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TITLE: ISOLATING AND CHARACTERIZING GENES OF LIVER STAGE ANTIGENS WITH VACCINE POTENTIAL

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A 175kd Plasmodium falciparum blood stage antigen with binds to erythrocytes and to merozoites, designated EBA-175, appears to be involved in the initial attachment of the merozoite to the red cell, and it has been postulated that antibodies to EBA could inhibit merozoite reinvasion. The gene encoding EBA-175 has been isolated, cloned and sequenced by using monospecific antibodies selected from malaria immune monkey serum by affinity purification with EBA-175 adsorbed to nitrocellulose. Peptides predictive of antigenic determinants were synthesised, conjugated to KLH and used to immunise mice and rabbits. Antibodies raised against peptide KIM4 recognized authentic EBA-175 in immunoblots, immunoprecipitated EBA-175 and reacted with free merozoites and segmenters. Furthermore, antibodies against peptide KIM4 inhibited the invasion of merozoites into erythrocytes in vitro. Sequence analyses of the region of the gene coding for peptide KIM4 showed that this region was strictly conserved in 7 strains of P. falciparum studied. These results confirm the importance of EBA-175 and identify a region of the protein upon which vaccine development efforts should focus.

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FOREWORD

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✓ In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (NIH Publication No. 86-23, Revised 1985).

N/A For the protection of human subjects, the investigator(s) have adhered to policies of applicable Federal Law 45CFR46.

✓ In conducting research utilizing recombinant DNA technology, the investigator(s) adhered to current guidelines promulgated by the National Institutes of Health.

Kim Lee Sim — 2/28/90

PI Signature — Date

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INTRODUCTION

Malaria disease which is estimated to directly account for more than 2 million deaths per year worldwide, is caused by the erythrocytic stages of parasites of the genus Plasmodium. It is thought by many to be the most important infectious disease in the world. Aiding the spread of the disease is the increasing incidence of drug resistant strains of the parasite. For these reasons there have been major efforts to develop malaria vaccines. The development of effective vaccines is complicated by the fact that potentially protective antigens are specific for each of the main developmental stages of this parasite. An effective vaccine is envisaged to contain a mixture of different stage antigens. The major focus of this research has been directed against the erythrocytic stages of the parasite; the stage responsible for the pathology and morbidity of the disease.

After release from mature schizonts, merozoites must invade erythrocytes within minutes to ensure further development and maintenence of the life cycle. The process of merozoite recognition and invasion of erythrocytes involves a sialic acid-dependent ligand (1-3). A previous study described a 175 K protein antigen in culture supernatants that binds to a sialic acid-dependent ligand on erythrocytes and to an unknown ligand on merozoites (4), acting as a bridge between erythrocyte and merozoite. This molecule, EBA-175, has obvious value as a target antigen for vaccine development as antibodies against it may interfere effectively with the invasion of merozoites into erythrocytes.

The purpose of the present work is to isolate and characterise the gene encoding EBA-175, select portions of the protein encoded by this gene for immunogenicity studies, and to determine if the antibodies elicited by such antigens inhibit the invasion of merozoites into erythrocytes.

The methods of approach used were to screen a genomic expression library with an affinity purified antibody to EBA-175, select approprate clones, sequence the clones to obtain the entire sequence of the gene encoding EBA-175, analyse the deduced amino acid sequence for potentially immunogenic domains, synthesise peptides from these domains, immunise mice and rabbits with these peptides, and determine if the antibodies induced by such immunizations recognized the peptides, blood stage parasites, EBA-175 on Western blots, and inhibited merozoite invasion of erythrocytes.

BODY

A) SUMMARY OF ANNUAL REPORT (FEBRUARY 1988 - FEBRUARY 1989)

It was reported in the annual report that a clone containing part of the gene encoding EBA-175 had been identified from the screening of an expression library. The following is a brief description of sections which have been described in detail in the annual report:

i) Reagent to screen library: This was a monospecific affinity purified antibody to EBA-175.

ii) Genomic library: A genomic expression library in gt11 was constructed and screened .

iii) Screening the library: A clone designated EBA9, containing an insert size of 1.8 kb was identified.

iv) Confirmatory studies: The clone with the 1.8 kb insert was introduced into an expression host and proteins expressed were analysed in Western blots to confirm that the 1.8 kb was actually part of the gene encoding EBA-175.

