Heterologous expression of Cysteine-rich Inter-Domain Region-1 (CIDR1) of *Plasmodium faciparum* erythrocyte protein 1 (P*f*EMP-1) in *Pichia pastoris*

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ABSTRACT

The aim of this study was to investigate the utility of a heterologous eukaryotic expression system, the yeast Pichia pastoris, for expression of one of the major structural domains of the modular and A T rich variant protein Plasmodium faciparum Erythrocyte Protein 1 (PfEMP1). The Cysteine-Rich Interdomain Region 1 (CIDR 1) of PfEMP1 was recodoned and expressed as a non-glycosylated recombinant protein of the expected molecular weight (28kDa). The immunoreactivity of the recombinant CIDR was evaluated using sera from Sudan and Ghana where malaria is endemic. Results from immunoblotting and immunofluorescence with rabbit polyclonal antibodies raised against the recombinant CIDR domain indicate that the Pichia expression system can be used for the expression of recodoned A T rich Plasmodial proteins. However, controlled fermentation is necessary to obtain to obtain large quantities of material for functional and structural analysis.

Key words: *CIDR1, DBLs, ELISA, His-Tagged Protein, Malaria vaccine, PfEMP1 Pichia pastoris, Plasmodium falciparum, recombinant protein and IFA.*

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INTRODUCTION

Malaria caused by *Plasmodium falciparum* remains a highly important parasitic disease even though it has been controlled in many previously endemic areas. About 40% of the world's population lives in areas of intense malaria transmission, mainly around the tropical and subtropical regions. Infection results in 1.1-2.7 million deaths per annum, the majority of which are amongst children under the age of five years (Baird, 1995 and 1998, WHO, 2005). Biologically, during the intra-erythrocytic cycle of the parasite, early immature developmental stages are present in the peripheral circulation. The more larger mature forms can adhere to the host endothelial surfaces and sequester, thus avoiding continuous circulation in the blood stream. This attachment is mediated mainly by P*f*EMP1 molecules. P*f*EMP1 proteins are highly immunogenic with a molecular mass of 200 to 400 kD. Each P*f*EMP1 contains 2-7 extracellular Duffy binding like (DBL) domains; one or two cysteine-rich interdomain

regions (CIDRs) distributed within the DBLs; a transmembrane region (TM) and an intracellular acidic segment (ATS). Each parasite clone appears to express a single P*f*EMP. (Roberts *et al.*, 1993; Smith, *et al.*, 2001). During the acquisition of clinical immunity to *falciparum* malaria, one target for immunoglobulins is the molecule PfEMP1. Antibodies to P*f*EMP1 show significant correlation with development of clinical immunity.*P. falciparum* is the major cause of human malaria morbidity and mortality .There is thus increasing recognition of the moral importance to create vaccines against diseases that primarily affect the poor in the under-developed world. There is therefore a need to find suitable expression systems for the production of malarial proteins required for basic pre-clinical and clinical vaccine research.

In spite of considerable research efforts, no effective vaccine against *P. falciparum* malaria is yet available. There is therefore an urgent need for basic pre-clinical and clinical research on vaccine development. This objective is not without scientific and technical problems. One obstacle in malaria vaccine development is that *P. falciparum* has one of the most A/T rich genomes known (typically 80% A/T rich genes and up to 85% A/T in its intragenic regions). Protein expression from A/T rich genomes is known to be highly problematic (Gardener *et al.*, 2002). Another obstacle is the codon usage as different organisms often show a strong preference for one of the numerous codons that encode the same amino acid in the 'degenerate' genetic code. One of the most recommended expression system is the *Pichia pastoris*, Phaff, 1956, is a methylotrophic budding yeast (Phylum: Ascomycota, Class: Hemiacomycetes, Order: Saccharomycetules, Family: Saccharomycetaceae). It is considered as non-conventional yeast compared with the conventional baker's yeast *S. cerevisiae* (Cereghino and Cregg, 1999). It seems to be most likely suitable for the production of malarial antigens required for diagnostic application or further characterisation, e.g. crystallisation and structure analysis. This system has a number of advantages for protein expression, including, the capability of performing many eukaryotic post-translational modifications, such as glycosylation, disulfide bond formation, and proteolytic processing. Eukaryotic proteins are produced in an active form and do not require refolding to give activity, as is the case for many eukaryotic proteins made in *Escherichia coli*. The system is relatively easy to scale up for bulk production particularly if the product can be secreted by the yeast cell (Cregg *et al.*, 1993; Sreekrishna *et al.*, 1997; Cereghino & Cregg, 1999, 2000). For these reasons the *Pichia* system therefore was chosen to express a domain of variant protein P*f*EMP1, namely, Cysteine-rich Interdomain Region (CIDR) domain. The reasons underlying this were that the CIDR region has been reported to encode a particular binding property, particularly that of binding to CD36 (Baruch *et al.*, 1995; Newbold *et al.*, 1997). Immunization of *Aotus* monkeys with the 'M2 region', a conserved motif of CIDR1 has elicited high titre anti-sera that were found to be primarily variant-specific. (Baruch *et al.*, 2003; Miller *et al.*, 2002). There are also reports that some variants of this region can mediate binding to CSA on the surface CHO cells (Degen *et al.*, 2000). The sequences of the CIDR1 domain have thus been considered to be potential vaccine candidates – a part of the so-called 'conserved head structure' of P*f*EMP-1 molecules (Gamain *et al.*, 2001). The aim of this work is then to determine whether the *Pichia* system can be used to produce large quantities of correctly folded recombinant CIDR1. Such conformationally correct antigens are needed in order to raise specific

