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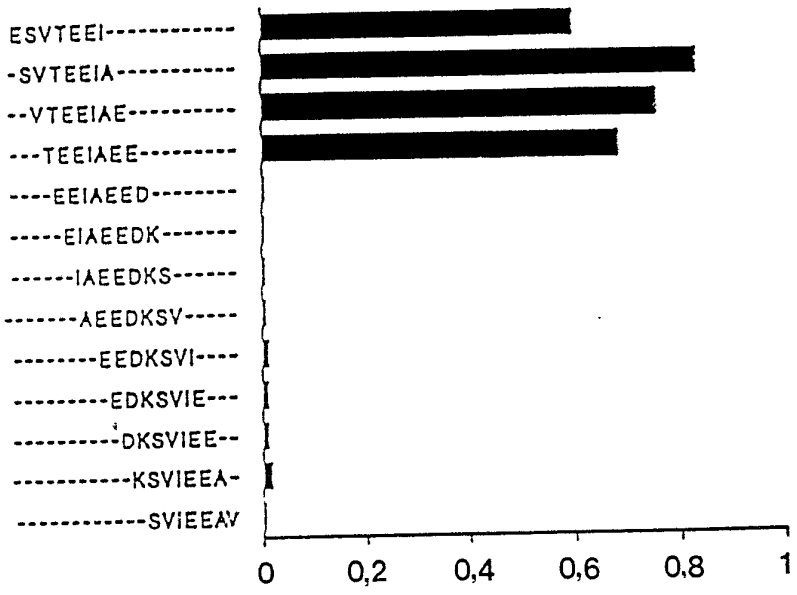

(57) Abstract

A peptide comprising the amino acid sequence: U-O-X-glu-Z or O-X-glu-Z-ala-glu, wherein: U is an amino acid residue selected from val and ile; O is an amino acid residue selected from ala and thr; X is an amino acid residue selected from asp and glu; and Z is an amino acid residue selected from ile and val; the use of the peptide in the preparation of a vaccine; a composition for vaccination against malaria induced by *Plasmodium falciparum*, comprising such peptide in admixture with a pharmaceutically acceptable carrier; and a method of inducing immunity against malaria induced by *Plasmodium falciparum*, which comprises administering to a person in need of such immunity an effective amount of said composition.

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New peptides and their use.

The present invention relates to new peptides, their use as active ingredients in vaccines and compositions containing same.

BACKGROUND OF THE INVENTION

Malaria is a wide spread disease, particularly in the developing countries, and scientists are constantly looking for new means to control the dangerous parasitic disease caused by plasmodial parasites. Among these parasites Plasmodium falciparum causes the most severe disease, responsible for the major part of the mortality due to malaria.

One strategy in combatting malaria resides in the use of a conventional vaccine based on attenuated or dead malaria parasites, but such approach has not been found to be practically feasible.

The alternatives are constituted by development of modern techniques, such as the manufacture of proteins by chemical synthesis or by DNA technology, such proteins as components in subunit vaccines being able to induce protective immunity against the parasite infection.

One strategy in the selection of antigenic sequences to be included in a potential subunit vaccine against Plasmodium falciparum malaria is to define the epitopes of antibodies which have the capacity to interfere with the parasite life cycle. Properly presented in immunogens these epitopes are expected to induce protective antibody responses. With regard to the asexual blood-stages of P.falciparum, the main attention in this context has been made to antibodies with capacity to inhibit merozoite reinvasion in vitro (Anders, R.F. (1985) Candidate antigens for an asexual blood-stage vaccine. Parasitol. Today 1, 152-155). However, antibodies which inhibit the cytoadherence of infected erythrocytes to endothelial cells (Howard, R.J. (1988) Malarial proteins at the membrane of Plasmodium falciparum-infected erythrocytes and their involvement in cytoadherence to endothelial cells. Prog.Allergy 41, 98-147; Udomsangpetch, R., Aikawa, M., Ber-

