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(54) Title: PEPTIDES INCLUDING BINDING DOMAIN OF PLASMODIUM FALCIPARUM PROTEINS (CBP1 AND CBP2) TO CHEMOKINE CX3CL1

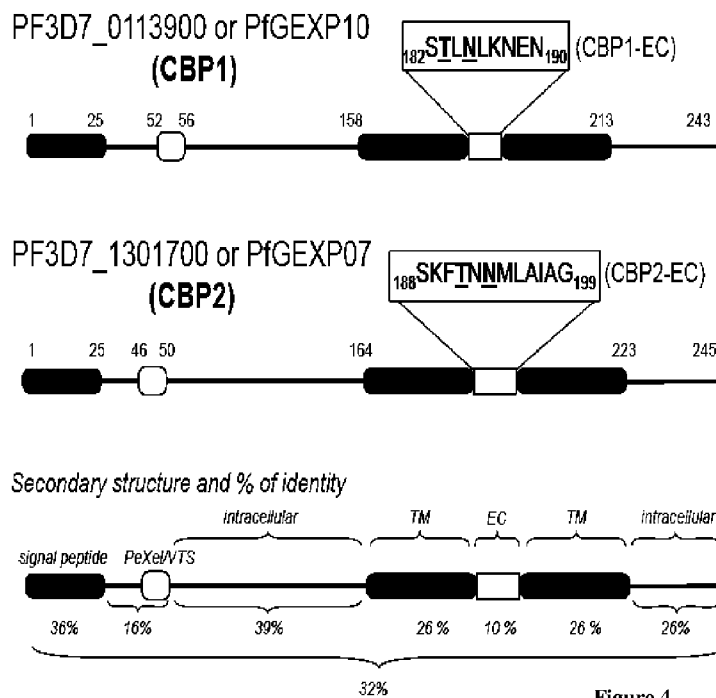


Figure 4

(57) Abstract: The invention relate to isolated peptides including binding domain of the *Plasmodium Falciparum* proteins (CBP1 and CBP2) to the chemokine CX3CL1 which has the property to inhibit the cytoadherence of infected red blood cells (iRBC) to endothelial cells. These isolated peptides (or their specific antibody) are used for the prevention or the treatment of Malaria and also acting as "CX3CL1 binding protein" for the treatment of inflammatory diseases or CX3CL1 -associated disease.



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**PEPTIDES INCLUDING BINDING DOMAIN OF PLASMODIUM FALCIPARUM
PROTEINS (CBP1 AND CBP2) TO CHEMOKINE CX3CL1**

FIELD OF THE INVENTION:

The invention relates to isolated peptides including binding domain of two *Plasmodium Falciparum* proteins (CBP1 and CBP2) to the chemokine CX3CL1 which have the property to inhibit the cytoadherence of infected red blood cells (iRBC) to endothelial cells. These isolated peptides (or their specific antibody) are used for the prevention or the treatment of Malaria and also acting as “CX3CL1 binding protein” for the treatment of inflammatory diseases or CX3CL1 associated diseases.

BACKGROUND OF THE INVENTION:

As the major causative agent of severe malaria, *Plasmodium falciparum* is responsible for the bulk of malaria-related mortality worldwide (WHO, 2011). Clinical manifestations of severe malaria result from a combination of high parasite burdens and sequestration of mature *P. falciparum*-infected red blood cells (iRBCs) in microvascular beds throughout the body, a phenomenon described more than a century ago and commonly referred to as cytoadherence (Bignami & Bastiannelli, 1890). Cytoadherence of iRBC causes obstruction of blood flow in small brain blood vessels - thereby contributing to cerebral malaria - and in the villous chamber of the placenta, leading to pregnancy-associated malaria (Brabin et al, 2004; Bray & Sinden, 1979). Sequestration of iRBC causes microvascular obstruction, leads to metabolic disturbances (Planche et al, 2005; Zougbede et al, 2010), and allows iRBC to escape splenic clearance, which is a selective advantage for the microorganism (Buffet et al, 2009; Mebius & Kraal, 2005).

The emergence of drug resistant strains has compounded the problem of treating the malaria disease. Unfortunately, no FDA-approved vaccine exists. Thus, there is a continued need to develop novel vaccines and therapies for treating malaria infection.

Numerous parasite molecules of *P. falciparum* have been identified as ligands for cytoadherence; however, only the surface antigen variant family called “*Plasmodium falciparum* Erythrocyte Membrane Protein 1” (*PfEMP1*) that is encoded by the *var* genes, is demonstrated as a bona fide adherence molecule (Smith et al, 2013). Conversely, several host molecules have been identified as receptors for iRBC adherence to human endothelium,

including CD36, ICAM1, P-selectin, thrombospondin, CSA (chondroitin sulfate A), and protein C receptor (Kirchgatter & Del Portillo, 2005; Rowe et al, 2009; Turner et al, 2013).

Importantly, clinical data have indicated that cytoadherence of iRBC may involve other molecules that have not yet been identified (Rowe et al, 2009). For example, iRBC
5 adhere to the chemokine CX3CL1 (Hatabu et al, 2003) and a recent study rules out the intervention of PfEMP1 in the adherence phenotype to CX3CL1 (Janes et al., 2011). So the P. *falciparum* ligand to CX3CL1 remains unknown.

Chemokines, or chemotactic cytokines, are secreted soluble molecules that are expressed by numerous cell types of the immune system, either constitutively or following the
10 induction of inflammatory responses. Chemokines have indeed played a pivotal role in recruitment, activation, and retention of immune and non-immune cells and specifically, their infiltration or egression at inflamed sites and immune organs (Baggiolini, 1998; Viola & Luster, 2008).

Chemokine receptors are essentially G-protein Coupled Receptors (GPCR), members
15 of the Rhodopsin class (Moser & Loetscher, 2001). The chemotactic activity of chemokines involves both activation of cell movement as well as adherence on various support. While chemokines generally operate through cell adherence by inducing or activating classical adhesive molecules like integrins (Moser & Loetscher, 2001; Rot & Von Andrian, 2004), CX3CL1 is an exception since it is natively expressed as a transmembrane protein that is
20 endowed with an adhesive function unique among chemokines. During inflammation, CX3CL1 is expressed by numerous endothelial cell types, including the cerebral (Tarozzo et al, 2002; Zujovic et al, 2000) and trophoblastic (Cammass et al, 2005; Shimoya et al, 2003) lineages.

Inflammation is the body's immediate response to damage to its tissues and cells by
25 pathogens, noxious stimuli such as chemicals, or physical injury. (U.Weiss, Nature Insight, 2008, Vol. 454, Issue no. 7203). Though this phenomenon is extremely important for the accomplishment of healing processes, dysregulated inflammation can have severe consequences.

Inflammation can be divided into two forms: acute inflammation, which is a short term
30 response, and chronic inflammation, which is a dysregulated and maladaptive reaction highly detrimental for the host. It is now well established that chronic inflammation is involved in the development of several diseases among which are cardiovascular diseases, obesity, diabetes, asthma, inflammatory bowel diseases, allergies and rheumatoid arthritis (Durgaprasad L. et al, Inflammation & Allergy- Drug Targets, 2013, 12, 349-361). Acute

inflammation is considered as mainly beneficial in the healing process, but when it is uncontrolled, it can lead to severe pathologies as for example the Systemic Inflammatory Response Syndrome (SIRS) and sepsis which are both common causes of morbidity and death in intensive care units (Fitting C. et al, PloS one 7.6, 2012, e38916). Nonsteroidal anti-inflammatory (NSAIDs) are the most commonly used anti-inflammatory drugs but have several side effects among which are gastrointestinal ulcers and bleeding (H. Süleyman et al., Pharmacological Reports, 2007, 59, 247-258).

Thus, there is a continued need to develop novel drugs and therapies for treating inflammatory disorders.

The inventors of the present invention have discovered that, surprisingly, two *P. falciparum* proteins named CBP1 and CBP2 which are involved in CX3CL1-mediated cytoadherence via a binding domain with CX3CL1, and accordingly, peptides derived from this binding domain, would represent a promising target of malaria infection but also for inflammation.

SUMMARY OF THE INVENTION:

The invention provides an isolated peptide comprising an amino acid sequence of formula (I):

Xaa1-Xaa2-Xaa3-Threonine -Xaa4-Xaa5-Xaa6-Xaa7-Xaa8-Xaa9-Xaa10-Xaa11

, wherein :

Xaa1 is serine (S) or absent;

Xaa2 is lysine (K), or absent;

Xaa3 is Phenylalanine (F) or serine (S);

Xaa4 is **asparagine (N), leucine (L)**, alanine (A), aspartic acid (D) or glutamic acid (E);

Xaa5 is **asparagine (N)**, alanine (A) or aspartic acid (D);

Xaa6 is methionine (M) or leucine (L);

Xaa7 is leucine (L) or lysine (K);

Xaa8 is alanine (A), asparagine (N) or aspartic acid (D),

Xaa9 is isoleucine (I) or glutamic acid (E);

Xaa10 is **alanine (A), asparagine (N) or** aspartic acid (D).

Xaa11 is Glycine (G) or absent;

In preferred embodiments, Xaa5 is **asparagine (N)**. In preferred embodiments, Xaa1 is serine (S) or absent, Xaa2 is lysine (K), or absent, Xaa3 is serine (S) or Phenylalanine (F), Xaa4 is leucine (L) or asparagine (N), Xaa5 is **asparagine (N)**, Xaa6 is leucine (L), or methionin (M), Xaa7 is lysine (K) or leucine (L), Xaa8 is alanine (A) or asparagine (N),
5 Xaa9 is glutamic acid (E) or isoleucine (I), Xaa10 is **asparagine (N)**, **alanine (A)**, Xaa11 is Glycine (G), or absent.

In a preferred embodiment, the isolated peptide comprises or consists of the following amino acid sequences

10 STLNLKNEN (SEQ ID NO:1) or
SKFTNNMLAIAG (SEQ ID NO:2)

The invention further relates to antibodies generated against the isolated peptides of the invention.

15 The invention further relates to a pharmaceutical and vaccinal composition comprising an isolated peptide of the invention, together with a pharmaceutically acceptable carrier, and to the use of the peptides or the pharmaceutical composition according to the invention for treating or preventing Malaria or inflammatory disease and disease related to CX3CL1.

20 DETAILED DESCRIPTION OF THE INVENTION:

Using a proteomic approach, inventors have identified two *P. falciparum* proteins involved in CX3CL1-mediated cytoadherence. These proteins - named CBP1 and CBP2 for CX3CL1-Binding-Proteins – were previously identified as potential surface proteins of the early gametocytes and called *P. falciparum* Gametocyte-Exported Proteins (PfGEXP10 and
25 PfGEXP07) (Silvestrini et al, 2010). They have no other known function, share 32 % of their residues, and contain a signal peptide and an export motif called “Plasmodium export element” (Pexel) (Marti et al, 2004) or Vacuolar Transport Signal (VTS) (Hiller et al, 2004). They have no homology with other proteins from *P. falciparum*, have no ortholog in other *Plasmodium* species and, unlike PfEMP1, PfMC-2TM, RIFIN or STEVOR (Lavazec et al,
30 2006), are coded by single non-polymorphic genes. CBPs are expressed at the surface of iRBC and mediate the cytoadherence of *P. falciparum*-infected RBC to mammalian cells, a process that can be inhibited by peptides and anti-CBP antibodies.

The inventors have identified peptides sequence of the binding domain of CBP1 and CBP2 to the chemokine CX3CL1, corresponding to their extracellular domain (CBP1-EC and

CBP2-EC), which has the property to inhibit the cytoadherence of infected red blood cells (iRBC) to endothelial cells (CBP1 and CBP2, previously known as PfGEXP10 and PfGEXP07, sequences deposited in PlasmoDB database under accession number **PF3D7_0113900 and PF3D7_1301700**). These binding domains of 9 and 12 amino-acids residues correspond to amino acid positions 182-190 of PfGEXP10 for CBP1-EC and to amino acid positions 188-199 of PfGEXP07 for CBP2-EC. These binding domains are able to inhibit the cytoadherence of *P. falciparum*-infected RBC to mammalian cells and consequently to treat Malaria disease. The inventors have obtained that these peptide CBP1-EC and CBP2-EC (and their specific antibody directed against CBP1-EC and CBP2-EC) competes with the native protein CBP1 and CBP2 for binding to the CX3CL1 chemokine.

So inventor's work identified new ligands for the human chemokine CX3CL1 and discovered a non-polymorphic family of proteins in *P. falciparum* involved in the cytoadherence, thus opening the way to an innovative vaccination approach. The inventors have thus found a new targeted therapy for treating malaria infection by the *Plasmodium falciparum* with no available targeted therapy.

Isolated peptides

The invention relates to novel isolated peptides derived from the extracellular domain of CBP1 and CBP2 proteins of *Plasmodium Falciparum*, which have capacity to inhibit the interaction between CBP1 or CBP2 of *Plasmodium Falciparum* with CX3CL1 and/or to inhibit the cytoadherence of infected red blood cells (iRBC) to endothelial cells;.

In one aspect, the invention provides an isolated peptide, comprising an amino acid sequence of formula (I):

Xaa1-Xaa2-Xaa3-Threonine -Xaa4-Xaa5-Xaa6-Xaa7-Xaa8-Xaa9-Xaa10-Xaa11

, wherein :

Xaa1 is serine (S) or absent;

Xaa2 is lysine (K), or absent;

Xaa3 is Phenylalanine (F) or serine (S);

Xaa4 is **asparagine (N)**, **leucine (L)**, alanine (A), aspartic acid (D) or glutamic acid (E);

Xaa5 is **asparagine (N)**, alanine (A) or aspartic acid (D);

Xaa6 is methionine (M) or leucine (L);

Xaa7 is leucine (L) or lysine (K);

Xaa8 is alanine (A) asparagine (N) or aspartic acid (D).

Xaa9 is isoleucine (I) or glutamic acid (E);

Xaa10 is **alanine (A), asparagine (N) or** aspartic acid (D).

5 Xaa11 is Glycine (G) or absent;

10 In preferred embodiments, Xaa5 is **asparagine (N)**. In preferred embodiments, Xaa1 is serine (S) or absent, Xaa2 is lysine (K), or absent, Xaa3 is serine (S) or Phenylalanine (F), Xaa4 is leucine (L) or asparagine (N), Xaa5 is **asparagine (N)**, Xaa6 is leucine (L), or methionine (M), Xaa7 is lysine (K) or leucine (L), Xaa8 is alanine (A), asparagine (N), Xaa9 is glutamic acid (E) or isoleucine (I), Xaa10 is **asparagine (N), alanine (A)**, Xaa11 is Glycine (G), or absent.

15 In a specific embodiment, the isolated peptide excludes the whole sequence of CBP1 and CBP2 proteins (PfGEXP10 (SEQ ID NO:9) and PfGEXP07 (SEQ ID NO:10)).

In particular embodiment, the invention provides an isolated peptide selected from the group comprising or consisting of:

- 20 i) the amino acids sequence consisting of STLNLKNEN (SEQ ID NO:1) ;
- ii) the amino acids sequence consisting of SKFTNNMLAIAG (SEQ ID NO:2);
- iii) the amino acids sequence consisting of STLALKAEA (SEQ ID NO:3)
- iv) the amino acids sequence consisting of STLDLKDED (SEQ ID NO:4)
- v) the amino acids sequence consisting of SKFTAAMLAIAG (SEQ ID
- 25 NO:5);
- vi) the amino acids sequence consisting of SKFTDDMLAIAG (SEQ ID NO:6);
- vii) an amino acid sequence substantially homologous to the sequence of (i), to (vi) preferably an amino acid a sequence at least 80% identical to the
- 30 sequence of (i) to (vi).

In a preferred embodiment, the isolated peptide comprises or consists of the following amino acid sequences STLNLKNEN (SEQ ID NO:1) or SKFTNNMLAIAG (SEQ ID NO:2)

As used herein, the term “amino acid” refers to natural or unnatural amino acids in their D and L stereoisomers for chiral amino acids. It is understood to refer to both amino acids and the corresponding amino acid residues, such as are present, for example, in peptidyl structure. Natural and unnatural amino acids are well known in the art. Common natural amino acids include, without limitation, alanine (Ala), arginine (Arg), asparagine (Asn), aspartic acid (Asp), cysteine (Cys), glutamine (Gln), glutamic acid (Glu), glycine (Gly), histidine (His), isoleucine (Ile), leucine (Leu), Lysine (Lys), methionine (Met), phenylalanine (Phe), proline (Pro), serine (Ser), threonine (Thr), tryptophan (Trp), tyrosine (Tyr), and valine (Val). Uncommon and unnatural amino acids include, without limitation, allyl glycine (AllylGly), norleucine, norvaline, biphenylalanine (Bip), citrulline (Cit), 4-guanidinophenylalanine (Phe(Gu)), homoarginine (hArg), homolysine (hLys), 2-naphthylalanine (2-Nal), ornithine (Orn) and pentafluorophenylalanine.