antibodies to this antigen and to develop assays to assess its immunogenicity.

MATERIALS AND METHODS

Pichia pastoris expression vector pPICZαA

The *Pichia* expression vector pPICZαA (Invitrogen, Fig. 1) was used and it has been designed for heterologous expression and secretion of recombinant proteins. This expression vector carries a C-terminal His-tag sequence to allow purification and detection of fusion proteins. The 'tag' consists of six histidine residues to allow purification on nickel affinity resins. Other features of this plasmid include an inducible AOX1 promoter, α-factor secretion signal for transport/secretion of proteins and the zeocin resistance gene for selection in *E. coli* and yeast .

Codon-optimised version of CIDR1

The CIDR1 domain used in this study was derived from the 3D7 strain of *P. falciparum*. The P*f*EMP1 *var* gene containing this particular CIDR1 was classified as a type 5 var gene organised into DBLα, CIDRα, DBLβ, C2, DBLγ and CIDR-nonα domains (Gardener *et al.*, 2002). A similar but somewhat differently organised classification scheme has been proposed by other workers (Lavstsen *et al.*, 2003; Stemmer *et al.*, 1995). The CIDR used in this study was re-codoned (T. Fagin and P. Bruce) to match the codon usage of *P. pastoris* and cloned into the pPICZαA expression vector (Fig. 1). Potential N-glycosylation sites were also mutated. Re-synthesis involved removal of restriction sites that may interfere with downstream cloning techniques. Predicted N-glycoslyation sites (N- X- S/T- N) in the amino acid sequence were identified using the web-based program 'NetNGlyc' at http://www.cbs.dtu.dk/services/NetNGlyc/. The recodoned gene was ligated into the plasmid vector and the construct was amplified in and then extracted from *E.coli* cells of the XL-10 Gold type (Stratagene). Zeocin resistant clones were sequenced and PCR-screening was used to confirm the presence of inserts, using yeast alcohol oxidase gene specific primers (AOX3 and AOX5). Resulting recombinant plasmids were linearized before transfection by electroporation

Induction, fermentation and immuno-detection experiments

CIDR1-pPICZαA expression was monitored by small-scale, mid-scale and fermentation induction experiments. Clones were initially grown in BMGY with vigorous shaking. Cells were harvested and re-suspended in BMMY, and cultured for 3 days in total. Expression levels were maintained by the addition of methanol to the culture every 24 hours. The fermentation run consisted of the following stages: Glycerol Batch Phase – 20 hours, Glycerol Feed Batch phase for 2 hours, Starvation phase for 1 hour, Methanol Feed Batch for 70 hours and Harvest. Expression was optimized for production using a New Brunswick BioFlo5000 fermentor according to the recommended protocol (Invitrogen). The pH was maintained at 5 with 14% ammonium hydroxide and the temperature was maintained at 30°C. Other fermentation parameters were as follows: the dissolved-oxygen level was maintained by airflow of 90 litres per minute, the air supplemented with oxygen. Supernatants were reduced and separated by SDS-PAGE on 4-12% polyacrylamide gels. Following SDS-PAGE of supernatant electrophoresis, one gel was stained and the other was transferred to a PVDF membrane for immuno-detection. Membranes were probed with His-tag-HRP antibody. The blot was visualised with the ECL Plus detection kit (Amersham Biosciences). Proteins were stained using Coomassie blue .