zins, K., Wahlgren, M. and Perlmann, P. (1989) Cytoadherence of knobless Plasmodium falciparum-infected erythrocytes and its inhibition by a human monoclonal antibody. Nature 338, 763-765) or inhibit rosette formation between uninfected and infected erythrocytes (Carlsson, J., Holmquist, G., Taylor, D.W., Perlmann, P. and Wahlgren, M. (1990) Antibodies to a histidine-rich protein (PfHRP1) disrupt spontaneously formed Plasmodium falciparum erythrocyte rosettes. Proc.Natl.Acad. Sci. USA 87, 2511-2515) may also be of interest. Such antibodies are expected to interfere in vivo with the sequestration of late-stage infected erythrocytes (Howard, R.J. (1988) Malarial proteins at the membrane of Plasmodium falciparum-infected erythrocytes and their involvement in cytoadherence to endothelial cells. Prog.Allergy 41, 98-147; Carlson, J., Holmquist, G., Taylor, D.W., Perlmann, P. and Wahlgren, M. (1990) Antibodies to a histidine-rich protein (PfHRP1) disrupt spontaneously formed Plasmodium falciparum erythrocyte rosettes. Proc.Natl.Acad.Sci. USA 87, 2511-2515).

The human monoclonal antibody (mAb) 33G2, obtained from an Epstein-Barr virus transformed B-cell originating from a Liberian P.falciparum-immune donor (Udomsangpetch, R., Lundgren, K., Berzins, K., Wåhlin, B., Perlmann, H., Troye-Blomberg, M., Carlsson, J., Wahlgren, M., Perlmann, P. and Björkman, A. (1986) Human monoclonal antibodies to Pf155, a major antigen of malaria parasite Plasmodium falciparum. Science 231, 57-59) has several interesting biological properties. It inhibits both P.falciparum merozoite reinvasion in in vitro cultures (Udomsangpetch, R., Lundgren, K., Berzins, K., Wåhlin, B., Perlmann, H., Troye-Blomberg, M, Carlsson, J., Wahlgren, M., Perlmann, P. and Björkman, A. (1986) Human monoclonal antibodies to Pf155, a major antigen of malaria parasite Plasmodium falciparum. Science 231, 57-59) as well as cytoadherence of infected erythrocytes to melanoma cells in vitro (Udomsangpetch, R., Aikawa, M., Berzins, K., Wahlgren, M. and Perlmann, P. (1989) Cytoadherence of knobless Plasmodium falciparum-infected erythrocytes and its in-

hibition by a human monoclonal antibody. Nature 338, 763--765). The mAb, thus, has the capacity to interfere with the parasite erythrocytic life cycle at two potential target sites for protective antibodies in vivo (Anders, R.F. (1985) Candidate antigens for an asexual blood-stage vaccine. Parasitol. Today 1, 152-155) which makes the epitope recognized by the mAb of great interest with regard to vaccine development.

The mAb 33G2 was initially selected due to its reactivity with Pf155/RESA as detected by erythrocyte membrane immunofluorescence (EMIF) and immunoblotting (Udomsangpetch, R., Lundgren, K., Berzins, K., Wåhlin, B., Perlmann, H., Troye-Blomberg, M., Carlsson, J., Wahlgren, M., Perlmann, P. and Björkman, A. (1986) Human monoclonal antibodies to Pf155, a major antigen of malaria parasite Plasmodium falciparum. Science 231, 57-59) but subsequent analyses with recombinant fusion proteins and synthetic peptides revealed that the antibody showed reactivity with a family of cross-reacting P.falciparum blood-stage antigens, including Pf155/RESA, Pf11.1 and Ag332 (Mattei, D., Berzins, K., Wahlgren, M., Udomsangpetch, R., Perlmann, P., Griesser, H.W., Scherf, A., Müller-Hill, B., Bonnefoy, S., Guilotte, M., Langsley, G., Pereira da Silva, L. and Mercereau-Puijalon, O. (1989) Cross--reactive antigenic determinants present on different Plasmodium falciparum blood-stage antigens. Parasite Immunol. 11, 15-30; Mercereau-Puijalon, O., Langsley, G., Mattei, D., Guilotte, M., Blisnick, T., Berzins, K., Griesser, H.W., Scherf, A., Müller-Hill, B. and Pereira da Silva, L. (1987) Presence of cross-reacting determinants on several blood--stage antigens of Plasmodium falciparum. UCLA Symp.Molec. Cell.Biol. 42, 343354; Udomsangpetch, R., Carlsson, J., Wåhlin, B., Holmquist, G., Ozaki, L.S., Scherf, A., Mattei, D., Mercereau-Puijalon, O., Uni, S., Aikawa, M., Perzins, K. and Perlmann, P. (1989) Reactivity of the human monoclonal antibody 33G2 with repeated sequences of three distinct Plasmodium falciparum antigens. J.Immunol. 142, 3620-3626). A