Amino acids are typically classified in one or more categories, including polar, hydrophobic, acidic, basic and aromatic, according to their side chains. Examples of polar amino acids include those having side chain functional groups such as hydroxyl, sulfhydryl, and amide, as well as the acidic and basic amino acids. Polar amino acids include, without limitation, asparagine, cysteine, glutamine, histidine, selenocysteine, serine, threonine, tryptophan and tyrosine. Examples of hydrophobic or non-polar amino acids include those residues having nonpolar aliphatic side chains, such as, without limitation, leucine, isoleucine, valine, glycine, alanine, proline, methionine and phenylalanine. Examples of basic amino acid residues include those having a basic side chain, such as an amino or guanidino group. Basic amino acid residues include, without limitation, arginine, homolysine and lysine. Examples of acidic amino acid residues include those having an acidic side chain functional group, such as a carboxy group. Acidic amino acid residues include, without limitation aspartic acid and glutamic acid. Aromatic amino acids include those having an aromatic side chain group. Examples of aromatic amino acids include, without limitation, biphenylalanine, histidine, 2-naphthylalanine, pentafluorophenylalanine, phenylalanine, tryptophan and tyrosine. It is noted that some amino acids are classified in more than one group, for example, histidine, tryptophan and tyrosine are classified as both polar and aromatic amino acids. Amino acids may further be classified as non-charged, or charged (positively or negatively) amino acids. Examples of positively charged amino acids include without limitation lysine, arginine and histidine. Examples of negatively charged amino acids include without limitation glutamic

acid and aspartic acid. Additional amino acids that are classified in each of the above groups are known to those of ordinary skill in the art.

The term CBP1-EC and CBP2-EC and Seq1 and Seq2 used in the present application are equivalent.

5 Preferably, an isolated peptide according to the invention has the capacity (i) to inhibit the interaction between CBP1 or CBP2 of *Plasmodium Falciparum* with CX3CL1; and/or (ii) to inhibit the cytoadherence of infected red blood cells (iRBC) to endothelial cells and/or CX3CL1.

10 The skilled in the art can easily determine whether isolated peptide is biologically active. For example, the capacity to inhibit the interaction between CBP1 or CBP2 protein of *Plasmodium Falciparum* with CX3CL1 can for example be determined by any routine test well known by the man skills in the art : Bimolecular fluorescence complementation (BiFC), affinity electrophoresis, label transfer, yeast two-hybrid, phage display, co-immunoprecipitation, microcalorimetry, Surface Plasmon Resonance, binding assay with
15 radioactive ligand.

The capacity to inhibit the cytoadherence of infected red blood cells (iRBC) to endothelial cells and/or CX3CL1 can for example be determined by using Static Adherence assay and or/ Flow adherence assay (e.g. as described in Example + Figures 1 and 2).

20 As used herein, a "biologically active" fragment refers to a fragment exhibiting at least one, preferably all, of the biological activities of a peptide of SEQ ID NO:1 or SEQ ID NO:2, provided the biologically active fragment retains the capacity of inhibiting cytoadherence of infected red blood cells (iRBC) to endothelial cells. The biologically active fragment may for example be characterized in that it is capable of inhibiting the interaction between CBP1 or CBP2 protein of *Plasmodium Falciparum* with CX3CL1 and/or inhibiting
25 the cytoadherence of infected red blood cells (iRBC) to endothelial cells and/or CX3CL1 (see Example and Figures 3b,c).

A peptide "substantially homologous" to a reference peptide may derive from the reference sequence by one or more conservative substitutions. Two amino acid sequences are
30 "substantially homologous" or "substantially similar" when one or more amino acid residue are replaced by a biologically similar residue or when greater than 80 % of the amino acids are identical, or greater than about 90 %, preferably greater than about 95%, are similar

(functionally identical). Preferably, the similar, identical or homologous sequences are identified by alignment using, for example, the GCG (Genetics Computer Group, Program Manual for the GCG Package, Version 7, Madison, Wisconsin) pileup program, or any of the programs known in the art (BLAST, FASTA, etc.). The percentage of identity may be
5 calculated by performing a pairwise global alignment based on the Needleman-Wunsch alignment algorithm to find the optimum alignment (including gaps) of two sequences along their entire length, for instance using Needle, and using the BLOSUM62 matrix with a gap opening penalty of 10 and a gap extension penalty of 0.5.

The term "conservative substitution" as used herein denotes the replacement of an
10 amino acid residue by another, without altering the overall conformation and function of the peptide, including, but not limited to, replacement of an amino acid with one having similar properties (such as, for example, polarity, hydrogen bonding potential, acidic, basic, shape, hydrophobic, aromatic, and the like). Amino acids with similar properties are well known in the art. For example, arginine, histidine and lysine are hydrophilic-basic amino acids and may
15 be interchangeable. Similarly, isoleucine, a hydrophobic amino acid, may be replaced with leucine, methionine or valine. Neutral hydrophilic amino acids, which can be substituted for one another, include asparagine, glutamine, serine and threonine.

By "substituted" or "modified" the present invention includes those amino acids that have been altered or modified from naturally occurring amino acids.

20 As such, it should be understood that in the context of the present invention, a conservative substitution is recognized in the art as a substitution of one amino acid for another amino acid that has similar properties.

According to the invention a first amino acid sequence having at least 80% of identity
25 with a second amino acid sequence means that the first sequence has 80; 81; 82; 83; 84; 85; 86; 87; 88; 89; 90; 91; 92; 93; 94; 95; 96; 97; 98; or 99% of identity with the second amino acid sequence. Amino acid sequence identity is preferably determined using a suitable sequence alignment algorithm and default parameters, such as BLAST P (Karlin and Altschul, 1990).

30 In some embodiments, the isolated peptide of the invention comprises at most 100 aminoacid (and at least 9). In some embodiments, the polypeptide of the invention comprises 100; 99; 98; 97; 96; 95; 94; 93; 92; 91; 90; 89; 88; 87; 86; 85; 84; 83; 82; 81; 80; 79; 78; 77; 76; 75; 74; 73; 72; 71; 70; 69; 68; 67; 66; 65; 64; 63; 62; 61; 60; 59; 58; 57; 56; 55; 54; 53; 52; 51; 50; 49; 48; 47; 46; 45; 44; 43; 42; 41; 40; 39; 38; 37; 36; 35; 34; 33; 32; 31; 30; 29;

28; 27; 26; 25; 24; 23; 22; 21; 20; 19; 18; 17; 16; 15; 14; 13; 12; 11; 10 or 9 amino acids. In some embodiments, the polypeptide of the invention comprises less than 50 amino acids. In some embodiments, the polypeptide of the invention comprises less than 30 amino acids. In some embodiments, the polypeptide of the invention comprises less than 25 amino acids. In
5 some embodiments, the polypeptide of the invention comprises less than 20 amino acids. In some embodiments, the polypeptide of the invention comprises less than 15 amino acids.

The N- and C-termini of the peptides described herein may be protected against proteolysis. For instance, the N-terminus may be in the form of an acetyl group, and/or the C-terminus may be in the form of an amide group. Internal modifications of the peptides to be
10 resistant to proteolysis are also envisioned, e.g. wherein at least a -CONH peptide bond is modified and replaced by a (CH₂NH) reduced bond, a (NHCO) retro-inverso bond, a (CH₂-O) methylene-oxy bond, a (CH₂-S) thiomethylene bond, a (CH₂CH₂) carba bond, a (CO-CH₂) cetomethylene bond, a (CHOH-CH₂) hydroxyethylene bond), a (N-N) bound, a E-alcene bond or also a -CH=CH-bond. The peptides described herein may also be protected
15 against proteolysis by the technique of stapled peptides as described by Walensky et al. (Science. 2004, 305, 1466-70).

In another aspect of the invention, peptides are covalently bound to a polyethylene glycol (PEG) molecule by their C- terminus or a lysine residue, notably a PEG of 1500 or 4000 MW, for a decrease in urinary clearance and in therapeutic doses used and for an
20 increase of the half-life in blood plasma. In yet another embodiment, peptide half-life is increased by including the peptide in a biodegradable and biocompatible polymer material for drug delivery system forming microspheres. Polymers and copolymers are, for instance, poly (D, L-lactide-co-glycol ide) (PLGA) (as illustrated in US2007/0184015, SoonKap Hahn et al).

25 The isolated peptides according to the invention may be produced using any method known in the art. They may for example be produced as recombinant peptides in a host cell (e.g. in a bacterial, yeast or eukaryotic host cell), or chemically synthesized (see for review Kent S.B.H. Chem. Soc. Rev., 2009,38, 338-351 and Bradley L. et al Annu Rev Biophys
30 Biomol Struct. 2005; 34: 91-118 or R. B. Merrifield (1969). "Solid-phase peptide synthesis." Advances in enzymology and related areas of molecular biology 32: 221-96.; R. B. Merrifield (1969). "The synthesis of biologically active peptides and proteins." JAMA 210(7): 1247-54. and Raibaut, L., O. El Mahdi and O. Melnyk (2015). "Solid Phase Protein Chemical Synthesis." Topics in current chemistry).

In a particular embodiment, isolated peptides of the invention can be conformationally constrained to enable the peptides to bind to CX3CL1 with a better affinity.

Cyclisation is well known in the art and generally involves the introduction of a disulfide bond between two cysteine residues. Typically, the cycle is formed through a side chain to side chain ring involving a monosulfide or disulfide bridge between pairs of cysteines, penicillamines, homocysteines, combinations of the foregoing, or other pairs of amino acids in which the side chains are linked with either one or two sulfur atoms. Methods for the synthesis of disulfide cyclic polypeptide are well known in the art and are described for example in US3,929,758, US4,216,141; and US4,102,877. Polypeptides of the invention may thus comprise cysteine residues at terminal ends to allow the cyclisation of the polypeptide.

Accordingly, isolated peptides of the invention may comprise an amino acid sequence of formula II consisting of:

Cysteine-PepX1-Xaa1-Xaa2-Xaa3-Threonine-Xaa4-Xaa5-Xaa6-Xaa7-Xaa8-Xaa9-Xaa10-Xaa11- PepX2 -Cysteine

, wherein :

Xaa1 is serine (S) or absent;

Xaa2 is lysine (K), or absent;

Xaa3 is Phenylalanine (F) or serine (S);

Xaa4 is **asparagine (N), leucine (L)**, alanine (A), aspartic acid (D) or glutamic acid (E);

Xaa5 is **asparagine (N)**, alanine (A) or aspartic acid (D);

Xaa6 is methionine (M) or leucine (L);

Xaa7 is leucine (L) or lysine (K);

Xaa8 is alanine (A), asparagine (N) or aspartic acid (D);

Xaa9 is isoleucine (I) or glutamic acid (E);

Xaa10 is **alanine (A), asparagine (N) or aspartic acid (D)**.

Xaa11 is Glycine (G) or absent;

- "PepX1" and "PepX2" each consists of, one independently from the other, a spacer polypeptide having an amino acid length varying from 0 to 10 amino acid residues,

In a particular embodiment, the isolated peptide of the invention comprises an amino acid sequence consisting of CSTLNLKNENC (SEQ ID NO: 11) and CSKFTNNMLAIAGC (SEQ ID NO: 12)

5 In another particular embodiment, the polypeptides as above described are cyclised via a disulfide bound between the two cysteine residues of the polypeptides.

Accordingly, the cyclic peptide of the invention may comprise an amino acid sequence having the formula (III):

10

C-PepX1-Xaa1-Xaa2-Xaa3-T-Xaa4-Xaa5-Xaa6-Xaa7-Xaa8-Xaa9-Xaa10-Xaa11-PepX2-C

, wherein:

15

Xaa1 is serine (S) or absent;

Xaa2 is lysine (K), or absent;

Xaa3 is Phenylalanine (F) or serine (S);

Xaa4 is **asparagine (N), leucine (L)**, alanine (A), aspartic acid (D) or glutamic acid (E);

20

Xaa5 is **asparagine (N)**, alanine (A) or aspartic acid (D);

Xaa6 is methionine (M) or leucine (L);

Xaa7 is leucine (L) or lysine (K);

Xaa8 is alanine (A), asparagine (N) or aspartic acid (D).

Xaa9 is isoleucine (I) or glutamic acid (E);

25

Xaa10 is **alanine (A), asparagine (N) or** aspartic acid (D).

Xaa11 is Glycine (G) or absent;

- "PepX1" and "PepX2" each consists of, one independently from the other, a spacer polypeptide having an amino acid length varying from 0 to 10 amino acid residues,

30

In a preferred embodiment, the cyclic polypeptide comprises an amino acid sequence consisting of:

CSTLNLKNENC (SEQ ID NO: 13) and CSKFTNNMLAIAGC (SEQ ID NO: 14)

Cyclisation can also be performed by peptide bonds between the amino-terminal of the first amino acid and the carboxy-terminal of the last amino acid.

Other methods of cyclization are contemplated by the invention. For example, those methods include but are not limited by those described by Marlowe (1993, *Biorg. Med. Chem. Lett.* 3:437-44) who describes peptide cyclization on TFA resin using trimethylsilyl (TMSE) ester as an orthogonal protecting group; Pallin and Tam (1995, *J. Chem. Soc. Chem. Comm.* 2021-2022) who describe the cyclization of unprotected peptides in aqueous solution by oxime formation; Algin et al (1994, *Tetrahedron Lett.* 35:9633-9636) who disclose solid-phase synthesis of head-to-tail cyclic peptides via lysine side-chain anchoring; Kates et al (1993, *Tetrahedron Lett.* 34:1549-1552) who describe the production of head-to-tail cyclic peptides by three-dimensional solid phase strategy; Tumelty et al (1994, *J. Chem. Soc. Chem. Comm.* 1067-1068) who describe the synthesis of cyclic peptides from an immobilized activated intermediate, wherein activation of the immobilized peptide is carried out with N-protecting group intact and subsequent removal leading to cyclization; McMurray et al (1994, *Peptide Res.* 7:195-206) who disclose head-to-tail cyclization of peptides attached to insoluble supports by means of the side chains of aspartic and glutamic acid; Hruby et al (1994, *Reactive Polymers* 22:231-241) who teach an alternate method for cyclizing peptides via solid supports; and Schmidt and Langer (1997, *J. Peptide Res.* 49:67-73) and those described by Davies JS (The cyclisation of peptides and depsipeptides *J Pept Sci* 2003,8:471-501); and Li and Roller (PPCyclisation strategies in peptide derived drug design. *Curr. Tp Med. Chem.* 2002, 3:325-41) and Boll, E., J. P. Ebran, H. Drobecq, O. El-Mahdi, L. Raibaut, N. Ollivier and O. Melnyk (2015). "Access to large cyclic peptides by a one-pot two-peptide segment ligation/cyclization process." *Organic letters* 17(1): 130-3.

Antibodies according to the invention

The inventors have generated specific antibodies directed against a peptide derived from the CBP1 and CBP2 protein (CBP1-EC and CBP2-EC), i.e. the isolated peptide of formula (I).

The anti-CBP1 and anti-CBP2 polyclonal antibodies were performed by immunizing rabbit with two synthetic peptides, LSTLNKKNEN (SEQ ID NO:7) and AATSKFTNNMLAIAGVG (SEQ ID NO:8) respectively. More precisely, the inventors have found that antibodies screened for their capacity to recognize specifically the isolated peptide of the invention (example 2 and figure 5A) and to stain iRBC samples from a Malaria patient.

It has also been found that an antibody directed against an isolated peptide of formula (I) above, inhibits iRBC binding to CX3CL1 (example 2 and figure 6A).

The invention provides an antibody that specifically binds to an isolated peptide of formula I.

5 The invention further provides an antibody that specifically binds to an isolated peptide comprising or consisting:

- i) the amino acids sequence consisting of STLNLKNEN (SEQ ID NO:1) ;
- ii) the amino acids sequence consisting of SKFTNNMLAIAG (SEQ ID NO:2);
- 10 iii) the amino acids sequence consisting of STLALKAEA (SEQ ID NO:3)
- iv) the amino acids sequence consisting of STLDLKDED (SEQ ID NO:4)
- v) the amino acids sequence consisting of SKFTAAMLAIAG (SEQ ID NO:5);
- vi) the amino acids sequence consisting of SKFTDDMLAIAG (SEQ ID NO:6);
- 15 vii) an amino acid sequence substantially homologous to the sequence of (i), to (vi) preferably an amino acid a sequence at least 80% identical to the sequence of (i) to (vi).

20 These antibodies can recognize an epitope located within, or comprising at least one amino acid located within, the fragment comprising or consisting of any one of isolated peptide (i) to (vii).

Preferably, said epitope is located within the fragment comprising or consisting of any one of isolated peptide (i) to (vii).

25 Most preferably said epitope is located within the fragment comprising or consisting of any one of (i) to (vii). Such antibodies are characterized in that they specifically bind to CBP1-EC and CBP2-EC

These antibodies can advantageously be used when selecting a patient to be treated by a peptide comprising CBP1-EC and CBP2-EC or a biologically active fragment thereof, e.g.
30 using the method described hereabove.

These antibodies can be polyclonal or monoclonal. When the antibodies are monoclonal, they can for example correspond to chimeric, humanized or fully human antibodies.

Methods for obtaining such antibodies are well known in the art. For example, polyclonal antibodies according to the invention can be obtained through immunization of a non-human mammal with said fragment comprising or consisting of any one of (i) to (vii). Starting from the polyclonal antibodies, one can then obtain monoclonal antibodies using standard methods.

This invention is also directed to the use of an antibody directed against an isolated peptide of formula (I) for manufacturing a pharmaceutical composition for treating malaria disease linked to the infection of an individual with plasmodium falciparum

Pharmaceutical compositions

The invention further relates to a pharmaceutical composition comprising a peptide of the invention, a cyclic peptide of the invention, or antibody of the invention together with a pharmaceutically acceptable carrier.

More particularly, the invention relates to a pharmaceutical composition comprising an isolated peptide of the invention or an antibody of the invention, together with a pharmaceutically acceptable carrier.

The peptide or antibody is formulated in association with a pharmaceutically acceptable carrier.

As used herein, the term "pharmaceutically acceptable" and grammatical variations thereof, as they refer to compositions, carriers, diluents and reagents, are used interchangeably and represent that the materials are capable of administration to or upon a mammal without the production of undesirable physiological effects such as nausea, dizziness, gastric upset and the like.