v) Sequencing the clone EBA9: The entire clone was sequenced by the dideoxy chain termination method of Sanger.

vi) Characterization of the 1.8 kb insert: This insert was used as a probe in genomic Southern analyses to show the strain conservation of the EBA-175 gene.

vii) Synthetic peptides predictive of antigenic determinants: Synthetic peptides were made from regions of the molecule with high hydrophilicity indices and large beta turns.

viii) Immunization with synthetic peptides: Peptides were conjugated to KLH acting as a carrier and used to immunise mice and rabbits.

ix) Isolating other regions of the EBA-175 gene: Attempts were made to isolate a 5.5 kb XbaI restriction fragment which contains further upstream sequences of the EBA-175 gene.

B) RESEARCH REPORT (FEBRUARY 1989 - FEBRUARY 1990)

1) Isolation of the complete gene encoding EBA-175.

The size selected XbaI genomic library was screened with for the clone containing the 5.5 kb fragment, using labeled 1.8 kb as probe. The library was plated at a concentration of 1,000 colonies per large Petri plate. The library was screened as described by Sim (4). Briefly, duplicate nitrocellulose filter lifts of the colonies were made and lysed in situ by placing them sequentially in puddles of 0.5M NaOH, 1M Tris pH7.4 and 1.5M NaCl, 0.5M Tris pH 7.4 prior to immobilization to filters and hybridization . More than 3,000 clones of the library were screened in this attempt. Two positive clones were identified, and both contained inserts of the expected 5.5 kb. Restriction analyses with enzymes Eco RI, DdeI and HindIII showed that these two clones were identical. As such only one of the clones, designated the Xba 5.5 kb clone was selected for further study. The strategy used to sequence further unidentified upstream sequences in this 5.5 kb fragment was as follows. The enzyme Exonuclease III was used to digest the 5.5 kb fragment sequentially generating deletion fragments. Hybridization analyses identified the deletion fragments which were located further upstream from the 1.8 kb fragment. These upstream fragments were cloned into the bidirectional sequencing vector pGEM 4Z, and sequencing was by method of Sanger (5). There was an additional 2.1 kb of new upstream open reading frame sequences.

The approach taken to obtain further upstream sequences were as follows. Oligonucleotide primers were synthesized and used to amplify a 500 bp fragment at the 5' region of the Xba 5.5 kb clone by the polymerase chain reaction (6). The amplification reaction was performed for 30 cycles in a Perkin-Elmer Cetus thermal cycler (programed for 1.5 mins.at 95°C, 2 mins. at 45°C, and 4 mins. at 72°C, followed by a final step of 5 mins. at 72°C. This amplified fragment was labeled and used as a probe to screen the previously mentioned gt11 library. Three positive plaques (9_2, 13_1 and 13_2)were identified and used as individual probes in genomic Southerns. The clone 9_2 was found to contain no new upstream sequences. The clones 13_1 and 13_2 were found to be identical and sequence analysis showed that they both contained an additional 400 bp of new upstream sequences.

The same strategy was used to identify fragments containing the rest of the gene encoding EBA-175. Essentially, 20 bp oligonucleotide primers at the 5' region of the 13_1 clone were synthesized and used to amplify a 250 bp fragment using the polymerase chain reaction. This amplified fragment was used as a probe to identify a 3.5 kb XbaI fragment on a genomic Southern. To obtain this 3.5 kb fragment, a size selected (2.3 kb - 4.4 kb) XbaI library was constructed in pGEM4Z, and screened using the 250 bp amplified fragment as probe. A positive clone (Xba 14) containing an insert of the correct size and hybridization profile was obtained. Clone Xba 14 was sequenced and found to contain the rest of the gene.

The gene encoding EBA-175 was found to be 4.281 kb and contained no introns (Fig.1) Unlike most P. falciparum genes which have been studied, the gene encoding EBA-175 does not contain repeats. The deduced amino acid sequence of EBA-175 contains regions that are similar *to the sialic acid binding residues of influenza virus hemagglutinin* (boxed residues in Fig. 1).

2) Synthetic peptides predictive of antigenic determinants.

Two peptides KIM2 and KIM4 were synthesized and used to immunise mice and rabbits as reported in the annual report. Antibodies raised against peptide KIM2 did not recognise P. falciparum parasites in immunofluorescence analyses, did not recognise authentic EBA-175 in Western blots nor did they immunoprecipitate EBA-175.

