park, Y. H.; Do, Y. R.; Shin, H. J.; Kim, M. K.; Hyun, M. S.; Sohn, S. K., FCGR3A gene polymorphisms may correlate with response to frontline R-CHOP therapy for diffuse large B-cell lymphoma. Blood 2006, 108, (8), 2720-5.
[17] Zhang, W.; Wang, X.; Li, J.; Duan, M. H.; Zhou, D. B., Fcgamma receptor IIIA polymorphisms and efficacy of rituximab therapy on Chinese diffuse large B-cell lymphoma. Chin Med J (Engl) 123, (2), 198-202.
[18] Warmerdam, P. A.; van de Winkel, J. G.; Gosselin, E. J.; Capel, P. J., Molecular basis for a polymorphism of human Fc gamma receptor II (CD32). J Exp Med 1990, 172, (1), 19-25.
[19] Ravetch, J. V.; Perussia, B., Alternative membrane forms of Fc gamma RIII(CD16) on human natural killer cells and neutrophils. Cell type-specific expression of two genes that differ in single nucleotide substitutions. J Exp Med 1989, 170, (2), 481-97.
[20] Asaoka, Y.; Hatayama, K.; Ide, T.; Tsumoto, K.; Tomita, M., The binding of soluble recombinant human Fcgamma receptor I for human immunoglobulin G is conferred by its first and second extracellular domains. Mol Immunol 2013, 54, (3-4), 403-7.
[21] Ierino, F. L.; Powell, M. S.; McKenzie, I. F.; Hogarth, P. M., Recombinant soluble human Fc gamma RII: production, characterization, and inhibition of the Arthus reaction. J Exp Med 1993, 178, (5), 1617-28.
[22] Sondermann, P.; Jacob, U.; Kutscher, C.; Frey, J., Characterization and crystallization of soluble human Fc gamma receptor II (CD32) isoforms produced in insect cells. Biochemistry 1999, 38, (26), 8469-77.
[23] Sanger, F.; Nicklen, S.; Coulson, A. R., DNA sequencing with chain-terminating inhibitors. Proceedings of the National Academy of Sciences of the United States of America 1977, 74, (12), 5463-7.
[24] Krause, M.; Ukkonen, K.; Haataja, T.; Ruottinen, M.; Glumoff, T.; Neubauer, A.; Neubauer, P.; Vasala, A., A novel fed-batch based cultivation method provides high cell-density and improves yield of soluble recombinant proteins in shaken cultures. Microb Cell Fact 9, 11.
[25] Panula-Perala, J.; Siurkus, J.; Vasala, A.; Wilmanowski, R.; Casteleijn, M. G.; Neubauer, P., Enzyme controlled glucose auto-delivery for high cell density cultivations in microplates and shake flasks. Microb Cell Fact 2008, 7, 31.
[26] Laemmli, U. K., Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 1970, 227, (5259), 680-5.
[27] Sinclair, G.; Choy, F. Y., Synonymous codon usage bias and the expression of human glucocerebrosidase in the methylotrophic yeast, Pichia pastoris. Protein Expr Purif 2002, 26, (1), 96-105.
[28] Jia, H.; Fan, G.; Yan, Q.; Liu, Y.; Yan, Y.; Jiang, Z., High-level expression of a hyperthermostable Thermotoga maritima xylanase in Pichia pastoris by codon optimization. Journal of Molecular Catalysis B: Enzymatic 2012, 78, 72-77.