The preparation of a pharmacological composition that contains active ingredients dissolved or dispersed therein is well understood in the art and need not be limited based on formulation. Typically such compositions are prepared as injectables either as liquid solutions or suspensions; however, solid forms suitable for solution, or suspensions, in liquid prior to use can also be prepared. The preparation can also be emulsified. In particular, the pharmaceutical compositions may be formulated in solid dosage form, for example capsules, tablets, pills, powders, dragees or granules.

The choice of vehicle and the content of active substance in the vehicle are generally determined in accordance with the solubility and chemical properties of the active compound, the particular mode of administration and the provisions to be observed in pharmaceutical practice. For example, excipients such as lactose, sodium citrate, calcium carbonate,

dicalcium phosphate and disintegrating agents such as starch, alginic acids and certain complex silicates combined with lubricants such as magnesium stearate, sodium lauryl sulphate and talc may be used for preparing tablets. To prepare a capsule, it is advantageous to use lactose and high molecular weight polyethylene glycols. When aqueous suspensions are used they can contain emulsifying agents or agents which facilitate suspension. Diluents such as sucrose, ethanol, polyethylene glycol, propylene glycol, glycerol and chloroform or mixtures thereof may also be used.

The dosing is selected by the skilled person so that a anti-infectious effect is achieved, and depends on the route of administration and the dosage form that is used. Total daily dose of a peptide administered to a subject in single or divided doses may be in amounts, for example, of from about 0.001 to about 100 mg/kg body weight daily and preferably 0.01 to 10 mg/kg/day. Dosage unit compositions may contain such amounts of such submultiples thereof as may be used to make up the daily dose. It will be understood, however, that the specific dose level for any particular patient will depend upon a variety of factors including the body weight, general health, sex, diet, time and route of administration, rates of absorption and excretion, combination with other drugs and the severity of the particular disease being treated.

Vaccine composition

As inventors identified new ligands for the human chemokine CX3CL1 and discovered a non-polymorphic family of proteins in *P. falciparum* involved in the cytoadherence, thus opening the way to an innovative vaccination approach.

Accordingly, another object of the invention is a vaccine composition comprising an isolated peptide of formula (I) or a cyclic peptide of formula (III) and an immunoadjuvant compound.

By “vaccine composition” it is herein intended a substance which is able to induce an immune response in an individual, and for example to induce the production of antibodies directed against the isolated peptide of formula (I).

A vaccine is defined herein as a biological agent which is capable of providing a protective response in an animal to which the vaccine has been delivered and is incapable of causing severe disease. The vaccine stimulates antibody production or cellular immunity against the pathogen causing the disease; administration of the vaccine thus results in immunity from the disease.

Preferably, said immunoadjuvant compound is selected in the group consisting of Freund complete adjuvant, Freund incomplete adjuvant, aluminium hydroxide, and calcium phosphate.

In order to enhance the immunogenicity of the vaccine composition according to the invention, the peptide of formula (I) or (III), can comprise from 2 to 12 peptides of formula (I) or (III), for instance “STLNLKNEN” (SEQ ID NO:1) or SKFTNNMLAIAG (SEQ ID NO:2). or CSTLNLKNENC (SEQ ID NO: 13) and CSKFTNNMLAIAGC(SEQ ID NO: 14)

In particular, said antigenic polypeptide, can have the following formula (IV) :

NH₂-PepNt-[(I)n-PepXn]_n-PepCt- COOH (III),

wherein :

- “PepNt” consists of a polypeptide having an amino acid length varying from 0 to 100 amino acid residues and located at the N-terminal end of the polypeptide of formula (IV);

- “[I)n-PepXn]” consists of a polypeptide unit wherein :

- “(I)1” to - “(I)n” each consists of, one independently from each other, a peptide of formula (I) and/or (III) “ with n being an integer from 1 to 12; and

- “PepX1” to “PepXn” each consists of, one independently from the other, a spacer polypeptide having an amino acid length varying from 0 to 30 amino acid residues, with n being an integer from 1 to 12; and

- n is the number of [(I)n-PepXn] polypeptide units in said polypeptide, with n being an integer from 1 to 12; and

- “PepCt” consists of a polypeptide having an amino acid length varying from 0 to 100 amino acid residues and located at the C-terminal end of the polypeptide of formula (IV).

In particular, embodiment, peptide (I)1 to (I)n of formula (I) or (III), are selected between the group of “STLNLKNEN” (SEQ ID NO:1) and/or “SKFTNNMLAIAG” (SEQ ID NO:2) and/or CSTLNLKNENC (SEQ ID NO: 13) and CSKFTNNMLAIAGC(SEQ ID NO: 14).

In particular, said antigenic isolated peptide, can have the following formula (I) (III) or (IV) as defined above.

More particularly, said antigenic isolated peptide, comprising or consisting:

- i) the amino acids sequence consisting of STLNLKNEN (SEQ ID NO:1) ;

- ii) the amino acids sequence consisting of SKFTNNMLAIAG (SEQ ID NO:2);
- iii) the amino acids sequence consisting of STLALKAEA (SEQ ID NO:3)
- iv) the amino acids sequence consisting of STLDLKDED (SEQ ID NO:4)
- 5 v) the amino acids sequence consisting of SKFTAAMLAIAG (SEQ ID NO:5);
- vi) the amino acids sequence consisting of SKFTDDMLAIAG (SEQ ID NO:6);
- vii) the amino acids sequence consisting of CSTLNLKNENC (SEQ ID NO: 13)
- 10 viii) the amino acids sequence consisting of CSKFTNNMLAIAGC (SEQ ID NO: 14);
- ix) an amino acid sequence substantially homologous to the sequence of (i), to (viii) preferably an amino acid a sequence at least 80% identical to the
- 15 sequence of (i) to (viii).

Said antigenic peptide can be covalently linked through an amino acid residue to a carrier protein or a synthetic polymer.

In order to enhance peptide immunogenicity, the peptide of formula (I) can be covalently linked ("conjugated") to a larger molecule which serves as a carrier.

20

Attachment of the peptide to the carrier can be by one of several methods, including linking through a peptide Lys using glutaraldehyde (Reichlin, Methods Enzymol. 70: 159-165, 1980) or DCC procedures (for example, Atassi et al., Biochem. Biophys. Acta 670: 300-302, 1981), through a Peptide Asp or Glu using DCC (Bauminger et al., Methods Enzymol 25 70: 151-159, 1980), through a peptide Tyr using bis-diazotized benzidine (Walter et al., Proc. Nat. Acad. Sci. USA 77: 5197-5200, 1980), through photochemical attachment sites (Parker et al., Cold Spring Harbor Symposium - Modern Approaches to Vaccines, Ed. Chanock & Lerner, Cold Spring Harbor Press, New York, 1984), or through a peptide Cys (Liu et al., Biochem. 18: 690-697, 1979).

30 Peptide carrier conjugates can be separated from excess free peptide by dialysis or gel filtration. The level of loading of the peptide on the carrier can be determined either using a radioactive tracer to establish the loading level in a particular procedure, or by quantitative amino acid analysis of the conjugate, in comparison with the unloaded carrier. It is convenient, when using the latter technique, to incorporate a unique non-natural amino acid

into the peptide, at the N-terminal or C-terminal side, such as Nle, which can then serve as a quantitative marker for peptide incorporation, as measured by amino acid analysis of the conjugate. This Nle can also function as a spacer between the antigenic site and any amino acid incorporated to facilitate attachment, such as Cys, Lys, or Tyr, as described above.

5 Preferably, said carrier protein is selected from the group consisting of keyhole limpet hemocyanin (KLH), bovine serum albumin, or diphtheria toxoid.

In a vaccine composition according to the invention, said synthetic polymer can be a multiple branch peptide construction comprising a core matrix comprised of lysine residues.

10 Radially branched systems using lysine skeletons in polymers have been used by J. P. Tam [Proc. Natl. Acad. Sci. U.S.A., 85, 5409-5413 (1988)] to develop antigens without the use of carriers. Those antigens were designed to generate vaccines against a variety of diseases. Specifically, antigens for generating vaccines against malaria disease are described in PCT patent application ser. no. WO93/10152, WO2006/029887, WO2007/003384, WO2009/021931 and WO2009/080715, WO2015038708

15 The core matrix is preferably a dendritic polymer which is branched in nature, preferably with each of the branches thereof being identical. The core matrix is based on a core molecule which has at least two functional groups to which molecular branches having terminal functional groups are covalently bonded. Exemplary for use to form the core matrix is lysine. A central lysine residue is bonded to two lysine residues, each through its carboxyl
20 group, to one of the amino groups of the central lysine residue. This provides a molecule with four amino groups, which may be a core matrix for a structure comprising four peptides of formula (I). The manufacture of the above structures, is known in the art. See, e.g., Tam et al., J. Immun. 148, 914-920 (1992) and Wang et al., Science, 254, 285-288 (1991).

25 Additionally, spacers between said peptide and said carrier protein or synthetic polymer can be added. A peptide linker sequence may be employed to separate the first and second polypeptide components by a distance sufficient to ensure that each polypeptide folds into its secondary and tertiary structures. Such a peptide linker sequence is incorporated into the fusion protein using standard techniques well known in the art. Suitable peptide linker sequences may be chosen based on the following factors: (1) their ability to adopt a flexible
30 extended conformation; (2) their inability to adopt a secondary structure that could interact with functional epitopes on the first and second polypeptides; and (3) the lack of hydrophobic or charged residues that might react with the polypeptide functional epitopes. Preferred peptide linker sequences contain Gly, Asn and Ser residues. Other near neutral amino acids, such as Thr and Ala may also be used in the linker sequence. Amino acid sequences which

may be usefully employed as linkers include those disclosed in Maratea et al., Gene 40:39-46, 1985; Murphy et al., Proc. Natl. Acad. Sci. USA 83:8258-8262, 1986; U.S. Pat. No. 4,935,233 and U.S. Pat. No. 4,751,180. The linker sequence may generally be from 1 to about 50 amino acids in length. Linker sequences are not required when the first and second polypeptides have non-essential N-terminal amino acid regions that can be used to separate the functional domains and prevent steric interference.

The invention concerns also a vaccine composition comprising a peptide comprising the amino acid sequence STLNLKNEN (SEQ ID NO:1) or SKFTNNMLAIAG (SEQ ID NO:2), and/or CSTLNLKNENC (SEQ ID NO: 13) and CSKFTNNMLAIAGC(SEQ ID NO: 14).

said peptide, being covalently linked through an amino acid residue to a carrier protein or to a synthetic polymer.

Preferably, said carrier protein is selected from the group consisting of keyhole limpet hemocyanin (KLH), bovine serum albumin, or diphtheria toxoid.

The synthetic polymer can be a multiple branch peptide construction comprising a core matrix comprised of lysine residues. Spacers between said polypeptide and said carrier protein or synthetic polymer can be introduced.

Preferably, in the vaccine composition cited immediately above, there are spacers between said polypeptide and said carrier protein or synthetic polymer.

Therapeutic applications

A) Malaria

The isolated peptide as defined above, the antibody of the invention, the pharmaceutical and vaccine composition of the invention may be used for treating Malaria Disease.

The invention thus also relates to a peptide, or an antibody of the invention for use for treating Malaria Disease.

As used herein, the term “*Malaria*,” is a mosquito-borne infectious disease of humans and other animals caused by parasitic protozoans (a group of single-celled microorganism) belonging to the genus *Plasmodium*. [1] Malaria causes symptoms that typically include fever, fatigue, vomiting and headaches. In severe cases it can cause yellow skin, seizures, coma or death [Caraballo H (2014). Emergency Medicine Practice 16 (5)]. The disease is transmitted by the biting of mosquitos, and the symptoms usually begin ten to fifteen days after being bitten. In those who have not been appropriately treated disease may recur months later [1].

Clinical manifestations of severe malaria result from a combination of high parasite burdens and sequestration of mature *P. falciparum*-infected red blood cells (iRBCs) in microvascular beds throughout the body, a referred to as cytoadherence (Bignami & Bastiannelli, 1890). Cytoadherence of iRBC causes obstruction of blood flow in small brain blood vessels - thereby contributing to cerebral malaria - and in the villous chamber of the placenta, leading to pregnancy-associated malaria (Brabin et al, 2004). Sequestration of iRBC causes microvascular obstruction, leads to metabolic disturbances (Planche et al, 2005; Zougbede et al, 2010), and allows iRBC to escape splenic clearance, which is a selective advantage for the microorganism (Buffet et al, 2009; Mebius & Kraal, 2005).

In particular, the isolated peptide, or the antibody of the invention may have the ability to decrease the parasite load in a subject of at least 40%, 50%, 60%, 70%, 80%, 90% or 100%.

The invention also provides a method of treatment of a Malaria disease in a patient in need thereof, which method comprises administering said patient with an isolated peptide, or antibody of the invention.

The term “patient” or “subject” refers to a human or non-human mammal, preferably a mouse, cat, dog, monkey, horse, cattle (i.e. cow, sheep, goat, buffalo), including male, female, adults and children.

As used herein, the term “treatment” or “therapy” includes curative and/or prophylactic treatment. More particularly, curative treatment refers to any of the alleviation, amelioration and/or elimination, reduction and/or stabilization (e.g., failure to progress to more advanced stages) of a symptom, as well as delay in progression of a symptom of a particular disorder. Prophylactic treatment refers to any of: halting the onset, reducing the risk of development, reducing the incidence, delaying the onset, reducing the development, as well as increasing the time to onset of symptoms of a particular disorder.

B) Inflammation disease

CX3CL1 and its receptor CX3CR1 play a major role in numerous inflammatory processes. The CX3CR1/CX3CL1 axis is known to contribute to the recruitment of monocytes to inflammatory sites [Fong AM et al 1998, Auffray C et al 2009, Poupel L et al 2013] to sequester monocytes in the bone marrow (BM) [Jacquelin S et al 2013] and to induce macrophage survival [Kim K-W et al 2011].

The CBP1-EC and CBP1-EC peptides correspond to the external domain of the two invariant parasite ligands of CX3CL1, were assayed as “neutraligands”, i.e. molecules binding the ligand CX3CL1 to inhibit its binding to its receptor CX3CR1.

As show in example 3 inventors tested here the 2 peptides in two preclinical models of non-infectious peritonitis, a short-term one (LPS-induced) and a long term one (thioglycolate-induced)

Thus, the present invention relates to a isolated peptide of the invention or an cyclic peptide of invention for use in the treatment of an inflammatory disease.

As used herein, the terms “inflammatory disease” or “inflammatory disorder” relates to a complex reaction of vascularized tissue to infection, toxin exposure, or cell injury that involves extravascular accumulation of plasma proteins and leukocytes (Abbas et al., Cellular and Molecular Immunology, 7th edition, 2011).

Inflammatory diseases can be divided into to two groups: acute inflammatory diseases and chronic inflammatory diseases.

By “acute inflammatory diseases” it is herein referred to a common result of innate immune responses, and local adaptive immune responses. Acute inflammation is the initial response of the body to harmful stimuli and is achieved by the increased movement of plasma and leukocytes from the blood into the injured tissues. Although inflammation serves a protective function in controlling infections and promoting tissue repair, it can also cause tissue damage and disease (Abbas et al., Cellular and Molecular Immunology, 7th edition, 2011). Examples of acute inflammatory diseases are Systemic Inflammatory Response Syndrome (SIRS), sepsis, peritonitis, reperfusion injury, pancreatitis, nephritis, myocarditis, encephalitis, pelvic inflammatory disease, vasculitis, uveitis, keratitis and acne vulgaris.

By “chronic inflammatory diseases” it is herein referred to prolonged inflammation that leads to a progressive shift in the type of cells present at the site of inflammation and is characterized by simultaneous destruction and healing of the tissue from the inflammatory process. Examples of chronic inflammatory diseases are Inflammatory Bowel Diseases (IBD) as Crohn’s disease and ulcerative colitis, celiac disease, chronic bronchitis, chronic obstructive pulmonary disease, chronic prostatitis, gastritis, atherosclerosis, rheumatoid arthritis, obesity, allergies, asthma, psoriasis, dermatitis, eczema.

In a particular embodiment the inflammatory disease is acute inflammatory disease. In a preferred embodiment the acute inflammatory disease is selected between Systemic Inflammatory Response Syndrome (SIRS), sepsis, peritonitis.

C) CX3CL1 associated disease

The CX3CL1/CX3CR1 pathway has been shown to be involved in the development of autoimmune diseases such as multiple sclerosis (Huang et al. 2006, *FASEB J* 20(7): 896-905),
5 rheumatoid arthritis (Sawai et al. 2007 *Arthritis Rheum* 56(10): 3215-25), lupus erythematosus (Inoue et al. 2005, *Arthritis Rheum* 52(5): 1522-33), cardiovascular diseases (Moatti et al. 2001, *Blood* 97(7): 1925-8; Combadiere et al. 2003, *Circulation* 107(7): 1009-16; Lesnik et al. 2003, *J Clin Invest* 111 (3): 333-40; McDermott et al. 2003, *J Clin Invest* 111 (8): 1241-50), neurodegenerative diseases such as macular degeneration (Combadiere et al.
10 2007, *J Clin Invest* 117(10): 2920-8) and Parkinson's disease (Cardona et al. 2006, *Nat Neurosci* 9(7): 917-24), graft versus host disease (Robinson et al. 2000, *J Immunol* 165(11): 6067-72), cancer (Andre et al. 2006, *Ann Oncol* 17(6): 945-51; Vitale et al. 2007, *Gut* 56(3): 365-72), viral infections such as HIV infections (Faure et al. 2000, *Science* 287(5461): 2274-7; Garin et al. 2003, *J Immunol* 171 (10): 5305-12), respiratory syncytial virus infections
15 (Tripp et al. 2001, *Nat Immunol* 2(8): 732-8) and West Nile Virus infections (Getts et al. 2008, *J Exp Med* 205(10): 2319-37). The CX3CL1/CX3CR1 pathway has also been shown to be involved in cicatrization (Ishida et al. 2008, *J Immunol* 180(1):569-79), pain (Holmes et al. 2008, *J Neurochem* 106(2): 640-9), behavioural disorders (Gordon Research Conferences, 21-26/09/2008), and nucleic acids encoding CX3CL1 are useful as adjuvant in vaccines (Iga
20 et al. 2007, *Vaccine* 25(23): 4554-63).