Antibodies against peptide KIM4 recognized free merozoites as well as merozoites in segmenters [Fig.2. Phase contrast a,c; immunofluorescence b,d)] in immunofluorescence analyses (7). Furthermore, antibodies to peptide KIM4 recognized authentic EBA-175 in Western blots and immunoprecipitated EBA-175. Immunoprecipitation and fluorography were performed as described (8,9).

3) The biological function of antibodies to peptide KIM4

Antibodies against peptide KIM4 were found to inhibit the invasion of merozoites into erythrocytes in vitro. Inhibition of merozoite invasion assays were performed as previously described (10) and anti peptide KIM4 sera were preabsorbed on human erythrocytes and heat inactivated ($56^{o}C$, 30 mins.) prior to use. Percent inhibition was calculated based on sera from rabbits immunized with KLH alone. The actual percentage of infected erythrocytes was significantly lower ($p<0.005$, student's t test) in the erythrocytes exposed to anti peptide KIM4 sera as compared to anti-KLH sera at serum dilutions of 1/5 (2.3 ± 0.33 vs 13.7 ± 0.33, mean $\pm$ SEM percentage of infected erythrocytes), 1/10 (4.3 ± 1.20 vs 13.7 ± 0.33), and 1/20 (8.3 ± 0.33 vs 14.0 ± 0.33). Inhibition of invasion was also seen in 3 separate experiments performed in triplicates.

4) Sequence conservation of the peptide KIM4 functional domain

A 492 bp fragment (amino acid residues 992-1155) inclusive of KIM4 was amplified in 7 strains of P. falciparum [Camp (Malaysia), FCR3 (Uganda), Thai TN (Thailand), ItG (Brazil), 7G8 (Brazil), Honduras, LE5 (Liberia)] using oligonucleotide primers by the polymerase chain reaction (6). Five ul of reaction mixture was fractionated on a 1% agarose gel to confirm the size of the amplified products, and the gel was Southern blotted and probed with the 1.8 kb fragment of EBA-175 which included the sequence corresponding to EBA-peptide KIM4, to further confirm the specificity of the amplified products. The amplified products were ligated into the bidirectional sequencing vector pGEM4Z (Promega). Clones containing inserts were sequenced (Sequenase, U.S. Biochemical). The sequencing primers used were SP6 and T7. At least 3 randomly picked clones from each parasite strain were sequenced to acertain the sequence obtain.