feature shared between these antigens is their contents of several tandemly repeated amino acid sequences containing regularly spaced pairs of glutamic acid (Mattei, D., Berzins, K., Wahlgren, M., Udomsangpetch, R., Perlmann, P., Griesser, H.W., Scherf, A., Müller-Hill, B., Bonnefoy, S., Guillotte, M., Langsley, G., Pereira da Silva, L. and Mercereau-Puijalon, O. (1989) Cross-reactive antigenic determinants present on different Plasmodium falciparum blood-stage antigens. Parasite Immunol. 11, 15-30; Favaloro, J.M., Coppel, R.L., Corcoran, L.M., Foote, S.J., Brown, G.V., Anders, R.F. and Kemp, D.J. (1986) Structure of the RESA gene of Plasmodium falciparum. Nucleic Acids Res. 14, 8265-8277; Scherf, A., Hilbich, C., Sieg, K., Mattei, D., Mercereau-Puijalon, O. and Müller--Hill, B. (1988) The 11-1 gene of Plasmodium falciparum codes for distinct fast evolving repeats. EMBO J. 7, 1129-1137). These dimers of glutamic acid were suggested to be the structures responsible for the antigenic cross-reactions seen between the three antigens (Mattei, D., Berzins, K., Wahlgren, M., Udomsangpetch, R., Perlmann, P., Griesser, H.W., Scherf, A., Müller-Hill, B., Bonnefoy, S., Guilotte, M., Langsley, G., Pereira da Silva, L. and Mercereau-Puijalon, O. (1989) Cross-reactive antigenic determinants present on different Plasmodium falciparum blood-stage antigens. Parasite Immunol. 11, 15-30; Merecereau-Puijalon, O., Langsley, G., Mattei, D., Guilotte, M., Blisnick, T., Berzins, K., Griesser, H.W., Scherf, A., Müller-Hill, B. and Pereira da Silva, L. (1987) Presence of cross-reacting determinants on several blood--stage antigens of Plasmodium falciparum. UCLA Symp.Molec. Cell.Biol. 42, 343-354; Udomsangpetch, R., Carlsson, J., Wåhlin, B., Holmquist, G., Ozaki, L.S., Scherf, A., Mattei, D., Mercereau-Puijalon, O., Uni, S., Aikawa, M., Berzins, K. and Perlmann, P. (1989) Reactivity of the human monoclonal antibody 33G2 with repeated sequences of three distinct Plasmodium falciparum antigens. J.Immunol. 142, 3620-3626). Inhibition with synthetic peptides of the mAb 33G2 binding in EMIF showed that peptides corresponding to Ag332 repeat se-

quences were the most efficient inhibitors, suggesting that Ag332 was the original target for the antibody (Udomsangpetch, R., Carlsson, J., Wåhlin, B., Holmquist, G., Ozaki, L.S., Scherf, A., Mattei, D., Mercereau-Puijalon, O., Uni, S., Aikawa, M., Berrins, K. and Perlmann, P. (1989) Reactivity of the human monoclonal antibody 33G2 with repeated sequences of three distinct Plasmodium falciparum antigens. J.Immunol. 142, 3620-3626).

The major object of the present invention is to provide new peptides capable of inducing immunity against malaria.

Another object of the invention is to provide new compositions for vaccination against malaria comprising such peptide.

Yet another object of the invention is to provide a method of inducing immunity against malaria.

SUMMARY OF THE INVENTION

It has been found that a peptide comprising the amino acid sequence:

U-O-X-glu-Z, wherein:

U is an amino acid residue selected from val and ile;
O is an amino acid residue selected from ala and thr;
X is an amino acid residue selected from asp and glu; and
Z is an amino acid residue selected from ile and val

is capable of providing protective immunity against malaria induced by Plasmodium falciparum. Such protective immunity can be provided also by a peptide comprising the amino acid sequence:

O-X-glu-Z-ala-glu, wherein

O, X and Z have the above meaning.