Purification of His-Tagged protein

After bulk fermentation run, the supernatant was precipitated with ammonium sulfate, and the proteinaceous pellet was re-suspended in 1X PBS. One ml Ni-NTA Hi Trap chelating column was used for the purification of the recombinant CIDR1. His-tagged protein was eluted as recommended in the manufacturer's protocol. Samples were quantified by reading the absorbance at 280nm of the fractions after the BioRad DC protein assay had been carried out. Samples with the greatest concentration of protein were pooled and the elution buffer exchanged by dialysis against 1X PBS .

Glycosylation testing

In designing the synthetic CIDR1 DNA coding sequence, mutations have been introduced to the sequence to remove sites that are generally N-glycosylated by eukaryotic expression systems. The Pro-Q Fuchsia Glycoprotein Gel Stain kit (Molecular Probes) was used to test for glycosylation. The SDS-PAGE gel was washed with dd H_2O and incubated in an oxidizing solution (20% periodic acid). The gel was initially incubated in the Pro-Q Fuchsia reagent in the dark, followed by incubation in the metabisulphite reducing agent. After staining with the glycoprotein staining kit, the recombinant CIDR1 expressed in *Pichia* was compared with the positive control provided with the kit.

Reactivity of malaria infection serum with the *Pichia* produced CIDR1

To investigate whether humans mount an immune response following malaria infection to this recombinant derived CIDR1, an ELISA assay was set up using plasma samples collected in Sudan. Plate wells were coated with 0.5, 1 and 5 µg/ml of the recombinant CIDR1 in 100µl of coating buffer. Serum antibodies (dilution of 1:500 in blocking buffer, 1% skimmed milk powder in washing buffer) to the recombinant CIDR1 were measured using enzyme-linked immunosorbent assay (ELISA). This was carried out according to the procedure described by Cavanagh *et al.* 1998. The wells were incubated for 3 hours at room temperature with horseradish peroxidase-conjugated rabbit anti-human IgG (diluted 1:5000 in washing buffer, 0.05% Tween 20 in PBS). The wells were incubated for 15 min at room temperature with the substrate (0.1 mg ml-1 O-Phenylendiamine +0.012% H_2O_2) in developing buffer. The reaction was stopped by addition of 25µl 2M H_2SO_4 and OD measured at 492nm. MSP119 was used as positive control and measure of that individual serum's reactivity to a defined *P. falciparum* merozoite antigen .

To further test the antigenicity, the CIDR1 protein was tested against pools of antisera from a more highly endemic malaria transmission zone. The serum consisted of a 'male serum pool' of 20 serum samples from men living in a malaria endemic region of Ghana. The 'pregnant female serum pool' consists of pooled serum from the placenta of 15 Ghanaian women, collected at delivery. Six Scottish malaria naïve individuals donated sera to create the 'malaria negative pool' serum .

Rabbit immunization to produce anti-CIDR1 antibodies

Immunisations experiments using the CIDR recombinant product were carried out by the Harlan Bio-Sera lab Company according to their rabbit immunisation protocols. The antigen concentrations required for each immunization was 50 - 200µg administered with complete Freund's adjuvant to ensure a high quality/quantity response. This was followed by three booster injections of 100 µg at days 14, 28, and 56 with Freund's incomplete adjuvant. Antibodies were purified on protein A columns. In ELISA, affinity

purified rabbit anti-CIDR1 immunoglobulin reacted strongly with the *Pichia* product, as they did in Western blot (the pre-immune rabbit was the negative control). Both ELISA and IFA assays tested antisera obtained 4 weeks after the last injection of adjuvanted protein for reactivity were carried out.

IFA assays using anti-CIDR1 antibody

Rabbit sera from the immunization study were tested for reactivity to *P. falciparum* 3D7 parasite(from which the *var* gene that contains the CIDR1 domain was isolated) infected RBCs. IFA was carried out on acetone-fixed iRBCs, infected with the 3D7 parasite. The corresponding rabbit pre-immune sera were used as a negative control. Panels of well-characterised monoclonal antibodies to the late trophozoites and MSP1 on the surface of the merozoites namely, mAbs 2.2, mAbs 7.5, mAbs 12.8, and mAbs 12.10 (Dr. Jana McBride, Edinburgh University, José *et al.*, 1997) were used as positive controls.