CONCLUSIONS

The aim of this project was to identify potential malaria vaccine candidates. An erythrocyte binding antigen of malaria with a molecular weight of 175 kd (EBA-175) identified in culture supernatants, was previously shown to bind to a sialic acid-dependent ligand on erythrocytes and appeared to function as a parasite invasion receptor. It was postulated that antibodies against EBA-175 would interfere effectively with the parasite invasion process into red cells. An affinity purified monospecific antibody to EBA-175 was used to screen an expression library for the gene encoding EBA-175. This gene was cloned and sequenced. A 43 amino acid peptide (KIM4) predictive of an antigenic determinant was selected from the deduced 1426 amino acid sequence of EBA-175. Antibodies raised against peptide KIM4 inhibited the invasion of parasites into erythrocytes. The nucleotide sequence encoding the peptide KIM4 was shown to be strictly conserved among 7 strains of P. falciparum studied. These results confirm the importance of EBA-175 in parasite invasion and identify a region upon which vaccine development efforts should focus.

```
 -55                                                                                                     TATATATATATATATATATAGATAA  TAACATATAAATATATTCAATGTGCATACA
      M K C N I S I Y F F         A S F F V L Y F A K         A R N E Y D I K E N         E K F L D V Y K E K         F N E L D K K K Y G  50
   1  ATGAAATGTAATATTAGTATATATTTTTTT  GCTTCCTTCTTTGTGTTATATTTTGCAAAA  GCTAGGAATGAATATGATATAAAAGAGAAT  GAAAAATTTTTAGACGTGTATAAAGAAAAA  TTTAATGAATTAGATAAAAAGAAATATGGA
      N V Q K T D K K I F         T F I E N K L D I L         N N S K F N K R W K         S Y G T P D N I D K         N M S L I N K H N N  100
 151  AATGTTCAAAAAACTGATAAGAAAATATTT  ACTTTTATAGAAAATAAATTAGATATTTTA  AATAATTCAAAATTTAATAAAAGATGGAAG  AGTTATGGAACTCCAGATAATATAGATAAA  AATATGTCTTTAATAAATAAACATAATAAT
      E E M F N N N Y Q S         F L S T S S L I K Q         N K Y V P I N A V R         V S R I L S F L D S         R I N N G R N T S S  150
 301  GAAGAAATGTTTAACAACAATTATCAATCA  TTTTTATCGACAAGTTCATTAATAAAGCAA  AATAAATATGTTCCTATTAACGCTGTACGT  GTGTCTAGGATATTAAGTTTCCTGGATTCT  AGAATTAATAATGGAAGAAATACTTCATCT
      N N E V L S N C R E         K R K G M K W D C K         K K N D R S N Y V C         I P D R R I Q L C I         V N L S I I K T Y T  200
 451  AATAACGAAGTTTTAAGTAATTGTAGGGAA  AAAAGGAAAGGAATGAAATGGGATTGTAAA  AAGAAAAATGATAGAAGCAACTATGTATGT  ATTCCTGATCGTAGAATCCAATTATGCATT  GTTAATCTTAGCATTATTAAAACATATACA
      K E T M K D H F I E         A S K K E S Q L L L         K K N D N K Y N S K         F C N D L K N S F L         D Y G H L A M G N D  250
 601  AAAGAGACCATGAAGGATCATTTCATTGAA  GCCTCTAAAAAAGAATCTCAACTTTTGCTT  AAAAAAAATGATAACAAATATAATTCTAAA  TTTTGTAATGATTTGAAGAATAGTTTTTTA  GATTATGGACATCTTGCTATGGGAAATGAT