Preferred embodiments of the peptide of the present invention are the following:

glu-ser-val-thr-glu-glu-ile;
ser-val-thr-glu-glu-ile-ala;
val-thr-glu-glu-ile-ala-glu;
ser-val-thr-glu-glu-ile;
val-thr-glu-glu-ile-ala;
val-thr-glu-glu-ile;
ile-thr-glu-glu-ile;
val-ala-glu-glu-ile; and
ile-ala-asp-glu-ile.

Particularly preferred are the following peptides:
val-thr-glu-glu-ile,
thr-glu-glu-ile-ala-glu-glu, and
thr-glu-glu-ile-ala-glu.

Accordingly, the peptides of the present invention find medicinal use, particularly as acive ingredients in vaccines, such as vaccines against malaria.

The peptides of the present invention are also useful in the preparation of vaccines, particularly vaccines for combatting malaria induced by Plasmodium falciparum.

The invention also covers compositions for vaccination against malaria induced by Plasmodium falciparum, said composition comprising a peptide selected among those defined or mentioned above in admixture with a pharmaceutically acceptable carrier. It is preferred to use carriers suitable for parenteral administration.

As is generally known within immunology the immunogenic response resulting from administration of a relatively small peptide can be enhanced in several ways.

First, it is conceivable to contain the active peptide or principal in a larger molecule, wherein said peptide is present in repeating units. Such polymerized form can be prepared using recombinant DNA techniques.

Second, the peptide can be coupled to a macromolecular carrier, such as bovine serum albumin or other immunogenic

carrier or adjuvant thus inducing a better immune response against the peptide in view of the increased size of the molecule. The antigenic presentation of a small peptide in accordance with the invention can be improved for example by conjugation to a preformed iscom as a carrier (for details see Journal of Immunological Methods, 98 (1987) 137-143, K. Löfgren et al.).

The composition of the present invention can be constituted by a solution, a suspension or other form of preparation. Such solutions or suspension may take the form of sterilized aqueous isotonic preparations, such as isotonic saline solution or glucose solution. As indicated above parenteral administration is preferred.

It goes without saying that although the peptides of the invention can be used alone, combinations of two or more of same can be contained in one and the same composition.

Finally, the present invention provides a method of inducing immunity against malaria induced by Plasmodium falciparum, said method comprising administering to a person in need of such immunity an effective amount of the composition as defined above. The method is particularly exercised in the form of parenteral injection.

In the present disclosure the abbreviations used have the following meanings:

A = ala = alanine;
D = asp = aspartic acid;
C = cys = cysteine
E = glu = glutamic acid;
I = ile = isoleucine;
L = leu = leucine
K = lys = lysine
F = phe = phenylalanine
P = pro = proline
S = ser = serine
T = thr = threonine; and
W = trp = tryptophan
Y = tyr = tyrosine
V = val = valine.

EXAMPLES

The present invention will be described more detailed in the following specific examples. Said examples are not to be construed to limit the scope of the invention otherwise as defined in the appended claims. The examples are given with reference to the appended drawings, wherein:

Fig. 1 shows antibody reactivity of peptides according to the invention expressed as absorbance at 405 nm;

Fig. 2 shows antibody reactivity to peptides according to the invention also expressed as the absorbance at 405 nm;

Fig. 3 shows a replacement set analysis of 33G2 reactivity to an octapeptide; and

Fig. 4 shows antibody reactivity to epitope analogs expressed as the absorbance at 405 nm.

EXAMPLE 1

Establishment of a human B-cell line producing the monoclonal antibody 33G2.

Periferal blood lymnphocytes (PBL) were isolated by standard procedures from heparinized blood of a Liberian donor known to be immune to malaria (Udomsangpetch, R., Lundgren, K., Berzins, K., Wåhlin, B., Perlmann, H., Troye-Blomberg, M., Carlsson, J., Wahlgren, M., Perlmann, P. and Björkman, A. (1986) Human monoclonal antibodies to Pf155, a major antigen of malaria parasite Plasmodium falciparum. Science 231, 57-59). The PBL were incubated for 3 days with P.falciparum parasite extract in order to activate the malaria specific B-lymphocytes (Lundgren, K., Wahlgren, M., Troye-Blomberg, M., Berzins, K., Perlmann, H. and Perlmann, P. (1983) Monoclonal anti-parasite and anti-RBC antibodies produced by stable EBV-transformed B cell lines from malaria patients. J.Immunol. 131, 2000-2003). For transformation of B-lymphocytes Epstein Barr virus (EBV), contained in culture medium from the EBV-producing marmoset cell line B95-8, was added to the PBL and incubated for 2hr at 37°C. After washing, the cells were suspended at $2x10^6$/ml in tissue culture medium