Therefore, the invention is directed to a peptide according to the invention for use as a medicament, more specifically for use for the treatment or the prevention of any disease described herein.

A preferred aspect of the invention is directed to:

- 25 - a method for treating or preventing a disease selected from the group consisting of an autoimmune disorder, a cardiovascular disease, a neurodegenerative disease, a graft versus host disease, a behavioral disorder, a cicatrization disorder, a viral infection, cancer and pain comprising the step of administering an effective amount of an isolated peptide according to the invention to an individual in need thereof; and/or
- 30 - a isolated peptide according to the invention for use in treating or preventing a disease selected from the group consisting of an autoimmune disorder, a cardiovascular disease, a neurodegenerative disease, a graft versus host disease, a behavioural disorder, a cicatrization disorder, a viral infection, cancer and pain.

More generally, the present invention is devoted to the generation of novel therapeutic compounds and drugs with increased efficacy and specificity for the treatment of mental or neurological diseases, or pathologies of the immune system, infectious diseases or cancer. These modulators could also be used as drugs for the treatment of pathological states for which specific therapeutic agents are now lacking. Published studies using a macaque model have already emphasized the potential of chemokine variants in topical drugs (Lederman, 2004, Science, 306, 485-487.).

By "effective amount" is meant an amount sufficient to achieve a concentration of peptide which is capable of preventing, treating or slowing down the disease to be treated. Such concentrations can be routinely determined by those of skilled in the art. The amount of the compound actually administered will typically be determined by a physician, in the light of the relevant circumstances, including the condition to be treated, the chosen route of administration, the actual compound administered, the age, weight, and response of the individual patient, the severity of the patient's symptoms, and the like. It will also be appreciated by those of skilled in the art that the dosage may be dependent on the stability of the administered peptide.

In a preferred embodiment, said disease is selected from the group consisting of an autoimmune disorder, a cardiovascular disease, a neurodegenerative disease, cancer and a graft versus host disease. Autoimmune disorders include, e.g., multiple sclerosis, rheumatoid arthritis, lupus erythematosus, inflammatory bowel disease and ulcerative colitis. Cardiovascular diseases include, e.g., atherosclerosis, thrombosis, atherothrombosis and heart failure. Cancer include, e.g., breast cancer, colon cancer, prostate metastasis dissemination, and lymphoma. The neurodegenerative disease may for example correspond to Parkinson's disease.

The invention will be further illustrated by the following figures and examples. However, these examples and figures should not be interpreted in any way as limiting the scope of the present invention.

FIGURES:

Figure 1. Adherence of 3D7-iRBC on the CX3CL1 chemokine.

A) Static adherence of 3D7-iRBC at early stages (12% parasitaemia, 10% ring) or at late stages (7% parasitaemia, 1% ring, 6% mature stages) in 96-well plates coated with 25 pmoles of CX3CL1 (grey bars) or not (control, empty bars). The number of adherent cells per

mm² was expressed as mean values and standard deviations from duplicate wells. Data are representative of three independent experiments.

B) Flow adherence of enriched 3D7-iRBC, on parental L929 cells or on CX3CL1 positive-L929 cells after pretreatment or not with 500 nM of CX3CL1 or 500 nM of CCL2.

5 The number of adherent cells was expressed as percent of the control, as mean values and standard deviations from three independent experiments.

C) Static adherence of normal RBC (black bars) and enriched 3D7-iRBC (grey bars) in 96-well plates coated with 25 pmoles of CX3CL1 or CCL2 per well or not (buffer) as indicated. The number of adherent RBC per mm² was expressed as in A from three
10 independent experiments.

D) Static adherence of enriched 3D7-iRBC in 96-well plates coated with various concentration of CX3CL1. The number of adherent cells per mm² was expressed as in A. The use of enriched iRBC difference in panels 1C and 1D explains the difference in iRBC adherent number as compared to panel 1A panel.

15

Figure 2. Electrophoresis and anti-CX3CR1 staining of 3D7-iRBC membranes

A. Static adherence of CX3CL1 of enriched 3D7-iRBC pretreated or not (control) with 0.5 µg/ml of anti-CX3CR1 or anti-DARC antibodies, in 96-well plates coated with 25 pmoles. The number of adherent cells was expressed as percent of the control, as mean values
20 and standard deviations from four independent experiments.

B. Membranes proteins of normal RBC and 3D7-iRBC were separated on SDS/PAGE and transferred to PVDF membrane, immunostained with anti-CX3CR1 (left panel) or stained with Coomassie blue (right panel). Arrows indicates the gel plug used for mass spectrometry analysis.

25

Figure 3. Characterization of HEK293 clones expressing CBP candidates.

A) HEK293 clones expressing the five CBP candidates as chimera with EYFP were visualized wide field EYFP fluorescence. Images indicate the membrane expression of the EYFP-fusion proteins.

30 B) The clones were subjected to static adherence in 96-well plates coated or not (control) with 25 pmoles of CX3CL1 or CCL2 per well, as indicated. The number of adherent cells per mm² was expressed as mean values and standard deviations from four replicates wells.

Figure 4. Schematic primary and secondary structure of CBP1 and CBP2

Grey box: signal peptide. Empty box: Pexel/VTs motif. Filled box: putative TM domain. The % number indicates the sequence identity between CBP1 and CBP2 domain by domain or as a whole (lower). EC means putative “extracellular domain”.

5

Figure 5. The antibodies raised against CBP1 and CBP2 stain the external membrane of RBC infected by mature 3D7 Plasmodium falciparum strain.

A) Membranes of untreated RBC and enriched 3D7-iRBC were subjected to Western blotting using anti-CBP1 (left panel) or with anti-CBP2 (right panel) antisera (1/500 dilution).

10

B and C) 3D7-iRBC were visualized by transmitted light, by staining with anti-CBP1 (B) or anti-CBP2 (C) antisera and by Hoechst staining using either RBC infected by 3D7 strain at mature stages (80% parasitaemia, after gel flotation) or at early stage (4,5% parasitaemia, 4% ring). Bar = 10µm.

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Figure 6. Static adherence of 3D7-iRBC on the CX3CL1 chemokine is decreased in the presence of anti-CBP1/2 antibodies and in the presence of CBP-EC peptides.

A) Static adherence in 96-well plates coated with 25 pmoles of CX3CL1 of enriched 3D7-iRBC pretreated or not (control) with 0.5 µg/ml of anti-CX3CR1 antibodies, of anti-CBP1 pAb, of anti-CBP2 pAb or addition of both anti-CBP1 and CBP2 pAb, both anti-CX3CR1 and anti-CBP1/2 or with a control pAb. The data are expressed as percent of the control, as mean values and standard deviations from four independent experiments.

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B) Static adherence in 96-well plates coated with 25 pmoles of CX3CL1 of enriched 3D7-iRBC pretreated or not (control) with 0.1 µM of various peptides as indicated. The data are expressed as percent of the control, as mean values and standard deviations from four independent experiments.

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Figure 7. Static adherence of 3D7-iRBC on the CX3CL1 chemokine in the presence of CBP-EC peptides and of their variants.

Static adherence in 96-well plates coated with 25 pmoles of CX3CL1 of enriched 3D7-iRBC pretreated or not (control) with 0.1 µM of various peptides as indicated. The data are expressed as percent of the control, as mean values and standard deviations from four independent experiments.

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Figure 8. LPS preclinical model.

Seq1 or Rd-Seq1 peptides (100 µg) are injected just before intraperitoneal injection of LPS in WT animals (grey bars). Only LPS is injected in CX3CR1^{-/-} animals (black bar) and only PBS is injected in control animals (left bar). Bars represent mean ± SD of data collected in three animals

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Figure 9. Thioglycolate pre-clinical model.

After thioglycolate injection at D0 and injection (3 x 30 µg) of Seq1/2 or Rd-Seq1/2 peptides at D1 either in PBS (Seq1) or in PBS plus DMSO (Seq2). The monocytes, either inflammatory (up) or patrolling (down) are analyzed at D2 in peritoneal liquid. Bars represent mean ± SD of data collected in three animals

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Figure 10. Chemotaxis of CHO-CX3CR1 cells towards 5 nM CX3CL1 in the presence of 0.5µM of CBP-EC peptides and of their variants.

Migration assays were performed in 24-transwell inserts (Corning Costar, Avon, France) with 8µm polycarbonate filter. Cells were resuspended in chemotaxis buffer (DMEM containing 0.5% BSA and 10 nM HEPES) in presence or not of 0.5µM CBP-EC peptides located in the top chamber. The bottom of each well was filled with 600µL pre-warmed chemotaxis buffer at indicated chemokine concentration. The plates were then incubated for 4 hours at 37°C in a 5% CO₂ atmosphere. Cells that passed the membrane were stained with DAPI (VectaShield, Peterborough, UK) and counted manually with a fluorescence microscope (BX51; Olympus, Rungis, France) as fluorescent spots. Results are expressed as a number of cells migrating in the presence of chemoattractant or peptide minus the number of cells migrating in presence of buffer only. Experiments were run in triplicate, and results are representative of at least three independent experiments.

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25**Figure 11. Specific and functional interaction between CX3CL1 and the CBP-EC peptides assayed by fluorescence.**

Solutions of 30µl of 500nM of each of the 5-TAMRA fluorescent peptides were prepared at 4°C in the presence or not of 2µM of CCL2 or CX3CL1 using the HBSS buffer supplemented with 1mM CaCl₂ and 1mM MgCl₂ in a 96-well white plate. After 20 minutes of incubation at RT the 5-TAMRA fluorescence was measured (excitation 520 nm, emission 580 nm). Experiments are made in triplicate. The background (fluorescence of the control without chemokine) was subtracted and the data were expressed as mean values and standard deviations from three independent experiments.

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EXAMPLE 1:**Materials & Methods****Chemicals, proteins and antibodies**

5 All chemicals are from Sigma-Aldrich (L'Isle d'Abeau, France) except when stated. Chemokine CCL2 are from Peprotech (Levallois-Perret, France) and chemokine domain of CX3CL1 from R&D Systems (Lille, France). The peptides CBP1-EC or Seq1 (STLNLKNEN (SEQ ID NO:1) and CBP2-EC or Seq2 (SKFTNNMLAIAG (SEQ ID NO:2)) as well as the random peptides CBP1-EC-rd or Seq1-rd (NLETNSKNL (SEQ ID NO:15)) and CBP2-EC-rd
10 or Seq2-rd (MATGSAKNLFNI (SEQ ID NO:16)) were synthesised in the Institut Biologie Paris Seine (UPMC). Anti-CX3CR1 polyclonal antibody (pAb 14-6093) was purchased from eBioscience (SAS, Paris, France). Anti-DARC antibody was a generous gift from O. Bertrand (Inserm U-1134). The anti-CBP1 and anti-CBP2 polyclonal antibodies were performed at Proteogenix (Schiltigheim, France) by immunizing rabbit with two synthetic peptides,
15 LSTLNLKNEN (SEQ ID N:7) and AATSKFTNNMLAIAGVG (SEQ ID N:8) respectively.

Cell cultures

Human embryonic kidney (HEK293) and the fibroblastic L929 cell lines were grown in DMEM medium supplemented with 10% fetal calf serum (Dutscher, Brumath, France), 1% sodium pyruvate and antibiotics. To obtain L929 clone expressing CX3CL1, the CX3CL1-
20 pEYFP plasmid (Hermant et al, 2008) was transfected into L929 cells with the cationic polymer transfection reagent JetPEI (Polyplus transfection, Ozyme, St Quentin-en-Yvelines, France). A stable transformant resistant to 0.5 mg/ml G418 (Invitrogen) was selected with a FACS Aria cell sorter (BD Biosciences, Le Pont de Claix, France).

The Plasmodium falciparum 3D7 cloned strain was cultured in human erythrocytes as
25 described previously (Trager & Jensen, 1976). Enrichment of late-stage infected erythrocytes (parasitaemia greater than 80 %) was obtained by gelatin flotation (Plasmion, Fresenius Kabi, France) according to already published protocol (Pasvol et al, 1978).

Identification of CX3CL1-Binding Protein (CBP) candidates by SDS-PAGE and mass spectrometry

30 Membranes from RBCs and iRBCs (parasitaemia greater than 80 %) were prepared by hypotonic lysis (Steck & Kant, 1974) by incubation in 5P8 buffer (5 mM NaH₂PO₄/Na₂HPO₄, 0.35 mM EDTA, pH 8) and centrifuged 5 min at 4000 xg at 4°C to remove parasite pellet. The supernatant containing RBC ghost was centrifuged 15 min at 25,000 xg at 4°C. Then the pellet was washed in 5P8 buffer by centrifugation several times

until the ghost pellet became white. Ghosts were lysed in PBS buffer containing 1 % Triton-X100, 0.5 % SDS and antiprotease cocktail Complete (Roche Diagnostics, Meylan France). After centrifugation for 45 min at 25,000 xg (4°C), protein concentration of supernatants was evaluated (BCA, Pierce, Thermo Fisher Scientific, Courtaboeuf, France). Proteins were separated by 4-20 % SDS-PAGE (Novex 4-20%, Life Technologies). The fractionated proteins were either stained with Coomassie Blue (120 µg of cell lysate per lane) or transferred to PVDF membranes (Dutscher) (50 µg of cell lysate per lane). Immune complexes were visualized with secondary peroxidase-conjugated antibodies using a chemiluminescent kit (GE Healthcare Europe, Saclay, France).

Gel fractions were washed three times with 25 mM NH₄HCO₃, 50 % ethanol and then dehydrated with pure acetonitrile. Reduction and alkylation of proteins were performed by incubating gel samples in 10 mM DTT, 100 mM NH₄HCO₃ for 30 min at 56°C and alkylation in 55 mM iodoacetamide, 100 mM NH₄HCO₃ for 45 min at RT. After wash in 25 mM NH₄HCO₃, 50 % acetonitrile, gel samples were dehydrated with acetonitrile before being rehydrated with 40 µl of trypsin solution (400 ng in 50 mM NH₄HCO₃, 10 % acetonitrile) for overnight digestion at 37°C. Supernatants were transferred in a new tube and peptides were extracted twice with 50µl of 50 % acetonitrile, 0.1 % TFA. Tryptic peptides were dried and resuspended in 10 µl of 50 % acetonitrile, 0.1 % TFA. Liquid Chromatography – Mass Spectrometry (LC-MS/MS) analysis were performed on an Ultimate 3000 Nano HPLC instrument (Thermo Fisher Scientific, Courtaboeuf, France) coupled to an Esquire HCTultra ion trap mass spectrometer (Bruker Daltonics, Marne-la-Vallée, France). The LC separation was performed on a PepMap100 column (0.075 mm ID, 3 µm particle size, 15 cm long; Thermo Fisher Scientific) at a flow rate of 200 nL/min, employing a linear gradient from 0% to 50% of H₂O/acetonitrile/formic acid (5/95/0.1 v/v) for 35 min. A search for protein identity was carried out with MASCOT software (<http://www.matrixscience.com>) using the PlasmoDB protein database (<http://plasmodb.org/plasmo/>). Confident matches were defined by a MASCOT score above 28 (threshold score corresponding to statistical significance p<0.05).

CBPs transfectants in the HEK293 cell line

The CBP-DNAs were synthesized by the GeneArt Plasmid Services (Life Technologies) with the CX3CL1 peptide signal and codons optimization for human cells. They were cloned in pMA gene art vector between SacI and KpNI restriction sites. These constructs were digested with HindIII and BamHI and the HindIII/BamHI fragments were then cloned in pEYFP-NI (Clontech, Ozyme, France). CBP-pEYFP constructs were

transfected into HEK293 using the cationic polymer transfection reagent jetPEI (Polyplus transfection, Illkirch, France). Stable transformants resistant to 1 mg/ml G418 (Life Technologies) were selected with FACS AriaII Cell Sorter (Becton-Dickinson, le Pont de Claix, France).

5 **Static adherence assay**

CX3CL1 or CCL2 were adsorbed overnight at 4°C on flat-bottom 96-well microtiter plates (Nunc A/S, Roskilde, Denmark) at the indicated concentrations (50 µl/well) diluted in 25 mM Tris pH 8 and 150 mM NaCl. Well surfaces were blocked for 2 h at room temperature (RT) with 1 % nonfat milk in the same buffer. Enriched iRBCs were suspended at 5x10⁶ ml⁻¹ in HBSS supplemented with 1 mM CaCl₂, 1 mM MgCl₂ and 10 mM Hepes (pH 6.8). The CBPs-HEK293 transfectants to be tested were first labelled with 1 mM 5(6) carboxyfluorescein diacetate succinimidyl ester (Interchim, Montluçon, France). Before addition, enriched iRBCs and CBPs-HEK293 transfectants were treated or not for 15 min at RT with 500 nM CX3CL1 or CCL2, or with 0.5 µg/ml of the different Abs. Alternatively, the wells with the immobilized CX3CL1 chemokine were incubated with peptides CBP1-EC and CBP2-EC as well as CBP1-ECrd and CBP2-ECrd at 0.1 µM.