      M D F G G Y S T K A         E N K I Q E V F K G         A H G E I S E H K I         K N F R K E W W N E         F R E K L W E A M L  300
 751  ATGGATTTTGGAGGTTATTCAACTAAGGCA  GAAAACAAAATTCAAGAAGTTTTTAAAGGG  GCTCATGGGGAAATAAGTGAACATAAAATT  AAAAATTTTAGAAAAGAATGGTGGAATGAA  TTTAGAGAGAAACTTTGGGAAGCTATGTTA
      S E H K N N I N N C         K N I P Q E E L Q I         T Q W I K E W H G E         F L L E R D N R S K         L P K S K C K N N T  350
 901  TCTGAGCATAAAAATAATATAAATAATTGT  AAAAATATTCCCCAAGAAGAATTACAAATT  ACTCAATGGATAAAAGAATGGCATGGAGAA  TTTTTGCTTGAAAGAGATAATAGATCAAAA  TTGCCAAAAAGTAAATGTAAAAATAATACA
      L Y E A C E K E C I         D P C M K Y R D W I         I R S K F E [W] H [T] L         S K E Y E T Q K V P         K E N A E N Y L I K  400
1051  TTATATGAAGCATGTGAGAAGGAATGTATT  GATCCATGTATGAAATATAGAGATTGGATT  ATTAGAAGTAAATTTGAATGGCATACGTTA  TCGAAAGAATATGAAACTCAAAAAGTTCCA  AAGGAAAATGCGGAAAATTATTTAATCAAA
      I S E N K N D A K V         S L L L N N C D A E         Y S K Y C D C K H T         T T L V K S V L N G         N D N T I K E K R E  450
1201  ATTTCAGAAAACAAGAATGATGCTAAAGTA  AGTTTATTATTGAATAATTGTGATGCTGAA  TATTCAAAATATTGTGATTGTAAACATACT  ACTACTCTCGTTAAAAGCGTTTTAAATGGT  AACGACAATACAATTAAGGAAAAGCGTGAA
      H I D L D D F S K F         G C D K N S V D T N         T K V W E C K N P Y         I L S T K D V C V P         P R R Q E L C L G N  500
1351  CATATTGATTTAGATGATTTTTCTAAATTT  GGATGTGATAAAAATTCCGTTGATACAAAC  ACAAAGGTGTGGGAATGTAAAAACCCTTAT  ATATTATCCACTAAAGATGTATGTGTACCT  CCGAGGAGGCAAGAATTATGTCTTGGAAAC
      I D R I Y D K N L L         M I K E H I L A I A         I Y E S R I L K R K         Y K N K D D K E V C         K I I N K T F A D I  550
1501  ATTGATAGAATATACGATAAAAACCTATTA  ATGATAAAAGAGCATATTCTTGCTATTGCA  ATATATGAATCAAGAATATTGAAACGAAAA  TATAAGAATAAAGATGATAAAGAAGTTTGT  AAAATCATAAATAAAACTTTCGCTGATATA
      R D I I G G T D Y W         N D L S N R K L V G         K I N T N S K Y V H         R N K K N D K L F R         D E W W K V I K K D  600
1651  AGAGATATTATAGGAGGTACTGATTATTGG  AATGATTTGAGCAATAGAAAATTAGTAGGA  AAAATTAACACAAATTCAAAATATGTTCAC  AGGAATAAAAAAAATGATAAGCTTTTTCGT  GATGAGTGGTGGAAAGTTATTAAAAAAGAT
      V W N V I S W V F K         D K T V C K E D D I         E N I P Q F F R W F         S E W G D D Y C Q D         K T K M I E T L K V  650
1801  GTATGGAATGTGATATCATGGGTATTCAAG  GATAAAACTGTTTGTAAAGAAGATGATATT  GAAAATATACCACAATTCTTCAGATGGTTT  AGTGAATGGGGTGATGATTATTGCCAGGAT  AAAACAAAAATGATAGAGACTCTGAAGGTT
      E C K E K P C E D D         N C K S K C N S Y K         E W I S K K K E E Y         N K Q A K Q Y Q E Y         Q K G N N Y K M Y S  700
1951  GAATGCAAAGAAAAACCTTGTGAAGATGAC  AATTGTAAAAGTAAATGTAATTCATATAAA  GAATGGATATCAAAAAAAAAAGAAGAGTAT  AATAAACAAGCCAAACAATACCAAGAATAT  CAAAAAGGAAATAATTACAAAATGTATTCT
      E F K S I K P E V Y         L K K Y S E K C S N         L N F E D E F K E E         L H S D Y K N K C T         M C P E V K D V P I  750