(RPMI 1640 supplemented with 10% fetal calf serum, 1% glutamine and 25µg/ml gentamicin) containing 0.2 µg/ml of cyclosporin A and then incubated in 10 ml roundbottomed tubes for 5 days at 37°C in air + 5% CO_2. The cells were then transferred to 50 ml tissue culture flasks for continued propagation for 9 days. Cyclosporin was present in all media during the first 2 weeks of cell propagation. Two weeks after transformation, the cells were seeded in 96-well tissue culture plates with $5x10^5$ irradiated (4500 rad) allogeneic PBL per well as feeder cells. Immunoglobulin producing cultures were detected by measuring in enzyme linked immunosorbent assay (ELISA) (Engvall & Perlmann, Immunochemistry 8, 871-874, 1971) the presence in the culture medium of the wells of immunoglobulin. Immunoglobulin containing culture supernatants were further analyzed for the presence of antibodies to the P.falciparum antigen Pf155/RESA by means of indirect immunofluorescence using glutaraldehyde fixed and air dried monolayers of infected erythrocytes from P.falciparum in vitro cultures (Perlmann et al. J.Exp.Med. 159, 1686-1704, 1984). Cultures scoring positive in the latter assay were submitted to repeated cloning by limited dilution. Monoclonality of the antibodies produced was assessed by isoelectric focusing. The antibody producing clone 33G2 thus obtained showed high growth rate and antibody production (10-15 µg IgM/ml in 72 hours) (Udomsangpetch, R., Lundgren, K., Berzins, K., Wåhlin, B., Perlmann, H., TroyeBlomberg, M., Carlsson, J., Wahlgren, J., Perlmann, P. and Björkman, A. (1986) Human monoclonal antibodies to Pf155, a major antigen of malaria parasite Plasmodium falciparum. Science 231, 57-59).

EXAMPLE 2

Inhibition in vitro of malaria parasite invasion into red blood cells.

The capacity of the monoclonal antibody (mAb) 33G2 to inhibit parasite development in P.falciparum in vitro cultures was assayed by a procedure described by Wåhlin et al.

(Proc.Nat.Acad.Sci. USA 81, 7912-7916, 1984). P.falciparum cultures were diluted with normal O+ erythrocytes to a parasitemia of 0.5% and a hematocrit of 2%.

Aliquots of the parasite suspension (100 µl) were seeded in quadruplicate in 96-well flat-bottomed microculture plates. The mAb 33G2 was added to the wells at differrent concentrations, either in culture supernatant or after purification on concanavalin A-Sepharose of ammonium sulfate precipitated culture supernatants (Kleine et al. Molec.Immunol. 16, 421--425, 1979). After incubation for 20 hr at 37°C in a candle jar (Trager & Jensen, Science 193, 673-675, 1976), the erythrocytes from each well were washed in tris-buffered Hanks solution (TH) and monolayers were prepared on eight-well multitest slides as follows. Erythrocyte suspensions were applied to slides treated with bicarbonate buffer (pH 9.6). Immediately after being washed in TH, the monolayers were fixed briefly (2x10 sec) in 1% glutaraldehyde in phosphate-buffered saline (pH 7.4), washed in distilled water and then air-dried extensively under a fan. The parasites were stained with acridine orange and the number of parasitized erythrocytes was obtained by counting $4x10^4$ erythorcytes per sample.

Both preparations of mAb 33G2 inhibited P.falciparum reinvasion efficiently in a concentration dependent manner, the culture supernatant contained mAb giving 50% inhibition of reinvasion at 14 µg/ml and the purified mAb giving 50% inhibition at 5.5 µg/ml (Udomsangpetch, R., Lundgren, K., Berzins, K., Wåhlin, B., Perlmann, H., Troye-Blomberg, M., Carlsson, J., Wahlgren, M., Perlmann, P. and Björkman, A. (1986) Human monoclonal antibodies to Pf155, a major antigen of malaria parasite Plasmodium falciparum. Science 231, 57-59).