RESULTS AND DISCUSSION

Advances in biotechnology (culture techniques, genetic engineering, Malaria Genomics), make a vaccine against malaria more feasible now than ever before. The lines of biological evidence that suggest that a malaria vaccine is possible are that people living in endemic areas who have been multiply exposed to malaria can develop immunity against sever disease (Baird, 1995). Demonstration that protection against malaria can be induced by infecting volunteers with irradiated malaria sporozoites has been reported (Egan, 2002). Antibodies purified from life long residents of endemic areas can be transferred into other individuals and confer some protection against the effects of malaria infection (Sabchareon *et al.*, 1991). Immunization studies show that the vaccines already in hand can protect against malaria infection in animal models and humans. Anti-sporozoites vaccines based on cicumsporozoite protein provided consistent protection in humans (Kester *et al.*, 2001). Protection has been achieved in new world monkeys of genus *Aotus* and *Saimiri* against blood stage by vaccination with the antigen merozoite surface protein1 (MSP1) (Stowers *et al.*, 2002). Against this there can be difficulties. These include: the complex life cycle of malaria parasites provide a number of targets for vaccination, and the parasite has evolved a series of strategies that allow it to confuse, hide and misdirect the immune system. Identifying which antigenic components are to be included is a problematic issue and the richness of *P. falciparum* in it's A+T makes it essential to find an optimal expression system. (Gardener *et al.*, 2002). A major obstacle is the codon usage as different organisms often show a strong preference for one of the numerous codons that encode the same amino acid in the 'degenerate' genetic code. Expression in heterologous systems is usually up-regulated and driven by a strong promoter and this increases the likelihood of 'draining the tRNA pool' for uncommon tRNAs. This could lead to premature chain termination and affect protein folding (Sreekrishna *et al.*, 1997; Sorensen *et al.*, 1989; Pizzi and Frontali, 2001). The codon bias problem, *E. coli* expression, has been addressed by Stratagene who have engineered novel bacterial expression lines (BL21-CodonPlus® RPIL line). This has had success in allowing over-expression of A/T codon rich Plasmodium genes (Makoff *et al.*, 1994 and 1989). However, this has not removed the need to synthesise a codon-optimized version of the sequence of interest (Yadava and Ockenhouse, 2003). The methylotrophic eukaryotic expression host, *P. pastoris*, has been successfully developed as an alternative to the *E. coli* system (Hollenberg and Gellessien,

1997: Cereghino and Cregg, 2000). The codon bias has not been addressed in the case of the *Pichia* expression system so recodoning is always needed.

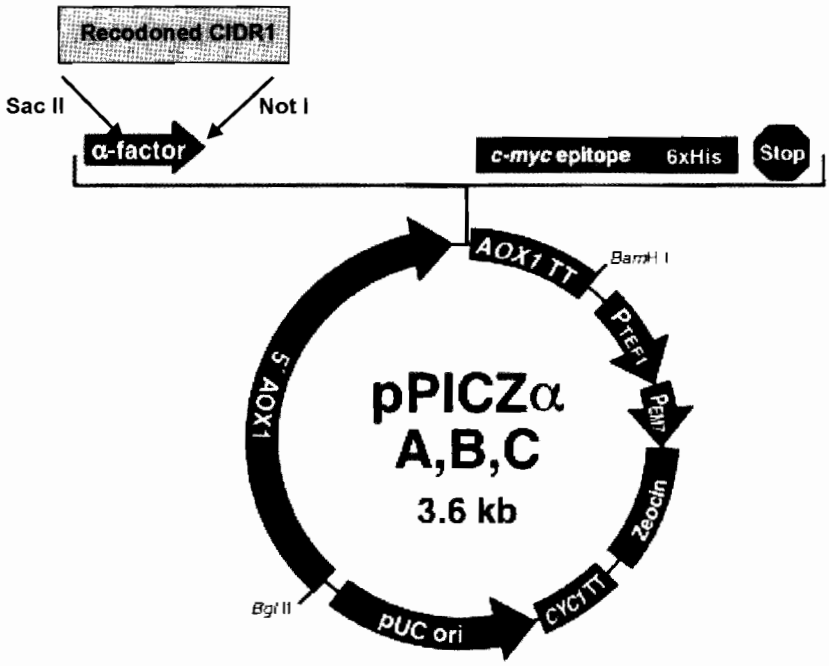

Fig.(1): The pPICZαA construct used for expression of the secreted version of the synthetic CIDR1. pPICZαA makes use of the AOX1 promoter and it has α-factor signal sequence for secretion of the expressed protein. It carries Zeocin resistance for selection of both yeast and E. coli in antibiotic media.