2101  GAATTTAAATCTATAAAACCAGAAGTTTAT  TTAAAGAAATACTCGGAAAAATGTTCTAAC  CTAAATTTCGAAGATGAATTTAAGGAAGAA  TTACATTCAGATTATAAAAATAAATGTACG  ATGTGTCCAGAAGTAAAGGATGTACCAATT
      S I I R N N E Q T S         Q E A V P E E N T E         I A H R T E T P S I         S E G P K G N E Q K         E R D D D S L S K I  800
2251  TCTATAATAAGAAATAATGAACAAACTTCG  CAAGAAGCAGTTCCTGAGGAAAACACTGAA  ATAGCACACAGAACGGAAACTCCATCTATC  TCTGAAGGACCAAAAGGAAATGAACAAAAA  GAACGTGATGACGATAGTTTGAGTAAAATA
      S V S P E N S R P E         T D A K D T S N L L         K L K G D V D I S M         P K A V I G S S P N         D N I N V T E Q G D  850
2401  AGTGTATCACCAGAAAATTCAAGACCTGAA  ACTGATGCTAAAGATACTTCTAACTTGTTA  AAATTAAAAGGAGATGTTGATATTAGTATG  CCTAAAGCAGTTATTGGGAGCAGTCCTAAT  GATAATATAAATGTTACTGAACAAGGGGAT
      N I S G V N S K P L         S D D V R P D K K E         L E D Q N S D E S E         E T V V N H I S K S         P S I N N G D D S G  900
2551  AATATTTCCGGGGTGAATTCTAAACCTTTA  TCTGATGATGTACGTCCAGATAAAAAGGAA  TTAGAAGATCAAAATAGTGATGAATCGGAA  GAAACTGTAGTAAATCATATATCAAAAAGT  CCATCTATAAATAATGGAGATGATTCAGGC
      S G S A T V S E S S         S S N T G L S I D D         D R N G D T F V R T         Q D T A N T E D V I         R K E N A D K D E D  950
2701  AGTGGAAGTGCAACAGTGAGTGAATCTAGT  AGTTCAAATACTGGATTGTCTATTGATGAT  GATAGAAATGGTGATACATTTGTTCGAACA  CAAGATACAGCAAATACTGAAGATGTTATT  AGAAAAGAAAATGCTGACAAGGATGAAGAT
      E K G A D E E R H S         T S E S L S S P E E         K M L T D N E G G N         S L N H E E V K E H         T S N S D N V Q [Q S]  1000
2851  GAAAAAGGCGCAGATGAAGAAAGACATAGT  ACTTCTGAAAGCTTAAGTTCACCTGAAGAA  AAAATGTTAACTGATAATGAAGGAGGAAAT  AGTTTAAATCATGAAGAGGTGAAAGAACAT  ACTAGTAATTCTGATAATGTTCAACAGTCT
      [G G] I V N M N V E K         E L K D T L E N P S         S S L D E G K A H E         E L S E P N [L S S] D         Q D M S N T P G P L  1050
3001  GGAGGAATTGTTAATATGAATGTTGAGAAA  GAACTAAAAGATACTTTAGAAAATCCTTCT  AGTAGCTTGGATGAAGGAAAAGCACATGAA  GAATTATCAGAACCAAATCTAAGCAGTGAC  CAAGATATGTCTAATACACCTGGACCTTTG
      D N T S E E T T E R         I S N N E Y K V N E         R E D E R T L T K E         [Y] E D I V L K S [H] M         N R E S D [D] G E [L Y]  1100
3151  GATAACACCAGTGAAGAAACTACAGAAAGA  ATTAGTAATAATGAATATAAAGTTAACGAG  AGGGAAGATGAGAGAACGCTTACTAAGGAA  TATGAAGATATTGTTTTGAAAAGTCATATG  AATAGAGAATCAGACGATGGTGAATTATAT
      D E N S D L S T V N         D E S E D A E A K M         K G N D T S E M S H         N S S Q H I E S D Q         Q K N D M K T V G D  1150
3301  GACGAAAATTCAGACTTATCTACTGTAAAT  GATGAATCAGAAGACGCTGAAGCAAAAATG  AAAGGAAATGATACATCTGAAATGTCGCAT  AATAGTAGTCAACATATTGAGAGTGATCAA  CAGAAAAACGATATGAAAACTGTTGGTGAT
      L G T T H V Q N E I         S V P V T G E I D E         K L R E S K E S K I         H K A E E E R L S H         T D I H K I N P E D  1200