EXAMPLE 3

Inhibition of cytoadherence of malaria infected erythrocytes to endothelial and melanoma cells in vitro.

The capacity of mAb 33G2 to inhibit the cytoadherence

of P.falciparum infected erythrocytes to endothelial cells was demonstrated in an assay using the melanoma cell line C32 as described by Udeinya et al. (Exp. Parasitol. 56, 207-214, 1983). Melanoma cells grown on cover slips were fixed with 1% formaldehyde in phosphate-buffered saline (pH 7.4) and then stored at 4°C until used. A suspension (2% hematocrit) of erythrocytes from a P.falciparum culture, containing mainly trophozoites and schizonts at 5-10% parasitemia, were incubated with the fixed melanoma cells at room temperature on a rotating platform for 1 hour. Unbound erythrocytes were flushed away with phosphate-buffered saline. The coverslips were then fixed with 1% glutaraldehyde in phosphate-buffered saline, stained with Giemsa and examined in the light microscope. For assaying antibody mediated inhibition of cytoadherence, pellets of infected erythrocytes (40-50 µl) were suspended in 100 µl of antibody solution (15-250 µg/ml) and incubated for 30 min at 37°C with agitation every 10 min. The erythrocytes were then diluted to a 2% hematocrit suspension and applied to the cytoadherence assay as described above. The number of bound erythrocytes per 100 melanoma cells was counted and expressed as the percentage of bound cells.

The mAb 33G2 inhibited cytoadherence in a dose dependent manner, giving about 45% inhibition at the highest antibody concentration tested (250 µg of purified mAb per ml) (Udomsangpetch, R., Aikawa, M., Berzins, K., Wahlgren, M. and Perlmann, P. (1989) Cytoadherence of knobless Plasmodium falciparum-infected erythrocytes and its inhibition by a human monoclonal antibody. Nature 338, 763-765).

EXAMPLE 4

Reactivity of monoclonal antibody 33G2 with cross-reactive P.falciparum antigens.

The mAb 33G2 was initially selected due to its reactivity with the P.falciparum antigen Pf155/RESA as detected by immunofluorescence and immunoblotting (see Ex. 1 and ref. 1). Analysis of antibody reactivity with different recombinant

P.falciparum blood stage antigens was performed by immunoblotting using recombinant bacterial (E.coli)plaques (Mattei, D., Berzins, K., Wahlgren, M., Udomsangpetch, R., Perlmann, P., Griesser, H.W., Scherf, A., Müller-Hill, B., Bonnefoy, S., Guillotte, M., Langsley, G., Pereira da Silva, L. and Mercereau-Puijalon, O. (1989) Cross-reactive antigenic determinants present on different Plasmodium falciparum blood--stage antigens. Parasite Immunol. 11, 15-30). The mAb showed binding to bacterial plaques expressing parts of the P.falciparum antigens Pf11.1, Ag332 and Pf155/RESA, showing the strongest reactivity with Ag332 expressing plaques (Mattei, D., Berzins, K., Wahlgren, M., Udomsangpetch, R., Perlmann, P., Griesser, H.W., Scherf, A., Müller-Hill, B., Bonnefoy, S., Guillotte, M., Langsley, G., Pereira da Silva, L. and Mercereau-Puijalon, O. (1989) Cross-reactive antigenic determinants present on different Plasmodium falciparum blood--stage antigens. Parasite Immunol. 11, 15-30). No binding was seen to bacterial plaques expressing the P.falciparum antigens FIRA or Ag281. The capacity of various synthetic peptides, corresponding to repeated sequences in the antigens Pf11.1, Ag332 and Pf11/RESA, to block the binding of mAb 33G2 to Pf11/RESA as detected by immunofluorescence was analysed (Udomsangpetch, R., Carlsson, J., Wåhlin, B., Holmquist, G., Ozaki, L.S., Scherf, A., Mattei, D., Mercereau-Puijalon, O., Uni, S., Aikawa, M., Berzins, K. and Perlmann, P. (1989) Reactivity of the human monoclonal antibody 33G2 with repeated sequences of three distinct Plasmodium falciparum antigens. J.Immunol. 142, 3620-3626). Different concentrations of the peptides (up to 200 µg/ml) were mixed with a fixed concentration of the mAb, which then was used in the immunofluoresce assay (see Ex. 1). The peptide Y $(SVTEEIAEEDK)_2$, corresponding to a dimer of amino acids 2-12 in antigen Ag332 (Mattei, D., Berzins, K., Wahlgren, M., Udomsangpetch, R., Perlmann, P., Griesser, H.W., Scherf, A., Müller-Hill, B., Bonnefoy, S., Guillotte, M., Langsley, G., Pereira da Silva, L. and Mercereau-Puijalon, O. (1989) Cross-reactive antigenic deter-