The cells (1.5x10⁶ iRBCs or 10⁴ HEK293 cells per well) were then added and incubated 1 h at 37°C and exactly quantified by measurements of nine different fields per well at 415 nm for iRBCs and 530 nm for HEK293 cells (Flexstation 3, Molecular Devices, Sunnyvale, USA). After washing to remove the non-adherent cells as described as previously (Hermand et al, 2000), the number of adherent cells was calculated as a ratio to the total number of cells loaded in each well, determined by the a new absorbance/fluorescence evaluation and expressed as number of adherent cells per mm², knowing that the well surface is 0.32 mm². In experiments evaluating inhibition of iRBC adherence by antibodies and peptides to normalize several experiments, the number of adherent cells was expressed as % of the control, i.e. the number of adherent iRBC on coated CX3CL1 (50 pmoles/well)..

Flow adherence assay

The day prior the assay, CX3CL1-L929 transfectant and parental cells (10⁴ cells in 20 µl DMEM containing 10% FCS) were plated in IbiTreat -15µ-SlideVI (Ibidi Biovalley, Marne La Vallée, France). The slide was set on the stage of an inverted microscope (TE300, Nikon, Champigny, France) equipped with a phase contrast 10× objective (Nikon, n.a. 0.25) and a cooled CCD camera (Sensicam, PCO, Kelheim, Germany). iRBC (parasitemia superior to 80 %) were resuspended in flow buffer HBSS (GE Healthcare Europe, Velizy-Villacoublay, France) supplemented with 1 mM CaCl₂, 1 mM MgCl₂ (pH 6.8) at 5x10⁶ mL⁻¹

and incubated 15 min at 37°C in the presence or not of 0,5 µM CX3CL1 or CCL2. A syringe pump (PHD 2000; Harvard Apparatus, Les Ulis, France) drove 0.5 ml of iRBC suspension through the IbiTreat slide at a wall shear stress of 0.27 dynes.cm⁻². The 37°C temperature was controlled all over the experiment. After a 10-min wash at 0.27 dynes.cm⁻², images of fifteen
5 0.57 mm² separate fields were recorded to count the adherent cells. The results were expressed as mean ± SD per mm².

Fluorescence imaging

To prevent nonspecific staining, the rabbit sera diluted were adsorbed on fresh normal human O⁺ red blood cells. In order to explore proteins at the membrane surface, unfixed
10 iRBCs were used in liquid phase Pellets (5 µl) of cultured iRBC were incubated for 45 min at RT with 100 µl of serum diluted 5 times in PBS supplemented with 2 % BSA and 100 µg/ml Hoechst reagent. After washing with PBS supplemented with 2 % BSA, antibodies were detected with Alexafluor 488 conjugated goat anti-rabbit IgG (Life Technologies). Immunofluorescence staining was analyzed with a direct microscope (BX51, Olympus,
15 Rungis, France).

Fluorescence imaging of the HEK293 clones expressing the CBP candidates as EYFP chimera was performed on Lab-Tek II slides (Nunc, Dutscher) after fixation (PFA 4%, 30 min 4°C) using an Olympus IX81-ZDC2 inverted microscope equipped for Total Internal Reflection fluorescence (TIRF). Digital images were captured on a cooled charge-coupled
20 device (CCD) camera (ORCA-ER, Hamamatsu, Massy, France).

Statistical analysis

Data are expressed as mean and standard deviations of replicates as indicated in the legend of figures. Analysis of variance (ANOVA) followed by post hoc analysis with Tukey test was performed using Prism 5.2 (GraphPad Software, San Diego, California, USA) to
25 establish the levels of significance: * $p \leq 0.05$, ** $p \leq 0.005$, *** $p \leq 0.005$.

EXAMPLE: 2

Results Malaria application

**Unselected, mature Plasmodium falciparum-infected RBC bind specifically to
30 CX3CL1**

RBC infected with mature forms (late trophozoite and schizont) of the 3D7 strain of *P. falciparum* significantly adhered to the immobilized CX3CL1 protein in a static adhesion assay (Figure 1A) while those infected with parasites at the early stages did not display specific adherence to CX3CL1. So we decided to work with enriched mature RBC infected

with the 3D7 strain (3D7-iRBC). Their adherence was also observed in a flow assay using the L929 fibroblastic cell line that either did or did not express CX3CL1. We saw that 3D7-iRBCs specifically adhered to the CX3CL1-positive L929 clone, while no adherence was observed on parental cells that did not express CX3CL1 (Figure 1B). Pre-incubating 3D7-iRBC with soluble CX3CL1 inhibited the adherence, while preincubation with the chemokine CCL2 had no effect (Figure 1B). To further investigate the specificity and selectivity of the 3D7-iRBC adherence, we used a static assay that allows numerous simultaneous conditions. Indeed, we found that 3D7-iRBC adhered to immobilized CX3CL1, while non-infected RBC did not (Figure 1C). Furthermore, this specific adherence was dosedependent (Figure 1D) and saturation was reached at 25 pmoles of CX3CL1 per well. Moreover, 3D7-iRBC did not bind to CCL2 (Figure 1C). Finally, we found that several rounds of selection of 3D7-iRBC on CX3CL1-expressing L929 did not result in the selection of a subpopulation of iRBC with greater adherence to CX3CL1 (data not shown).

Identification of two CBPs expressed at the surface of iRBC

As shown by Hatabu et al. (Hatabu et al, 2003), 3D7-iRBC adherence is significantly decreased in the presence of an anti-CX3CR1 antibody. In comparison, an antibody raised against DARC (Duffy Antigen Receptor for Chemokines), which is an antigen expressed by both uninfected and infected RBC (Tournamille et al, 2004), had no effect on adherence (Figure 2A). We therefore hypothesized that the anti-CX3CR1 antibody could recognize a parasitic component expressed on the surface of 3D7-iRBC, which likely shares structural features with CX3CR1. Several proteins were recognized by the anti-CX3CR1 antibody in the extract of 3D7-iRBC membranes; however only one band at 20-25 kDa (Figure 2B left, arrow) was absent from the membranes of uninfected RBC. Peptides from trypsin-digested extracts of the corresponding 1D gel band from both uninfected and infected RBC (Figure 2B right, arrow) were analyzed by mass spectrometry (LC-MS/MS) and their sequences were compared to those of proteins encoded by Plasmodium genomes in PlasmoDB. Among the hits with significant scores found only in the 1D gel band from iRBC, we selected the corresponding genes coding for proteins with a 20-35 kDa molecular mass and containing a Pexel motif (Marti et al, 2004) or VTS (Vacuolar transport signal) (Hiller et al, 2004), assuming that the potential CX3CL1 ligand should be expressed at the external iRBC membrane (Table 1). These five genes have only one intron but different chromosomal locations. Three of these genes encode for proteins containing a signal peptide and two potential transmembrane domains, similar to most of the Pexel/VTS-containing proteins (Lavazec et al, 2006).

The five candidate genes were then cloned as fusion proteins with YFP (Yellow fluorescent protein) in HEK293 cells that do not adhere to CX3CL1 or CCL2. The five HEK293 clones expressed the YFP fusion proteins at their cell surface (Figure 3A), as observed using wide field fluorescence (Figure 3A.). Functional adhesion tests revealed that two candidate cell clones, PF3D7_0113900 and PF3D7_1301700, specifically adhered to immobilized CX3CL1 while three others did not (Figure 3B). For simplicity, PF3D7_0113900 and PF3D7_1301700 are herein referred to CBP1 and CBP2. Adherence of the CBP1- and CBP2-expressing HEK293 clones was dose-dependent (data not shown) and specific for CX3CL1 (data not shown). In addition to the 3D7 reference strain, transcripts of both *cbp1* and *cbp2* genes were present in samples prepared from the peripheral blood samples of seven patients infected in various African locations (data not shown).

CBP1 and CBP2 are unique, homologous, monogenic exported proteins with two transmembrane domains, and are expressed on the external surface of iRBC

According to PlasmoDB, CBP1 and CBP2 are monogenic proteins of 243 and 245 amino acids in length, respectively. They are encoded by single-copy genes and their sequences share 32% identity (Table 1, Figure 4), making them the only two members of the hypothetical family of Plasmodium exported protein called “hyp8” (PlasmoDB) (Frech & Chen, 2013). Similar to all Pexel-containing genes, *cbp1* and *cbp2* contain one intron flanked by two exons; there are no homologs in the 3D7 genome and no orthologs in genomes of other Plasmodium strains/species. Both CBP1 and CBP2 have a signal peptide (hence their calculated molecular masses without signal peptide of 25320 Da and 25328 Da, respectively) and two short 9- and 12-amino acid extracellular domains. Their topology may be similar to STEVOR, RIFIN and PfMC-2TM, in that both N- and C-termini are intracellular (Lavazec et al, 2006). Of all the available sequences in the PlasmoDB, the *cbp1* gene has 49 singlenucleotide polymorphisms (SNP) and among them 41 are synonymous; *cbp2* has 15 SNP among them 13 are synonymous. Remarkably, the non-synonymous SNP of both genes exist outside the regions encoding for the transmembrane and extracellular domains.

We next raised rabbit polyclonal antibodies against peptides corresponding to the sequences of the short central domains of CBP1 and CBP2 located between the two transmembrane domains (Figure 4). In 3D7-iRBC membrane lysates, both anti-CBPs antibodies specifically recognized a 25 kDa band (Figure 5A, arrows), that matched in size to the band stained by the anti-CX3CR1 serum (Figure 2 left, arrow). RBC infected with mature - but not immature -forms of 3D7 using the anti-CBP1 and anti-CBP2 antibodies showed a

peripheral staining (Figure 5B and 5C) reminiscent of a typical PfEMP1 surface staining on iRBC.

Finally, these anti-CBP1 and anti-CBP2 antibodies were used to stain iRBC samples from a patient after a maturation period of 24 hours in vitro (Figure E4). This showed that CBP1 and CBP2 are expressed at the surface of iRBC during natural infection. Taken together, these data strongly suggest that CBP1 and CBP2 parasitic proteins are expressed at the external membrane of the iRBC and that the antibodies raised against their potential extracellular domain recognized their respective target.

Anti-CBP antibodies and CBP-EC peptides inhibit iRBC binding to CX3CL1

Anti-CBP1 and anti-CBP2 antibodies inhibited the adherence of 3D7-iRBC to immobilized CX3CL1 by 33% and 38% respectively i.e. at a similar level to the anti-CX3CR1 antibody (Figure 6A), while a control rabbit antibody did not have any effect on adherence. When anti-CBP antibodies were incubated simultaneously, the inhibitory effect on adherence was not enhanced. Similarly, incubating the anti-CX3CR1 with either of the CBP antibodies, we did not observe any change in the inhibitory effect (Figure 6A). Along with surface staining (Figures 5B and 5C), these observations provided additional evidence to support that CBP1 and CBP2 were both expressed at the surface of iRBC, and that the short central domains were accessible to antibodies and can act as external ligands to host receptors.

We thus hypothesized that peptides corresponding to the predicted extracellular domain (EC) of each CBP (called CBP1-EC and CBP2-EC, Figure 4) could directly bind to CX3CL1 thereby inhibiting iRBC adherence. Both CBP1-EC and CBP2-EC peptides significantly inhibited iRBC adherence to CX3CL1 by 41-48% (Figure 6B), while their random counterparts (CBP1-EC-rd, CBP2-ECrd, i.e. peptides with the same residues in a different order) had no such effect. Lastly the simultaneous addition of both CBP1-EC and CBP2-EC peptides did not enhance this inhibition.

Knowing that chemokines are of basic nature, we infer their binding to the CBP-EC could be facilitated by the presence of additional acidic residues. So we mutated the basic residues of CBP-EC (Xaa4, Xaa5, Xaa8 and Xaa10) in either acidic residues (D) or neutral ones (A). These replacements did not change significantly the potency of the CBP-EC peptides on the iRBC adhesion to CX3CL1 (Figure 7).

DISCUSSION

We report here on two parasitic proteins CBP1 and CBP2 that act as *P. falciparum* ligands to CX3CL1, thereby contributing to cytoadherence of iRBC. CBPs are coded by

genes of the *P. falciparum* 3D7 reference strain and also in field isolates, as demonstrated by the detection of mRNA transcripts in all patient samples analyzed to date (data not shown). Anti-CBP1 and anti-CBP2 antibodies stained the surface of iRBC from a patient as well as 3D7-iRBC (Figures 5B and 5C), suggesting that both CBP1 and CBP2 are expressed at the surface of iRBC.

The intensity of iRBC adherence to immobilized CX3CL1 reached a plateau at 25 pmol of CX3CL1 per well. This concentration is very close to that of CD36 and ICAM-1, which supports optimal in vitro static adherence of 3D7-iRBC (Gray et al, 2003). This CX3CL1-mediated cytoadherence is at least partially mediated by CBP1 and CBP2, since peptides corresponding to their predicted extracellular domains (called CBP1-EC and CBP2-EC), inhibit the binding of iRBC to CX3CL1 (Figure 6B) as do anti-CBP1 and anti-CBP2 antibodies directed against these short domains (Figure 6A). Similar to the majority of described *P. falciparum* Pexel/VTS-containing genes, the *cbp1* and *cbp2* transcripts are present early in erythrocyte development, while proteins are expressed later at trophozoite and schizont stages, according to PlasmoDB. We showed here that both are expressed at iRBC surface at the late stages (Figures 5B and 5C). Our work confirms and extends a previous proteomics approach showing the expression of CBP1 (PF3D7_0113900) at the surface of iRBC (Florens et al, 2004). This expression profile was consistent with the iRBC adherence to CX3CL1 primarily observed by Hatabu et al. (Hatabu et al, 2003), so it is likely that CBP1 and CBP2 are at least partially responsible of the cytoadherence phenotype observed by this group.

Finally, both proteins have been shown to be putatively exported protein in early gametocyte proteome (Silvestrini et al, 2010), are hence named GEXP10 and GEXP07 (Table 1) and could contribute to the gametocyte sequestration. Interestingly, immature gametocytes accumulate in the extravascular spaces of the human bone marrow (Farfour et al, 2012; Joice et al, 2014; Sinton et al, 1926) and it is known that CX3CL1 is well expressed in bone marrow stromal cells (Honczarenko et al, 2006), where it contributes to the retention of monocytes (Jacquelin et al, 2013). So CX3CL1 could arguably contribute to the gametocyte accumulation in specific body sites, including bone marrow.

The exact motif responsible for CBPs binding to CX3CL1 of CBPs remains to be indisputably identified. Interestingly however, cytoadherence-inhibiting-EC domains of CBP1 and CBP2 short TXN motif (TEN in CX3CR1, TLN in CBP1 and TNN in CBP2). The multiple asparagine residues in the extracellular domain of CBPs are not expected to bind to basic proteins like chemokines. The replacements of these basic residues by acidic ones

(Figure 7) did not induce significant changes in the inhibitory activity of the peptides. Furthermore simultaneous incubation with both CBP1-EC and CBP2-EC peptides (Figure 6B) did not lead to any synergistic inhibition (compared to the single-peptide effect) and suggested that both peptides bind to the same site on the CX3CL1 protein. Beyond the context of malaria infection, CBP1-EC and CBP2-EC peptides are very interesting potential hits to initiate the identification of a CX3CL1-specific “neutraligand” (a molecule directly neutralizing the chemokine but not antagonizing the cognate receptor), as recently described for CXCL12 (Galzi et al, 2010; Hachet-Haas et al, 2008).

In analogy with ICAM1 as a host-receptor for *P. falciparum* cytoadherence, CX3CL1 expression is induced on endothelia by inflammation (Fong et al, 1998; Harrison et al, 1999). This could facilitate the cytoadherence of additional iRBC through CBP-CX3CL1 interaction once the malaria infection has triggered inflammation (Avril et al, 2012; Claessens et al, 2012; Lavstsen et al, 2012). Whether CBPs act independently from the dominant *P. falciparum* ligand for cytoadherence PfEMP1, or rather in combination with it as a co-ligand, remains to be elucidated.

Although CBP1 and CBP2 induce an iRBC phenotype similar to that induced by PfEMP1 (translation at the ring stage, surface expression and cytoadherence at the mature stage), they differ markedly regarding their structural and genetic features. Our staining (Figure 5B) and functional data (Figure 6A) using antibodies strongly suggest that the N- and the C-terminus of CBP1 and CBP2 are intracytoplasmic (Figure 4) leaving only short 9- or 12-amino acid domains exposed at the external side of the host red blood cell. In comparison, the PfEMP1 ectodomain is large (250-350 kDa) and built from a combination of multiple domains (Smith et al, 2000). By Western blot (Figure 5A, left panel), anti-CBP antisera recognized proteins markedly smaller than 200 kDa, thus unequivocally different from PfEMP1. This is consistent with binding assays (Janes et al, 2011) ruling out the intervention of PfEMP1 in the adherence phenotype to CX3CL1. Our experiment showing that selection produced no increase in binding (data not shown) supports the same conclusion. The simplest explanation for the Western blot results with anti-CBP antisera on iRBC membrane lysates (Figure 5A) is that the 50, 70 and 110 kDa bands represent multimers of the 20-25 kDa CBPs.