Recodoning of CIDR1 was successfully obtained and the resulting recombinant insert was sequenced. The native and recodoned sequences are compared in Fig. (2). Hyper-glycosylation can cause antigen hyper-immunogenicity which can be lethal in experimental animals. The serine (S) or threonine (T) in the motif was replaced with an alanine (A), as it is the smallest amino acid, predicted to have the minimum effect on secondary structure. It is appropriate to remove the N-glycosylation sites as *P. pastoris* is known to glycosylate (and sometimes hyper-glycosylate) at asparagine residues and can therefore interfere with correct folding and tertiary structure of the protein (Bretthauer and Castellino 1999). In general, taxonomically close organisms use similar codons for their protein synthesis whereas taxonomically distant organisms tend to utilize different codons (Ikemura, 1982). All heterologous expression systems thus experimented with, apart from Dictyostelium (Howard *et al.*, 1988) and Tetrahymena (Peterson *et al.*, 2002) have more G+C rich codon-usage than *P. falciparum*. Condon optimization has proven successful for the expression of other malaria genes (Nagata *et al.*, 1999; Narum *et al.*, 2001). The production of recombinant *P. falciparum* proteins in *Pichia* has been shown in MSP-1 (Morgan *et al.*, 2004) and AMA-1 (Kocken *et al.*, 2002), Listeria monocytogenes (Uchijima *et al.*, 1998), tetanus (Stratford *et al.*, 2001) and HIV (Deml *et al.*, 2001).

Small-scale and mid-scale expression of CIDR1 in *P. pastoris* resulted in secretion of protein of the expected size showing little degradation (Fig. 3). The hexa-histidine tagged protein was detected using an antibody specific for the histidine tag.

Fig. (2): Nucleotide Sequence alignment of native and codon-optimised CIDR1. * indicates unchanged nucleotides while gaps indicate where substitutions have been made. The A+T content was reduced from 67.6% to 48.6%.

The positive control used in Western blot analysis of the *Pichia* derived recombinant protein was a His–tagged protein, serpin, a ~55KD *Brugia malayi* serine protease inhibitor expressed in *E. coli*. When the induction was scaled up, a secreted band of the expected molecular weight (28 KDa) was detected by Western blotting. Controlled fermentation is the most effective way for the recombinant protein to be expressed in the *Pichia* system because the parameters for maximally effective expression can be met. These include control of the temperature, dissolved oxygen, constant pH, agitation, aeration and methanol concentration. A time dependent product of the expected MW

(~28kDa) was consistently produced after 24 hours. This product was confirmed as CIDR1 in Western Blot using antibodies to the polyhistidine tag. Detection of CIDRI during fermentation peaked and then decreased with time, suggesting that some degradation was taking place. Measures were taken to protect and maintain the integrity of the *Pichia* expressed protein; protease inhibitors were added to supernatants to prevent degradation and to protect against aggregation. Some evidence of aggregation was observed in case of the *Pichia* supernatants analysed here, which quickly took on a cloudy appearance. Aggregates are common in the production of other P*f*EMP-1 domains and this makes their handling and analyses problematic (Dr. Graham Bentley, Institute Pasteur, personal communication). Estimated purified recombinant protein yields were 50 mg/litre (Fig. 4). These results confirm our expectation that the controlled fermentation is a much more effective method for the expression of the recombinant protein than induction in flasks. These results indicate that the *Pichia* expression system expresses recombinant CIDR1 protein, although controlled fermentation is necessary to obtain good quantities of material for functional and structural analysis. Using this system, a time dependent product of the expected MW (~28kDa) was consistently produced (Fig.4).