3451  TTGGGAACCACACATGTACAAAACGAAATT  AGTGTTCCTGTTACAGGAGAAATTGATGAA  AAATTAAGGGAAAGTAAAGAATCAAAAATT  CATAAGGCTGAAGAGGAAAGATTAAGTCAT  ACAGATATACATAAAATTAATCCTGAAGAT
      R N S N T L H L K D         I R N E E N E R H L         T N Q N I N I S Q E         R D L Q K H G F H T         M N N L H G D G V S  1250
3601  AGAAATAGTAATACATTACATTTAAAAGAT  ATAAGAAATGAGGAAAACGAAAGACACTTA  ACTAATCAAAACATTAATATTAGTCAAGAA  AGGGATTTGCAAAAACATGGATTCCATACC  ATGAATAATCTACATGGAGATGGAGTTTCC
      E R S Q I N H S H H         G N R Q D R G G N S         G N V L N M R S N N         N N F N N I P S R Y         N L Y D K K L D L D  1300
3751  GAAAGAAGTCAAATTAATCATAGTCATCAT  GGAAACAGACAAGATCGGGGGGGAAATTCT  GGGAATGTTTTAAATATGAGATCTAATAAT  AATAATTTTAATAATATTCCAAGTAGATAT  AATTTATATGATAAAAAATTAGATTTAGAT
      L Y E N R N D S T T         K E L I K K L A E I         N K C E N E I S V K         Y C D H M I N E E I         P L K T C T K E K T  1350
3901  CTTTATGAAAACAGAAATGATAGTACAACA  AAAGAATTAATAAAGAAATTAGCAGAAATA  AATAAATGTGAGAACGAAATTTCTGTAAAA  TATTGTGACCATATGATTCATGAAGAAATC  CCATTAAAAACATGCACTAAAGAAAAAACA
      R N L C C A V S D Y         C M S Y F T Y D S E         E Y Y N C T K R E F         D D P S Y T C F R K         E A F S S M I F K F  1400
4051  AGAAATCTGTGTTGTGCAGTATCAGATTAC  TGTATGAGCTATTTTACATATGATTCAGAG  GAATATTATAATTGTACGAAAAGGGAATTT  GATGATCCATCTTATACATGTTTCAGAAAG  GAGGCTTTTTCAAGTATGATATTCAAATTT
      L I T N K I Y Y Y F         Y T Y K T A K V T I         K K I N F S L I F F         F F F S F *                                 1435
4201  TTAATAACAAATAAAATATATTATTATTTT  TATACTTACAAAACTGCAAAAGTAACAATA  AAAAAAATTAATTTCTCATTAATTTTTTTT  TTCTTTTTTTCTTTTTAGGTATGCCATATT  ATGCAGGAGCAGGTGTGTTATTTATTATAT
4351  TGGTTATTTTTAGGTGCTTCACAAGCCAAAT  ATCAAAGGTTAGAAAAAATAAATAAAAATA  AAATTGAGAAGAATGTAAATTAAATATAGA  ATTCGAGCTCGG
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Figure 1. Nucleotide and deduced amino acid sequences of EBA-175. Nucleotides are numbered on the right and amino acids are numbers on the left. EBA-peptide 4 (r-sidues 920-962 on this figure) is underlined. Amino acid residues which are homologous to the sialic acid binding site residues of influenza virus hemagglutinin are boxed.

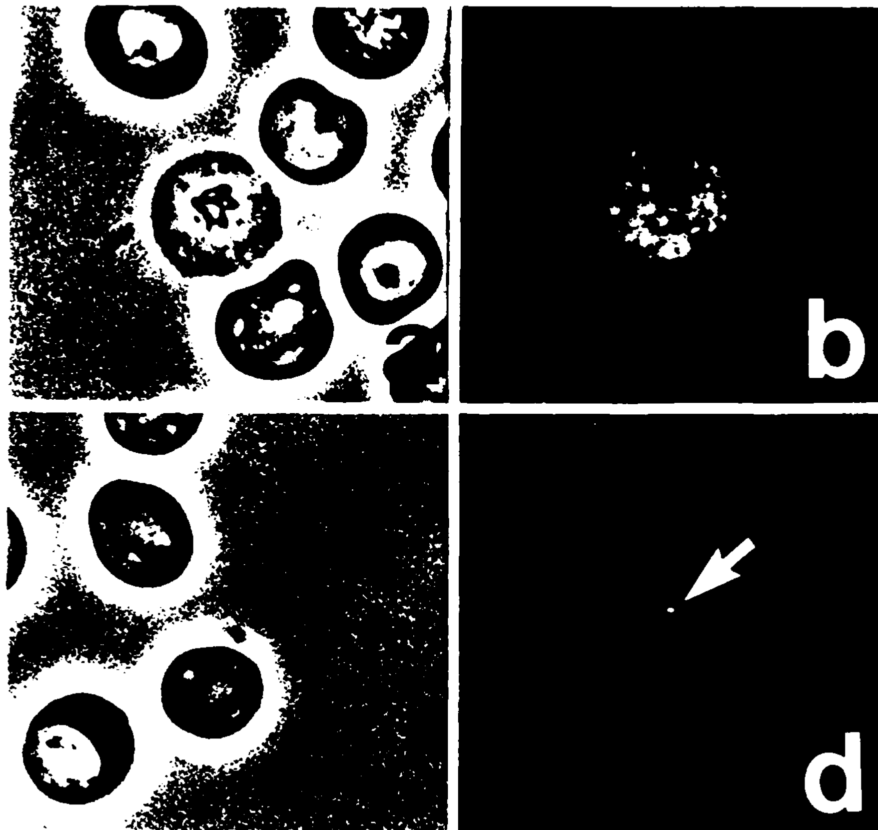

Figure 2. Immunofluorescence localization of EBA-175 by mouse antibodies against EBA-peptide 4 were performed with methanol-fixed thin blood films of Camp strain parasites as previously described. a. schizont-infected erythrocyte (phase contrast); b. schizont-infected erythrocyte showing localization of EBA-175; c. free merozoite (phase contrast); d. free merozoite showing localization of EBA-175. Magnification is 1000X. Identical immunofluorescence results were obtained with rabbit anti-peptide 4 antibody as well as with parasites of the FCR3, ItG and 7G8 strains.

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