The PfEMP1 family is encoded by the highly diverse and polymorphic var gene family associated with very broad antigenic diversity. In striking contrast, CBPs are monogenic members of a small family and exhibit no variant in their extracellular domain (PlasmoDB), which is consistent with the fact that we could not select for a subpopulation of

iRBC with higher binding affinity even after several cycles of panning(data not shown). PfEMP1 antigens are highly immunogenic; however intra-clonal diversity and poorly overlapping antigenic repertoires between clones/strains is a major drawback in vaccine development. Conversely, CBPs are constitutively expressed and monogenic and, as such, expected to be poorly immunogenic. The existence of a constitutively expressed, highly immunogenic antigen would have indeed turned *P. falciparum* malaria, an infection against which immunity is acquired very slowly, into a single-episode disease. If the putative poor immunogenicity of CBPs can be circumvented by an innovative vaccine design, the final outcome may have a major impact on vaccine-induced control of *P. falciparum* malaria.

In conclusion, the identification of parasite ligands for the endothelial CX3CL1 offers new ways to prevent malaria, and provides a novel, non-polymorphic target on iRBC that could be used for vaccination, a long-awaited weapon in times of increasing parasite resistance to antimalarial agents (Ariey et al, 2014; Dondorp et al, 2011).

Table 1: Characteristics of the first five parasite proteins (20-35 kDa, PEXEL positive) selected using proteomics as potential CBPs

Name (old name)	Acronym	Chromosome number	mRNA (bp)	Intron number	Peptide Signal	TM number	Sequence (AA)	MW (Da)
PF3D7_0113900 (PFA0670c)	CBP1	1	732	1	Yes	2	243	28234
PF3D7_1301700 (MAL13P1.61)	CBP2	13	738	1	Yes	2	245	28255
PF3D7_0501000 (PFE0050w)		5	783	1	No	1	260	30716
PF3D7_1478100 (PF14_0753)		14	744	1	Yes	2	247	28657
PF3D7_1102600 (PF11_0038)		11	816	1	No	3	271	32328

EXAMPLE 3: Effects of CBP-EC peptides *in vivo* in inflammatory disease

The Seq1 and Seq2 peptides correspond to the external domain of the two invariant parasite ligands of CX3CL1, a chemokine mediating cytoadherence of *Plasmodium falciparum*-infected erythrocytes. These peptides were assayed as “neutraligands”, i.e. molecules binding the ligand CX3CL1 to inhibit its binding to its receptor CX3CR1. The CX3CR1/CX3CL1 axis is known to contribute to the recruitment of monocytes to inflammatory sites [Fong AM et al 1998, Auffray C et al 2009, Poupel L et al 2013] to sequester monocytes in the bone marrow (BM) [Jacquelin S et al 2013] and to induce macrophage survival [Kim K-W et al 2011].

We tested here Seq1 and Seq2 peptides in two preclinical models of non-infectious peritonitis, a short-term one (LPS-induced, Figure 8) and a long term one (thioglycolate-induced, Figure 9))

LPS-induced peritonitis

This model consists in injecting LPS or PBS for control in the peritoneum immediately after the intravenous injection of the peptides (100µg) or PBS for control. After 4h, the mice are sacrificed and the inflammatory monocytes in peritoneal fluid are analysed by flow cytometry.

When injected intraperitoneally (IP), LPS induced after 4h, the recruitment of inflammatory monocytes from the BM. As show in Figure 8, this recruitment is significantly increased in the presence of Seq1 peptide and reaches a level similar to the recruitment observed in the CX3CR1-/- animals. This recruitment results in a greater accumulation of inflammatory monocytes in the peritoneum as compared to mice treated with the control peptide (Rd-Seq1) i.e. the Seq1 peptide with the same residues in a different order.

Thioglycolate-induced peritonitis

This model consists in injecting thioglycolate in the peritoneum at D0. At D1, the peptides are IP injected (3 x 30 µg) and at D2, the mice are sacrificed and the monocytes in peritoneal liquid are analysed by flow cytometry.

In the presence of Seq1 or Seq2, the number of inflammatory and patrolling monocytes is significantly decreased as compared to the number of corresponding monocytes in the presence of the Rd version of the same peptides (see Figure 9). Consequently Seq1 and Seq2 peptides reduce the CX3CR1-driven accumulation of monocytes in the inflammatory site and could probably act on the survival of macrophages.

Both of these *in vivo* effects of the CBP-EC peptides are presumably caused by a decrease CX3CL1-driven chemotaxis. We finally checked directly this activity *in vitro* (Figure 10) using CHO clone cells stably expressing CX3CR1. We indeed found that the cognate chemotaxis was significantly inhibited by the CBP-EC peptides, while their “random” versions had no effect.

In conclusion, we have found short peptides that are able to specifically inhibit the function of the CX3CL1/CX3CR1 by binding to the CX3CL1 ligand, hence their generic

name "neutraligand". Beyond of dissecting the exact nature of the CX3CL1-CX3CR1 binding sites, these peptides represent useful potential anti-inflammatory compounds, that could complement the CX3CR1 antagonists (Dorgham, K., A. Ghadiri, P. Hermand, M. Roderio, L. Poupel, M. Iga, O. Hartley, G. Gorochoy, C. Combadiere and P. Deterre (2009). "An engineered CX3CR1 antagonist endowed with anti-inflammatory activity." J Leukoc Biol 86(4): 903-11; Karlstrom, S., G. Nordvall, D. Sohn, A. Hettman, D. Turek, K. Ahlin, A. Kers, M. Claesson, C. Slivo, Y. Lo-Alfredsson, C. Petersson, G. Bessidskaia, P. H. Svensson, T. Rein, E. Jerning, A. Malmberg, C. Ahlgen, C. Ray, L. Vares, V. Ivanov and R. Johansson (2013). "Substituted 7-amino-5-thio-thiazolo[4,5-d]pyrimidines as potent and selective antagonists of the fractalkine receptor (CX3CR1)." Journal of Medicinal Chemistry 56(8): 3177-90 ; Poupel, L., A. Boissonnas, P. Hermand, K. Dorgham, E. Guyon, C. Auvynet, F. S. Charles, P. Lesnik, P. Deterre and C. Combadiere (2013). "Pharmacological inhibition of the chemokine receptor, CX3CR1, reduces atherosclerosis in mice." Arterioscler Thromb Vasc Biol 33(10): 2297-305.)

15

Table 2: Useful amino acid sequences for practicing the invention

SEQ ID NO	Nucleotide or amino acid sequence
1 (CBP1-EC)	STLNLKNEN
2 (CBP2-EC)	SKFTNNMLAIAG
3 (CBP1-EC-AA)	STLALKAEA
4 (CBP1-EC-DD)	STLDLKDED
5 (CBP2-EC-AA)	SKFTAAMLAIAG
6 (CBP1-EC-DD)	SKFTDDMLAIAG
7 (synthetic peptide CBP1-EC for immunisation)	LSTLNLKNEN
8 (synthetic peptide CBP2-EC for immunisation)	AATSKFTNNMLAIAGVG
9 (CBP1 polypeptide or PfGEXP10)	MNIYIRTIFFAPFICLLIFSSNHGLFQKKDNQNC NSHKFCLNCSGSYLKNSRWLSETSVSYSDEGEP LKYGANDNNYDSNLLKSEEQKQEISEHMKMLI KLYIDSSHDSAKEEKLFFQQVKKYFKNNEYKDF MRTFIKVIKIHNDLTHLKKQMASTEKRYRIISC VAAFLSTVSLAFTVVMLSTLNLKNENVIVTIIL GLLGFIISLPVISYILSPYLNSSLEKGYRIADLYSE KILRQILHFQ
10 (CBP2 polypeptide or PfGEXP07)	MSFCYVRTISIALFLFIFLFLNNGNFKNNFYRKD NKYSSIKIRSFRLAENQKVETEQSTPAKPEPTE FVNNDIHQNKNTFKKLKNNEKKKEILEEKMKD FVKLYVNKSSGECQRYRLQQHLKNYSSSNEYK KFMNKFVKFLKVHNDLNALKSQLHFNNVRAA TIIFIAGFLSIFAILLTISAVAATSKFTNNMLAIA

	GVGALGSALSLPGMALLFVPAMLYVLNRKNEI TNHYSERIIKQVSNF
11 (CBP1-EC with cysteine)	CSTLNLKNENC
12 (CBP2-EC with cysteine)	CSKFTNNMLAIAGC
13 cyclic CBP1-EC	CSTLNLKNENC —————
14 cyclic CBP2-EC	CSKFTNNMLAIAGC —————
15 random peptides CBP1-EC	NLETNSKNL
16 random peptides CBP2-EC	MATGSAKNLFNI
17 random peptides CBP1-EC-AA	ALETASKAL
18 random peptides CBP1-EC-DD	DLETDSKDL
19 random peptides CBP2-EC-AA	MATGSAKALFAI
20 random peptides CBP2-EC-DD	MATGSAKDLFDI

EXAMPLE 4 :**CBP extracellular peptides directly and specifically interact with CX3CL1.**

The inventors demonstrated that peptides corresponding to the predicted extracellular domain (EC) of each CBP (called CBP1-EC and CBP2-EC) could directly bind to CX3CL1. This assertion was borne out using fluorescent version of the CBP-EC peptides. While the fluorescence of CBP1-EC and CBP2-EC only slightly increased in the presence of the control chemokine (CCL2, empty bars), it was significantly enhanced in the presence of CX3CL1 (filled bars) (Figure 11). By contrast, this increase was minimal using the random counterparts of the CBP-EC peptides (CBP1-EC-rd, CBP2-ECrd, i.e. peptides with the same residues in a different order) (Figure 11). So the interaction between CBP and CX3CL1 appears to be direct and specific of the chemokine on one hand and of the CBP-EC sequence on the other hand.

15 The anti-CBP1 and anti-CBP2 antibodies stain specifically the gamatocytes.

The inventors also demonstrated that anti-CBP1 and anti-CBP2 stain specifically the gamatocytes. The inhibition of the interaction between CBP1/CBP2 and CX3CL1 by the peptides and antibodies of the invention may inhibit gamatocytes maturation by inhibiting their sequestration in the bone marrow. These results demonstrate that the peptides and antibodies of the invention may be used in the treatment of Malaria, as well as in the inhibition of human-to-mosquito transmission.

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Throughout this application, various references describe the state of the art to which this invention pertains. The disclosures of these references are hereby incorporated by reference into the present disclosure.

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CLAIMS:

1. An isolated peptide comprising an amino acid sequence of formula (I):

Xaa1-Xaa2--Xaa3-Threonine-Xaa4-Xaa5-Xaa6-Xaa7-Xaa8-Xaa9-Xaa10-Xaa11

5 , wherein :

Xaa1 is serine (S) or absent;

Xaa2 is lysine (K), or absent;

Xaa3 is serine (S) or Phenylalanine (F);

10 Xaa4 is leucine (L), asparagine (N), alanine (A), aspartic acid (D) or glutamic acid (E);

Xaa5 is asparagine (N), alanine (A) or aspartic acid (D);

Xaa6 is leucine (L), or methionin (M);

Xaa7 is lysine (K) or leucine (L);

Xaa8 is alanine (A), asparagine (N) or aspartic acid (D).

15 Xaa9 is glutamic acid (E) or isoleucine (I);

Xaa10 is asparagine (N), alanine (A), aspartic acid (D).

Xaa11 is Glycine (G), or absent;

20 2. The isolated peptide according to Claim 1, wherein the peptide is selected from the group consisting of :

(i) the amino acids sequence consisting of STLNLKNEN (SEQ ID N O:1) ;

(ii) the amino acids sequence consisting of SKFTNNMLAIAG (SEQ ID N0:2);

(iii) the amino acids sequence consisting of STLALKAEA (SEQ ID N O:3)

(iv) the amino acids sequence consisting of STLDLKDED (SEQ ID N O:4)

25 (v) the amino acids sequence consisting of SKFTAAMLAIAG (SEQ ID N0:5);

(vi) the amino acids sequence consisting of SKFTNNMLAIAG (SEQ ID N0:6);

(vii) an amino acid sequence substantially homologous to the sequence of (i), to (vi) preferably an amino acid a sequence at least 80% identical to the sequence of (i) to (vi).

30

3. The isolated peptide of any one of Claims 1-2 of at most 100 amino-acid.

4. An antibody that specifically binds to an isolated peptide of any one of claims 1 to 3.

5. The antibody according to claim 4 wherein the antibody is polyclonal or monoclonal.

6. A cyclic peptide comprising an amino acid sequence of formula (III):

C-PepX1-Xaa1-Xaa2-Xaa3-T-Xaa4-Xaa5-Xaa6-Xaa7-Xaa8-Xaa9-Xaa10-Xaa11-PepX2-C

, wherein:

Xaa1 is serine (S) or absent;

Xaa2 is lysine (K), or absent;

Xaa3 is Phenylalanine (F) or serine (S);

Xaa4 is asparagine (N), leucine (L), alanine (A), aspartic acid (D) or glutamic acid (E);

Xaa5 is asparagine (N), alanine (A) or aspartic acid (D);

Xaa6 is methionine (M) or leucine (L);

Xaa7 is leucine (L) or lysine (K);

Xaa8 is alanine (A), asparagine (N) or aspartic acid (D).

Xaa9 is isoleucine (I) or glutamic acid (E);

Xaa10 is alanine (A), asparagine (N) or aspartic acid (D).

Xaa11 is Glycine (G) or absent;

“PepX1” and “PepX2” each consists of, one independently from the other, a spacer polypeptide having an amino acid length varying from 0 to 10 amino acid residues.

7. The cyclic peptides according to claim 6 wherein the cyclic peptide is selected from the group consisting of :

CSTLNLKNENC (SEQ ID NO: 13) and

CSKFTNNMLAIAGC(SEQ ID NO: 14)

8. A pharmaceutical composition, comprising an isolated peptide according to any one of claims 1 to 3 or an antibody according to claim 4-5, or cyclic peptide according to claim 6-7 and one or more pharmaceutically acceptable excipient.

9. A vaccine composition comprising an isolated peptide according to claims 1-3 or a cyclic peptide according to claim 6-7 and an immunoadjuvant compound.
10. The vaccine composition according to claim 9, wherein said antigenic compound has the following formula (IV) :
- NH₂-PepNt-[(I)_n-PepX_n]_n-PepCt- COOH (IV),
wherein :
- “PepNt” consists of a polypeptide having an amino acid length varying from 0 to 100 amino acid residues and located at the N-terminal end of the polypeptide of formula (III) ;
 - “[I]_n-PepX_n” consists of a polypeptide unit wherein :
 - “(I)₁” to - “(I)_n” each consists of, one independently from each other, a isolated peptide according to any one of claims 1 to 3 and/or cyclic peptide according to claim 6-7 with n being an integer from 1 to 12; and
 - “PepX₁” to “PepX_n” each consists of, one independently from the other, a spacer polypeptide having an amino acid length varying from 0 to 30 amino acid residues, with n being an integer from 1 to 12; and
 - n is the number of [I]_n-PepX_n polypeptide units in said polypeptide, with n being an integer from 1 to 12; and
 - “PepCt” consists of a polypeptide having an amino acid length varying from 0 to 100 amino acid residues and located at the C-terminal end of the polypeptide of formula (II).
11. The isolated peptide according to claim 1 to 3, the antibody according to claim 4-5, the pharmaceutical according to claim 8, the vaccine composition according to claim 7 to 9, for use for preventing and/or treating Malaria disease.
12. The isolated peptide according to claim 1 to 3, the cyclic peptide according to claim 6, for use for treating inflammatory disease.
13. The isolated peptide for use according to claim 11, the cyclic peptide according to claim 6, wherein the inflammatory disease is selected between acute inflammatory diseases and chronic inflammatory diseases.

14. The isolated peptide for use according to claim 12, wherein said acute inflammatory disease is selected between Systemic Inflammatory Response Syndrome (SIRS), sepsis, peritonitis.
- 5 15. The isolated peptide according to claim 1 to 3, the cyclic peptide according to claim 6, for use for treating CX3CL1 associated disease.