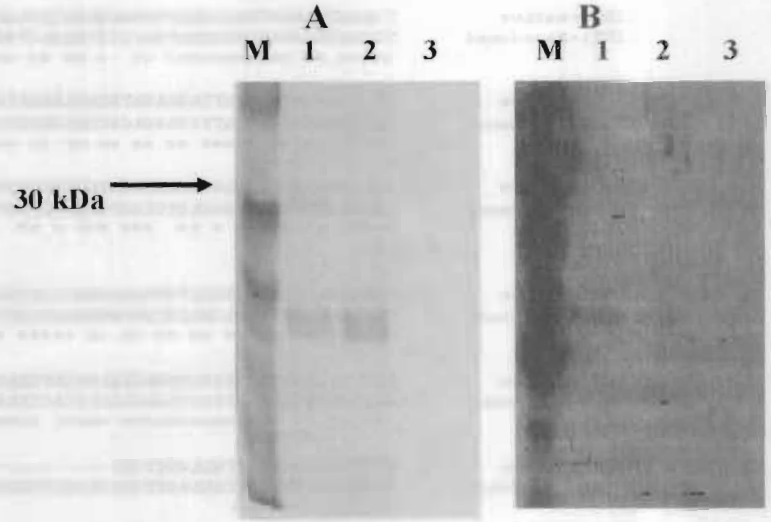

Fig. (3): Analysis of Pichia supernatants from small-scale cultures .A - Coomassie blue stained polyacrylamide gel. M - Molecular weight marker. 1 - P. pastoris culture supernatant, 24 hours after methanol induction. 2 - Culture supernatant after 48 hours of induction. 3 - Culture supernatant prior to induction. B - Duplicate gel analysed by Western blot using the anti- His tag antibody.

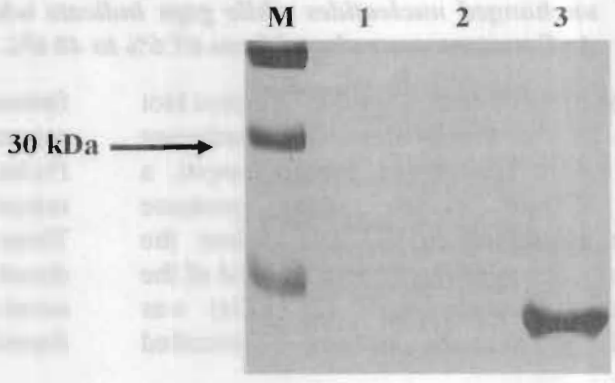

Fig. (4):Coomassie stained polyac - rylamide gel showing the results obtained after two-step purification of the recombinant CIDR1. Lane M is MW marker (Bio-Rad). 1 and 2 are early fractions and lane 3 is the protein after elution .

As shown in Fig. (5), no glycosylation of the 28-kD was detected although some glycosylated high molecular weight material appears to be present in the supernatant. For expression in *P. pastoris*, the codons of *P. falciparum* antigens need to be optimized and potential glycosylation sites have to be removed. Stowers *et al.* (2002) evaluated glycosylated and unglycosylated forms of *P. falciparum* MSP142 expressed in the milk of transgenic mice has been evaluated as an erythrocytic stage malaria vaccine designed to prevent invasion of RBCs by merozites. The glycosylated product was expressed at higher levels and was easier to purify, making it a seemingly preferable vaccine candidate to the non-glycosylated version. In the *Aotus* challenge model, the gycosylated form of MSP142 gave no protection, whilst the non-glycosylated form protected monkeys from lethal challenge with *P. falciparum* (Stowers *et al.*, 2002).