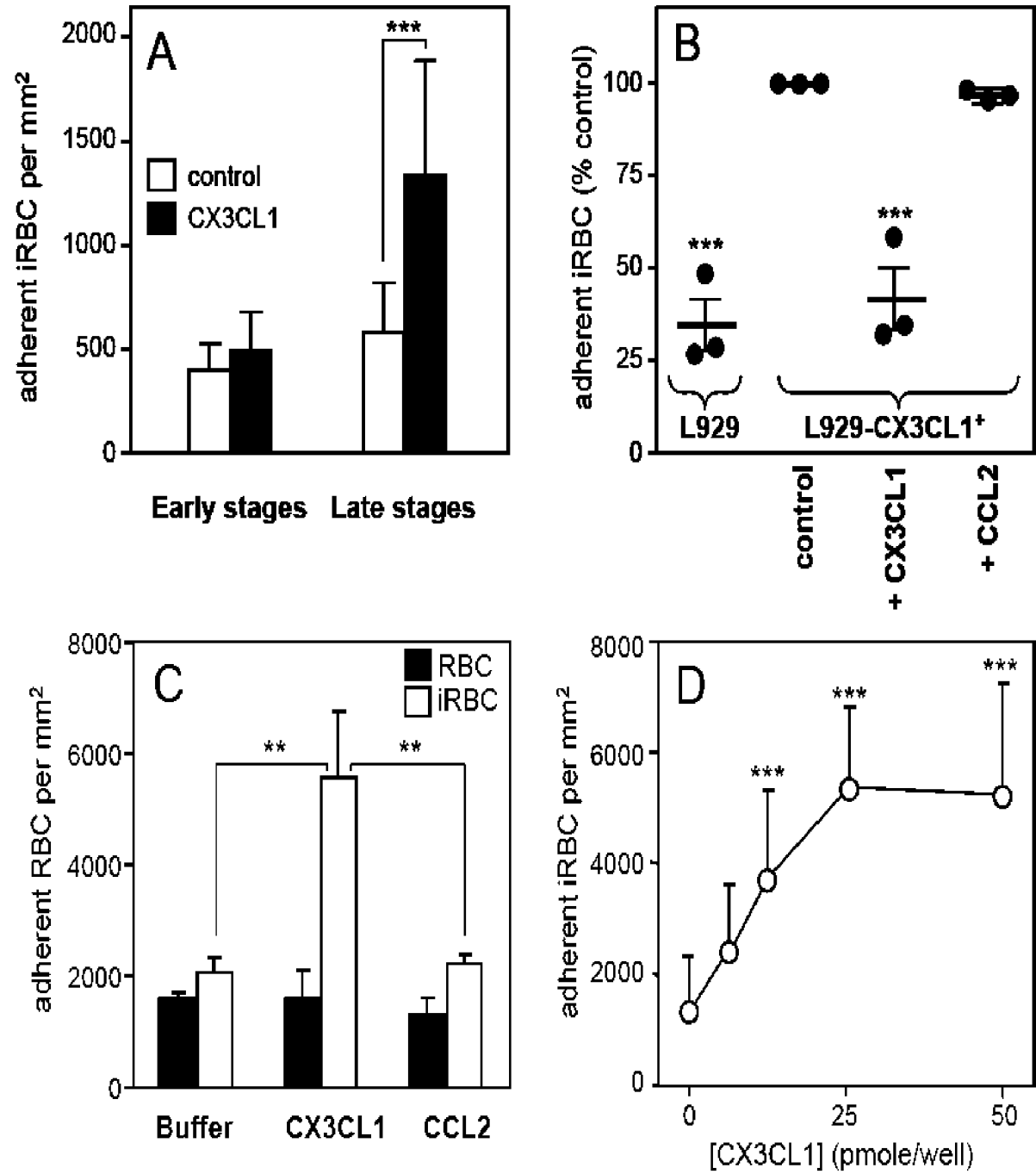
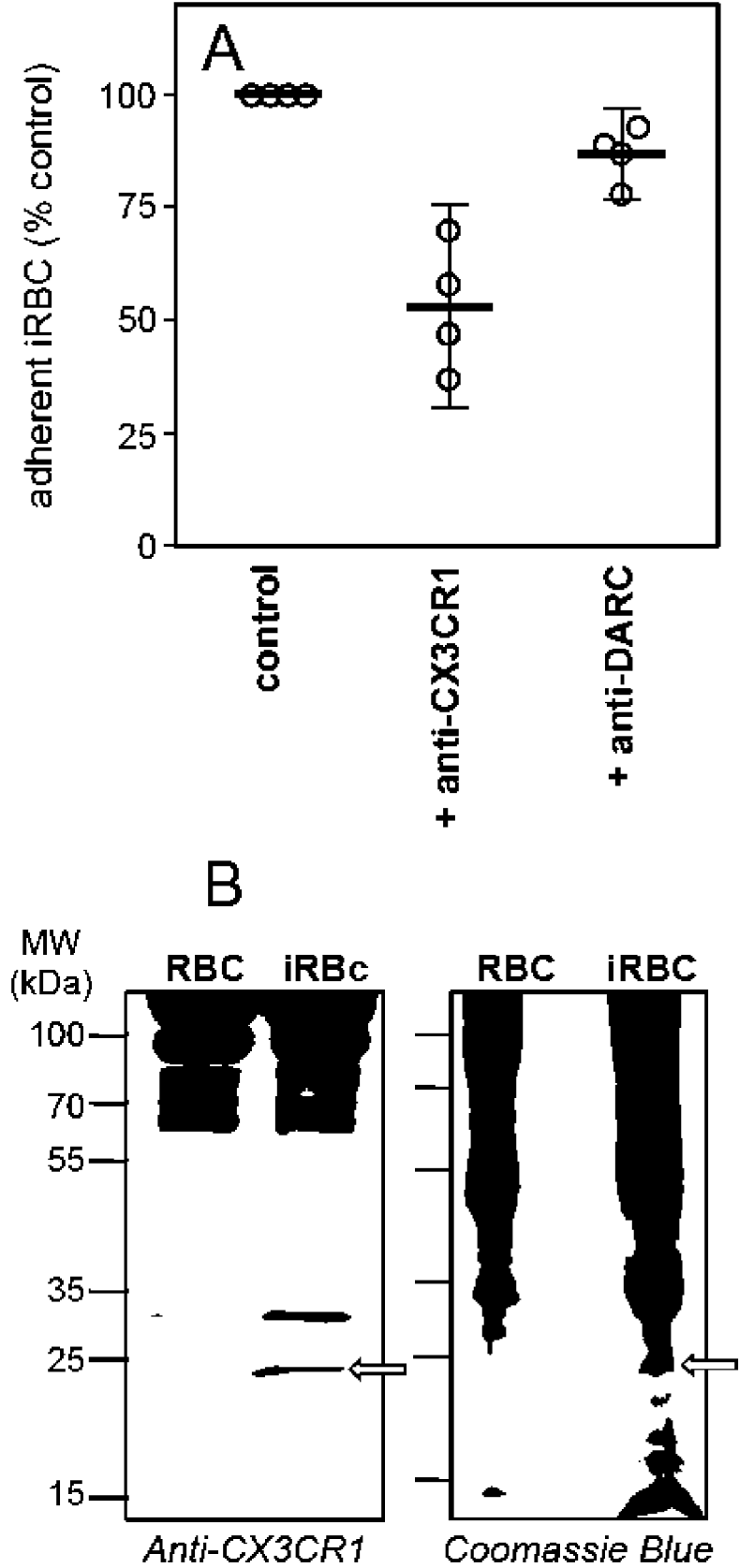


Figure 1

2/11



3/11

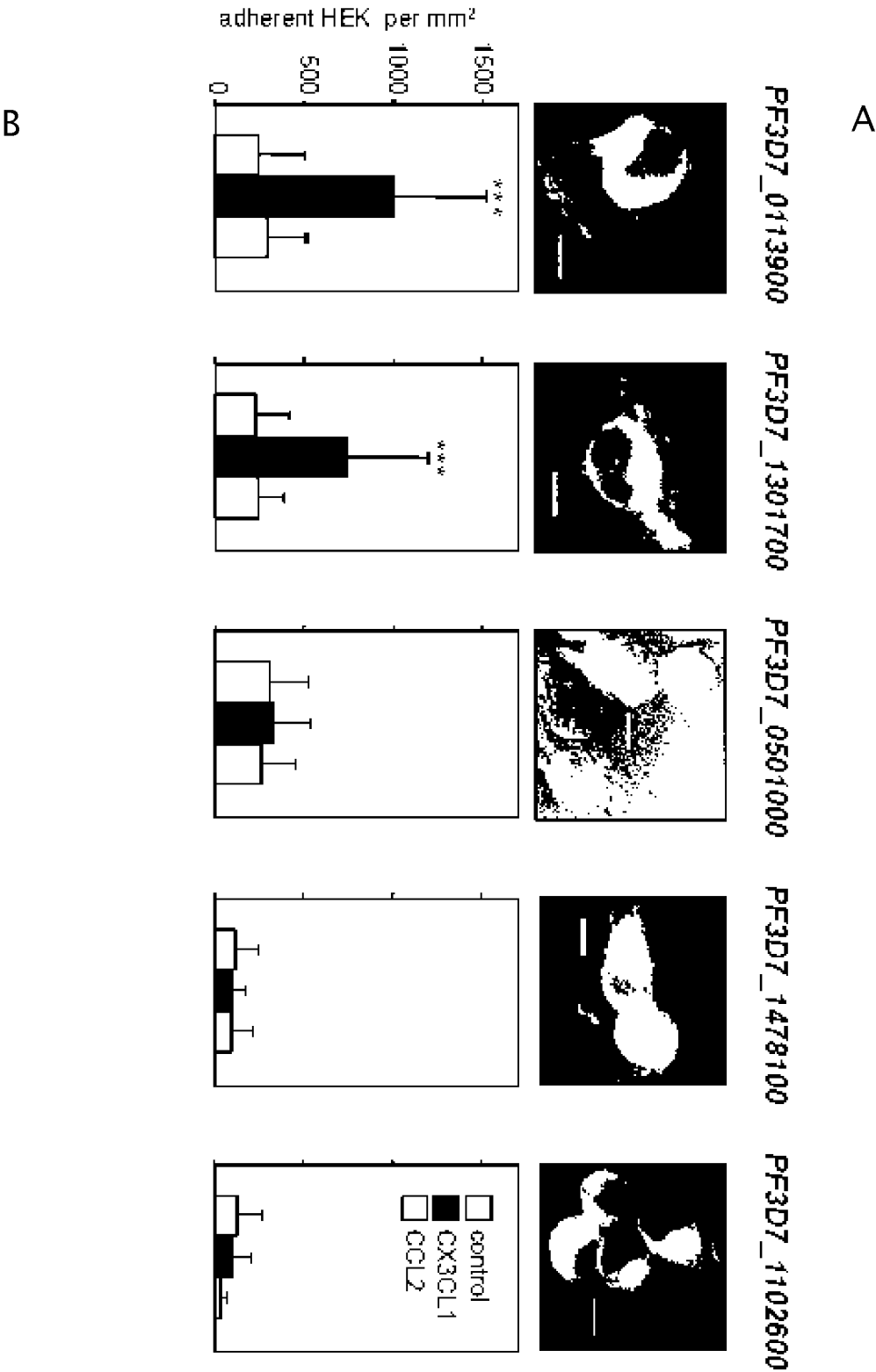


Figure 3

4/11

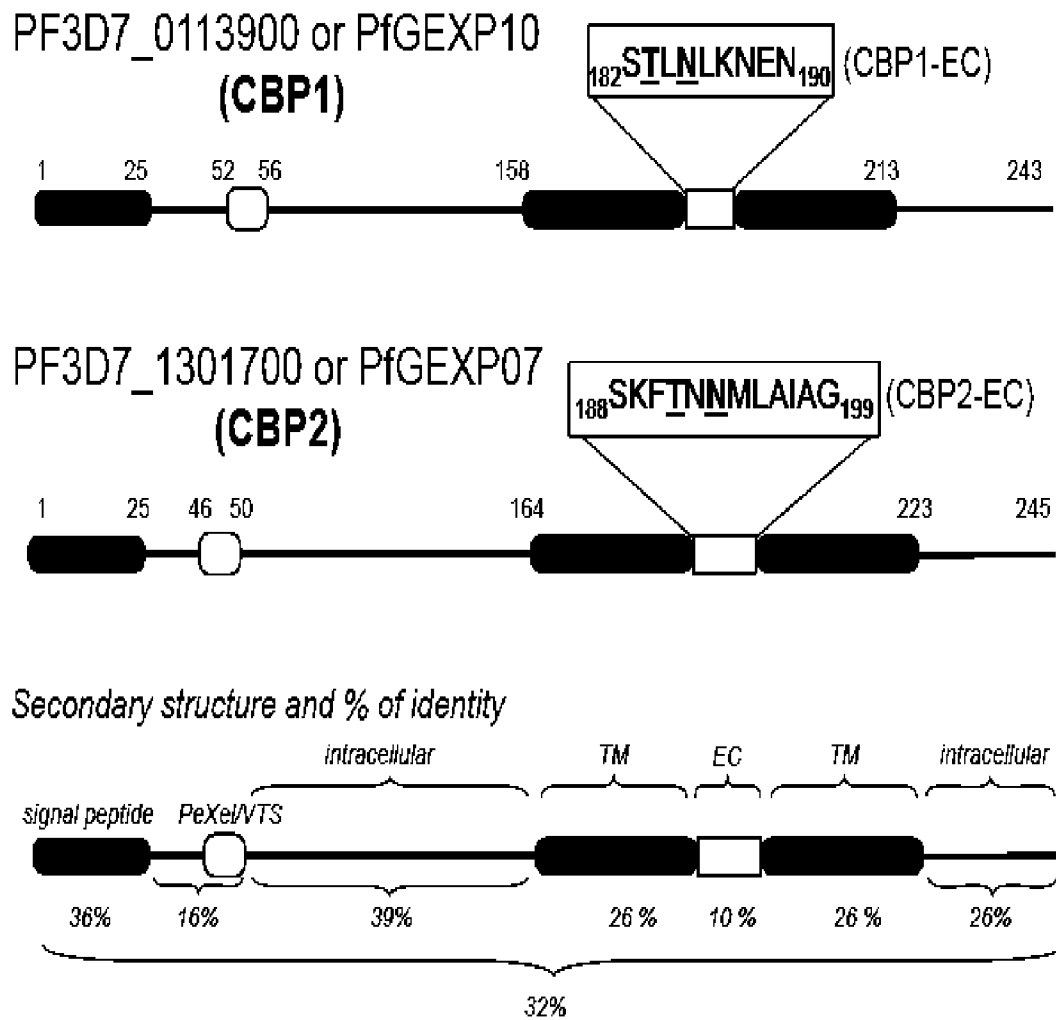


Figure 4

5/11

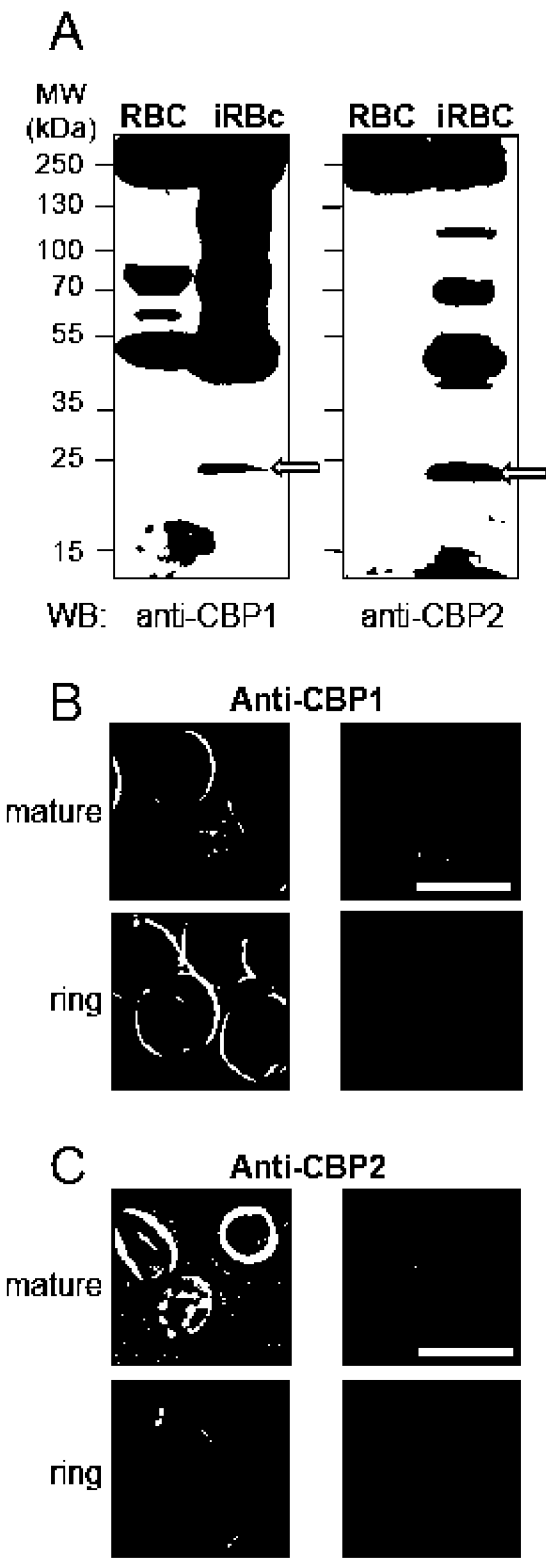


Figure 5

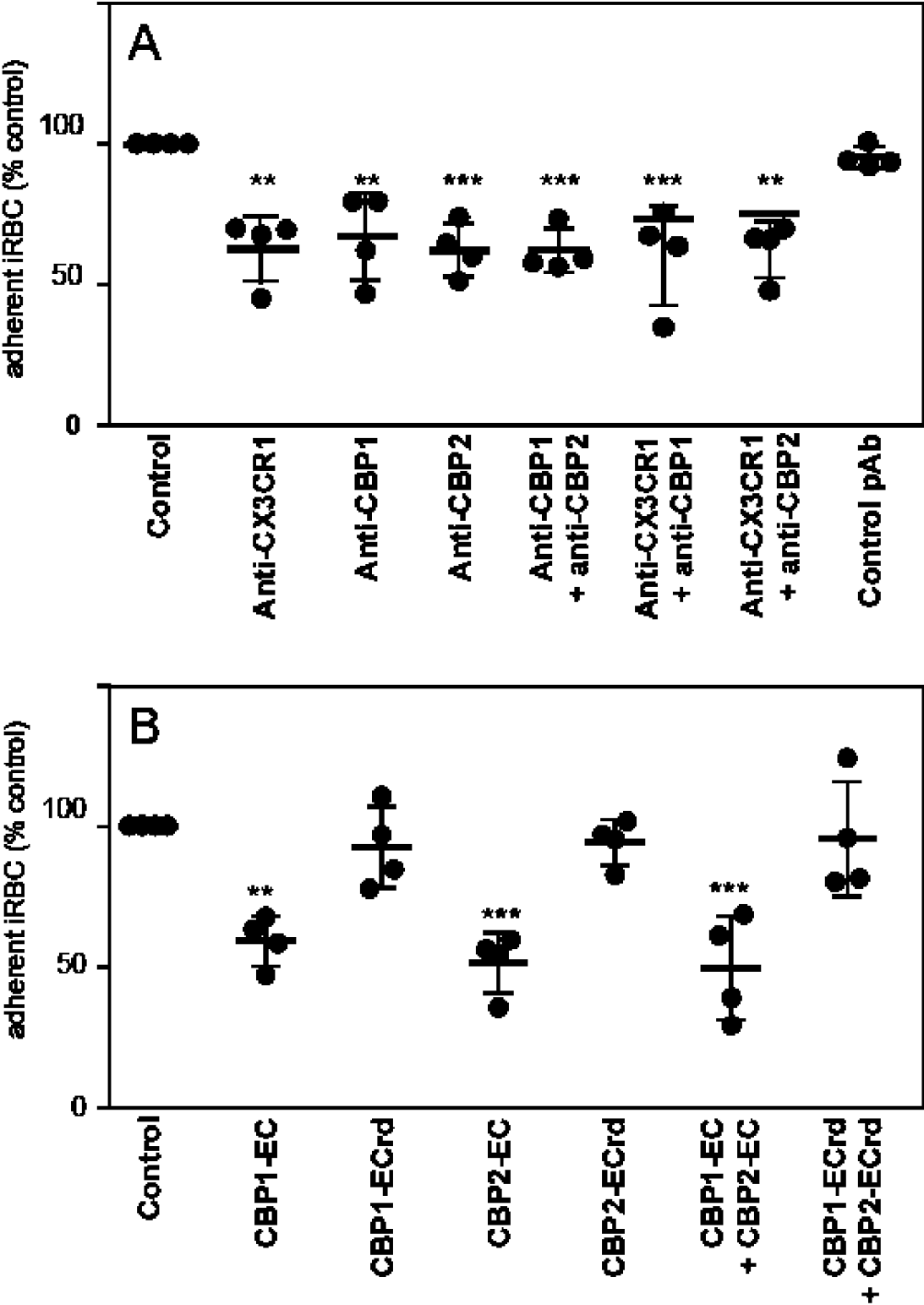
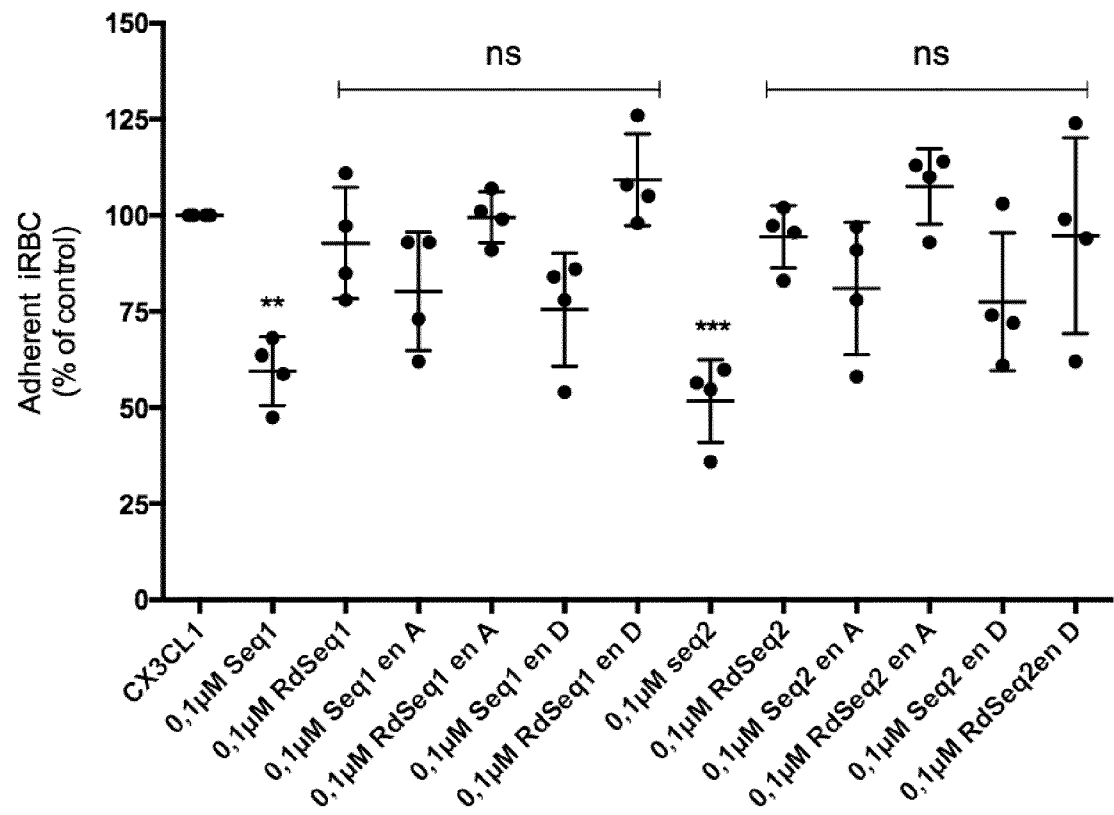


Figure 6

7/11



Seq 1 : STLNLKNEN

Rd Seq 1 : NLETNSKNL

Seq 1-A : STLALKAEA

Rd Seq 1-A : ALETASKAL

Seq 1-D : STLDLKDED

Rd Seq 1-D : DLETDSKDL

Seq 2 : SKFTNNMLAIAG

Rd Seq 2 : MATGSAKNLFNI

Seq 2-A : SKFTAAMLAIAG

Rd Seq 2-A : MATGSAKALFAI

Seq 2-D : SKFTDDMLAIAG

Rd Seq 2-D : MATGSAKDLFDI

Figure 7

8/11

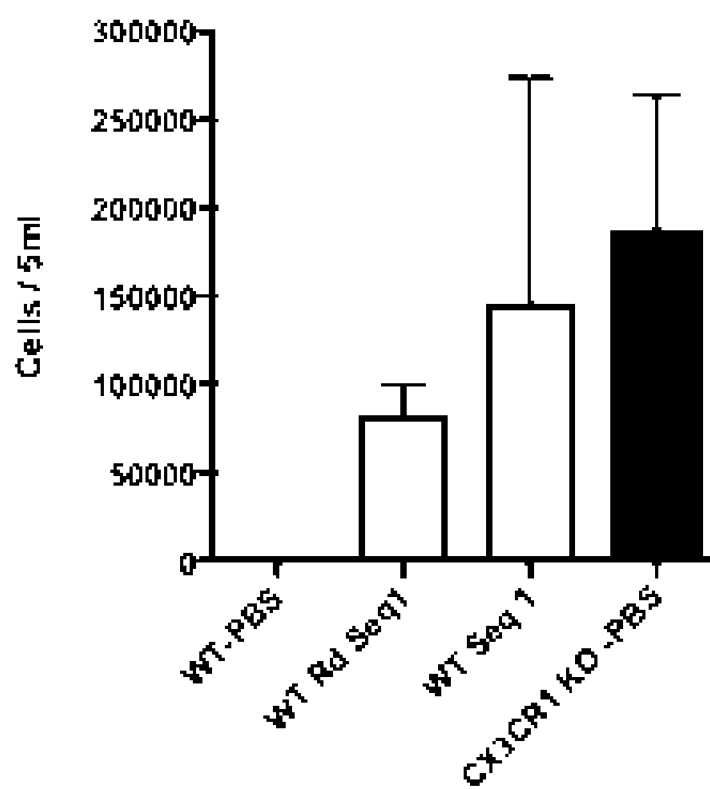
LPS Model - Early stage**Inflammatory monocytes in the peritoneum**

Figure 8

9/11

Thioglycolate Model - Late stage
Inflammatory monocytes in the peritoneum

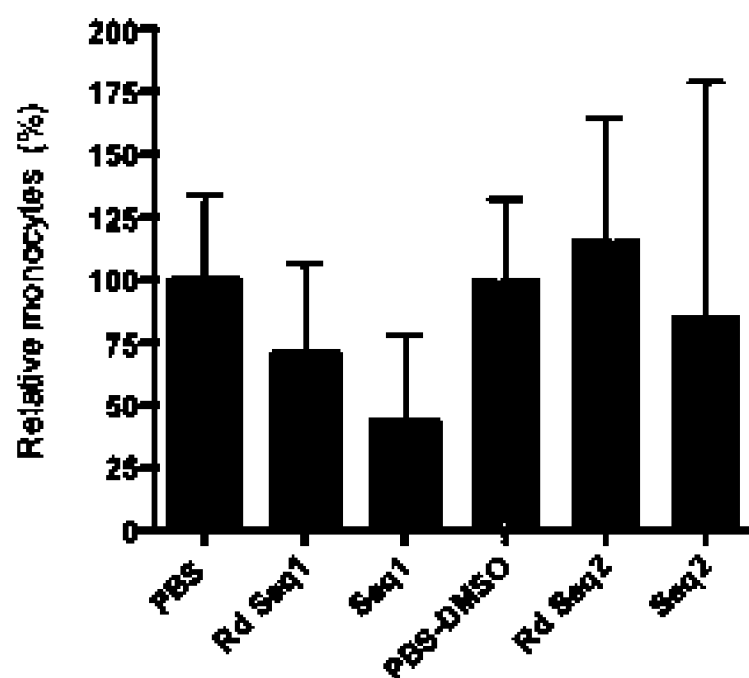


Figure 9

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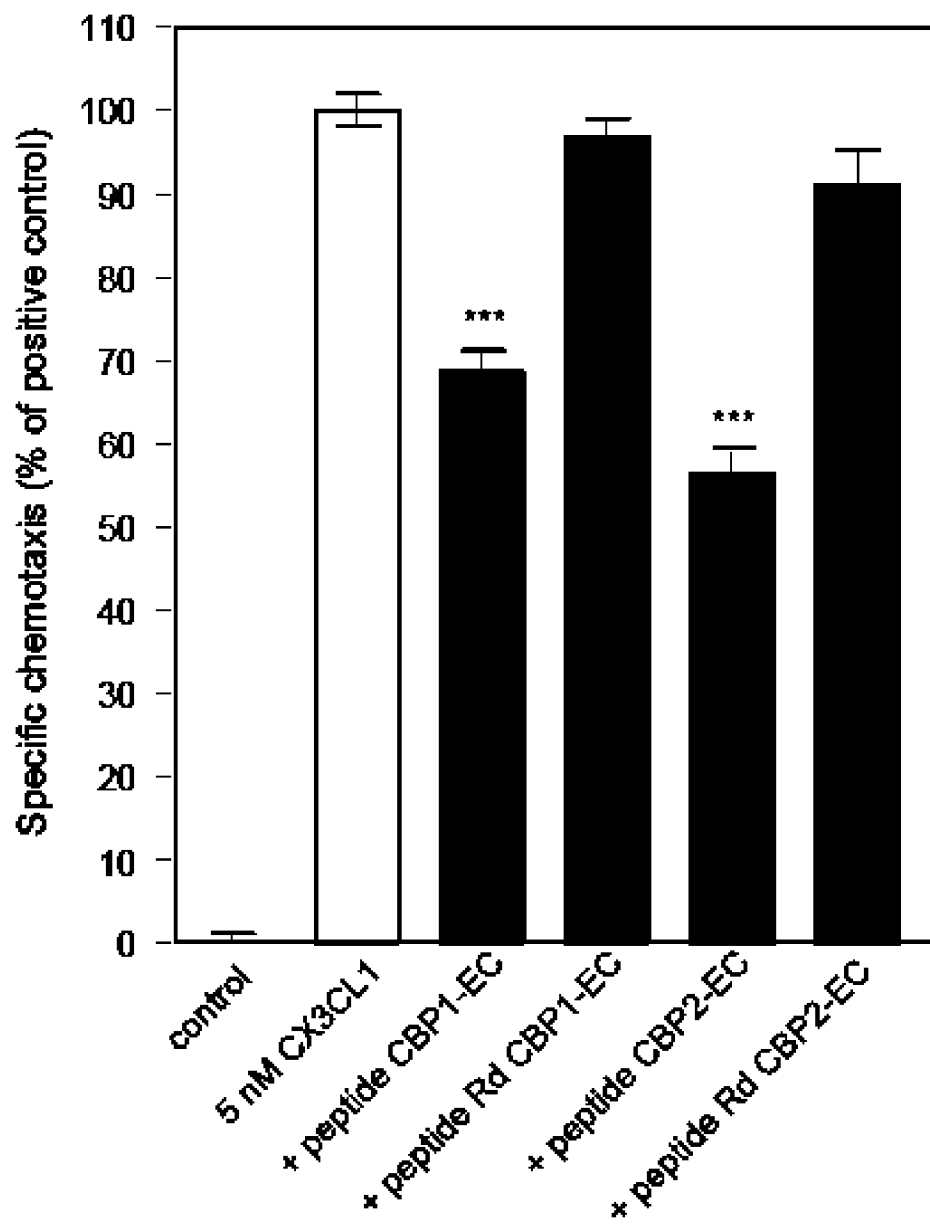


Figure 10

11/11

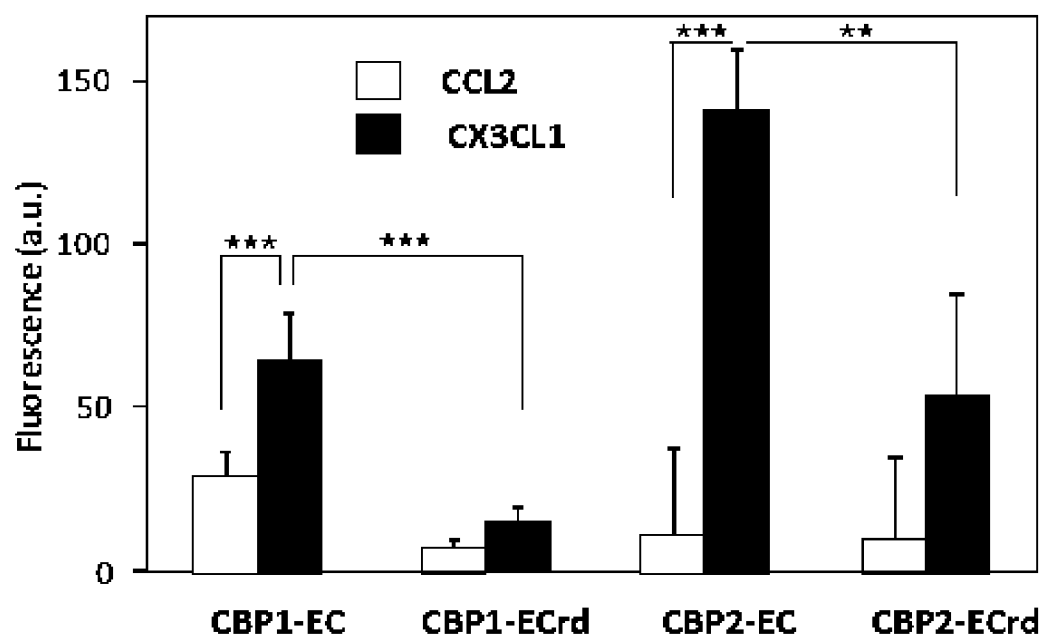


Figure 11

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2016/060755

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K14/445 C12N15/30 C07K16/20 A61P33/06
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, Sequence Search, CHEM ABS Data, WPI Data, EMBASE, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X A	WO 2009/094726 A1 (INST MEDICAL W & E HALL [AU]; COWMAN ALAN [AU]; BEESON JAMES [AU]; MAI) 6 August 2009 (2009-08-06) page 59, line 12 - page 60, line 34; claims 19-23; sequences 37,43 page 10, line 10 - line 28 page 9, line 11 - line 17 -----	1,2,4,5, 8,9, 11-13,15 6,7,14
X	WO 03/079746 A2 (SQUIBB BRISTOL MYERS CO [US]; FEDER JOHN [US]; RAMANATHAN CHANDRA [US]) 2 October 2003 (2003-10-02) page 32, paragraph 1; sequence 19 page 58, line 21 - line 26; sequence 27 page 90, line 27 - page 110, line 20 ----- -/--	1-5,8-10



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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Date of the actual completion of the international search

16 June 2016

Date of mailing of the international search report

24/06/2016

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Wiame, Ilse

INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2016/060755

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE UniProt [Online] 2 August 2005 (2005-08-02), "SubName: Full=Uncharacterized protein {ECO:0000313 EMBL:AA91466.1}"; XP002746032, retrieved from EBI accession no. UNIPROT:Q4KEN4 Database accession no. Q4KEN4 the whole document	1-5
A	----- T. HATABU ET AL: "Binding of Plasmodium falciparum-infected erythrocytes to the membrane-bound form of Fractalkine/CX3CL1", PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES, vol. 100, no. 26, 23 December 2003 (2003-12-23), pages 15942-15946, XP055220146, US ISSN: 0027-8424, DOI: 10.1073/pnas.2534560100 cited in the application the whole document	1-15
A	----- BRAD E. SLEEBES ET AL: "Inhibition of Plasmeprin V Activity Demonstrates Its Essential Role in Protein Export, PfEMP1 Display, and Survival of Malaria Parasites", PLOS BIOLOGY, vol. 12, no. 7, 1 July 2014 (2014-07-01), page e1001897, XP055219926, US ISSN: 1544-9173, DOI: 10.1371/journal.pbio.1001897 page 8, column 2, last paragraph - page 11, column 1, paragraph 2; figure 6 page 12, column 2, paragraph 3	1-15
A	----- DORGHAM KARIM ET AL: "An engineered CX3CR1 antagonist endowed with anti-inflammatory activity", JOURNAL OF LEUKOCYTE BIOLOGY, FEDERATION OF AMERICAN SOCIETIES FOR EXPERIMENTAL BIOLOGY, US, vol. 86, no. 4, 1 October 2009 (2009-10-01), pages 903-911, XP009126257, ISSN: 0741-5400 cited in the application abstract -----	12-15

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/EP2016/060755

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