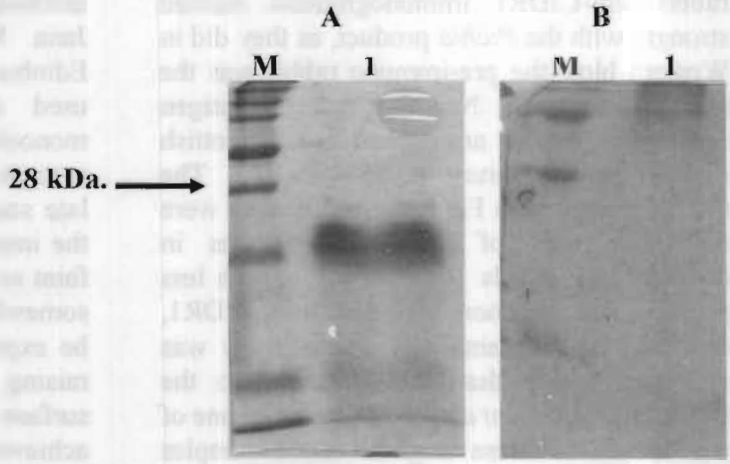

Fig. (5): ***A 15 % SDS-PAGE Coomassie stained gel of recombinant CIDR1 (A) and its duplicate gel (B) after glycoprotein staining. Lane M. Molecular weight marker. Lane 1. Purified proteins stained by Coomassie stain (A) and its duplicate stained with glycoprotein staining kit (B) to detect both N-linked and O-linked oligosaccharides. The positive controls in the marker in gel (B) stained with the Pro-Q Fuschia reagent.***

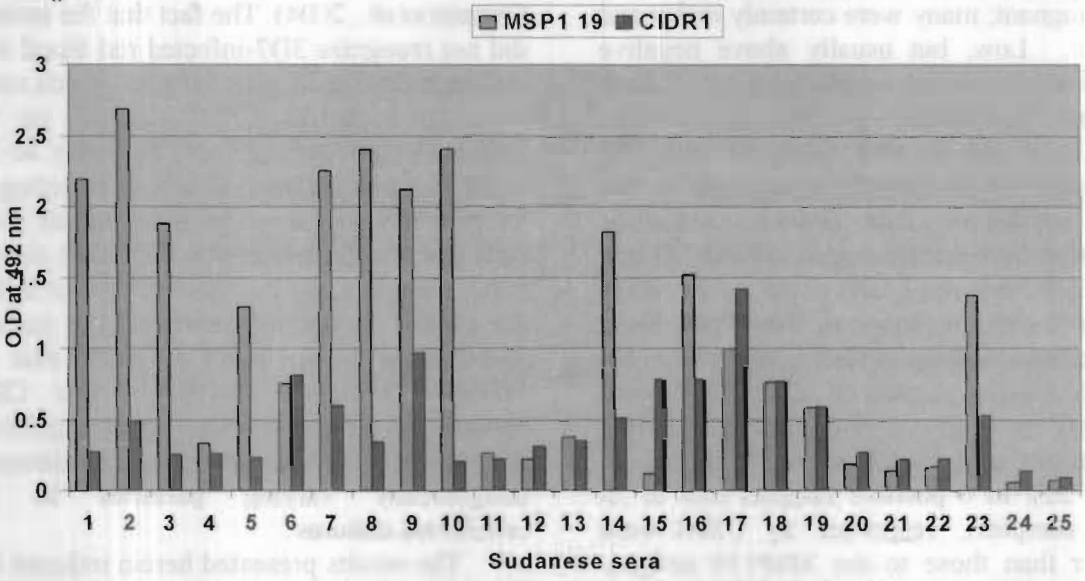

Fig. (6): Detection of recombinant CIDR1 and MSP-119 by individual Sudanese acute infection sera. Columns 1-24 show individual Sudanese sera reacted with the two malaria antigens coated to microtitre plate wells. Column 25 is the Scottish malaria naïve serum pool reaction with these antigens .

The affinity purified CIDR1 protein was used in ELISA using sera from malaria endemic areas and comparing this with the reaction obtained with malaria native sera using an MSP119 control. There were different levels of reactivity; the MSP-1 was often recognised more strongly than the P*f*EMP-1 derived product. Following rabbit immunizations an anti-CIDR antiserum was also tested for reactivity to *P. faciparum* infected RBCs. In ELISA, affinity purified rabbit anti-CIDR1 immunoglobulin reacted strongly with the *Pichia* product, as they did in Western blot (the pre-immune rabbit was the negative controls). No such malaria antigen reactive antibodies are present in the Scottish malaria native serum pools (Fig. 6). The results presented in Fig.6 show that there were different levels of reactive antibodies in different individuals. As MSP119 is both less polymorphic and more abundant than CIDR1, this probably explains why its reactivity was generally higher than that observed to the CIDR. (Cavanagh *et al.*, 1998). Few or none of the donors of these original blood samples were pregnant; many were certainly males and children. Low, but usually above negative control responses were made by most of these Sudanese individuals to the recombinant CIDR1. In one or two cases (9 and 17), moderately high antibody responses to the CIDR1 antigen are made. There is no absolute correlation between responses to MSP119 and the P*f*EMP1 derived CIDR domain; but where there is a clear response to the CIDR, there tends to be a medium to strong response to the MSP119 antigen. A ratio of 7/25 samples gave very low or negative responses to the two recombinant antigens (serum 4, 11, 12, 13, 20, 21 and 22). In 6 positive samples (out of 25 tested samples), responses to CIDR1were stronger than those to the MSP119 antigen. When the P*f*EMP-1 CIDR1 derived antigens were probed with both the male and the pregnant female serum pools sera, there was strong a reaction with both sera, again stronger to MSP-1, but no gender specific reactivity was observed. This was not expected in any case, as neither protein has been implicated in placental adhesion (Data not shown).

Immunoflorecence assay (IFA) was performed using the rabbit purified anti-CIDR1 antibodies and panel of previously characterised cross-reactive monoclonal antibodies to iRBC surface (Gifted from Dr Jana McBride and Dr David Cavanagh, Edinburgh University, José *et al.*, 1997) was used as a positive control. Whilst the monoclonal antibody controls strongly recognized acetone-fixed RBCs infected with late stage 3D7 *P. falciparum* strain parasites, the immunised rabbit sera showed either very faint or no recognition at all. This finding was somewhat disappointing although probably to be expected as it is commonly observed that raising polyclonal antibodies to the iRBC surface antigens is difficult and has been achieved by few groups (Chen *et al.*, 2004; Gamain *et al.*, 2004). The fact that the antisera did not recognize 3D7-infected red blood cells indicates that we have no reagent, which reacts with this specific CIDR domain on the infected erythrocyte surface. This may be due to the fact that we have no way of selecting for an enriched population of infected red blood cells specifically expressing the CIDR domain that we have raised the antisera against or that the CIDR1 is not expressed on the surface. Thus, there is no direct evidence that the PfEMP-1 molecule containing this CIDR antigen is on the surface of a significant proportion, or indeed any of the continuously antigenically varying parasites in our unselected cultures .

The results presented herein indicate that the *Pichia* expression system can express a *P.*

falciparum CIDR-domain derived cysteine-rich recombinant protein fragment. The quantity and quality of product obtained are notably improved after performing a scaled-up automated fermentation run. This product was provisionally confirmed as the intended CIDR product following in Western blot using antibodies to the engineered poly-histidine tag epitope .

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الملخص العربي

استخدام خلايا الخميرة من النوع *Pichia pastoris* المعاد برمجتها وراثيا لإنتاج انتجينات لطفيلي الملاريا من النوع *Plasmodium flaciparrum*

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كان الهدف من هذه الدراسة التحقق من فائدة الخميرة من النوع *Pichia pastoris* ككائن حقيقي النواة لإنتاج البروتينات المعاد تركيبها لطفيلي الملاريا من النوع *Plasmodium falciparum* . تم استخدام هذه الخميرة لإنتاج واحد من الانتيجينات الرئيسية و الغنية المحتوى من القواعد النيتروجينية A+T وهو الجزء المسمى Cysteine-Rich Interdomain Region (CIDR1) احد مكونات البروتين المتغير على سطح كريه الدم الحمراء المصابة بطفيلي الملاريا والمسمى *Plasmodium faciparum* Erythrocyte Protein 1 (P*f*EMP1). وقد أعيد تشفير هذا الجزئ recodoned وعبر عنه جينيا وانتج كانتيجين خالي من السكريات وبوزن الجزيئي (kDa٢٨). تم اختبار مدى انتيجينية immunoreactivity هذا المنتج المعاد تركيبه باستخدام امصال من السودان وغانا التي يتوطن فيها مرض الملاريا. ان النتائج المتحصل عليها هنا من اختبار immunoblotting و اختبار immunofluorescence باستخدام الأجسام المضادة عديدة النسيلة polyclonal من أرانب تم حقنها بانتيجين CIDR المعاد تركيبه تشير إلى أن الخميرة من النوع *Pichia pastoris* يمكن استخدامها للتعبير عن الانتيجينات والبروتينات الغنية المحتوى من القواعد النيتروجينية A+T كما هو الحال في طفيلي الملاريا. وللحصول على كميات كبيرة منتجة من الانتيجينات والبروتينات واللازمة للدراسات والتركيبة والوظيفية فانه ينصح بعملية تخمير متحكم في كل الظروف المواتية لإنتاجها بالشكل الوظيفي المطلوب.