minants present on different Plasmodium falciparum blood-stage antigens. Parasite Immunol. 11, 15-30), was the most efficient inhibitor of mAb binding, giving complete inhibition of immunofluorescence at 0.2 µg/ml (Udomsangpetch, R., Carlsson, J., Wåhlin, B., Holmquist, G., Ozaki, L.S., Scherf, A., Mattei, D., Mercereau-Puijalon, O., Uni, S., Aikawa, M., Berzins, K. and Perlmann, P. (1989) Reactivity of the human monoclonal antibody 33G2 with repeated sequences of three distinct Plasmodium falciparum antigens. J.Immunol. 142, 3620-3626). Also some peptides corresponding to sequences in Pf11.1 and PF155/RESA inhibited mAb 33G2 immunofluorescence but with considerably less efficiency, the Pf11.1 peptide $(EEVVEEVVP)_2$ and the Pf155/RESA peptide both giving complete inhibition at 100 µg/ml. The results show that mAb 33G2 recognizes a family of cross-reactive P.falciparum antigens including Pf11.1, Pf155/RESA and Ag332, the latter antigen being the optimal target for the mAb (Udomsangpetch, R., Carlsson, J., Wåhlin, B., Holmquist, G., Ozaki, L.S., Scherf, A., Mattei, D., Mercereau-Puijalon, O., Uni, S., Aikawa, M., Berzins, K. and Perlmann, P. (1989) Reactivity of the human monoclonal antibody 33G2 with repeated sequences of three distinct Plasmodium falciparum antigens. J.Immunol. 142, 3620-3626).

EXAMPLE 5

Determination of the epitope specificity of the monoclonal antibody 33G2.

The detailed epitope specificity on the single amino acid level for the mAb 33G2 was performed using the multiple peptide synthesis technique (PEPSCAN) developed by Geysen et al. (J.Immunol.Methods 102, 259-274, 1987). Peptides were synthesized on polyethylene rods on which polymers of polyacrylic acid had been formed by irradiation. Polyethylene rods and Fmoc L-amino acids performed as active esthers (Cambridge Research Biochemicals, UK) were used for synthesis according to instructions of the manufacturer. The N-termi-

nals of all peptides were acetylated. As a basis for the mAb 33G2 epitope analysis, the sequence of amino acid residues 1-19 (ESVTEEIAEEDKSVIEEAV) of Ag332 (Mattei, D., Berzins, K., Wahlgren, M., Udomsangpetch, R., Perlmann, P., Griesser, H.W., Scherf, A., Müller-Hill, B., Bonnefoy, S., Guillotte, M., Langsley, G., Pereira da Silva, L. and Mercereau-Puijalon, O. (1989) Cross-reactive antigenic determinants present on different Plasmodium falciparum blood-stage antigens. Parasite Immunol. 11, 15-30) was used, containing sequences of the peptides with the highest reactivity with the mAb (Udomsangpetch, R., Carlsson, J., Wåhlin, B., Holmquist, G., Ozaki, L.S., Scherf, A., Mattei, D., Mercereau-Puijalon, O., Uni, S., Aikawa, M., Berzins, K. and Perlmann, P. (1989) Reactivity of the human monoclonal antibody 33G2 with repeated sequences of three distinct Plasmodium falciparum antigens. J.Immunol. 142, 3620-3626). All possible overlapping heptapeptides, hexpeptides, pentapeptides and tetrapeptides covering the mentioned sequence were synthesized and their reactivity with mAb 33G2 was analysed by ELISA as described by Geysen et al. (J.Immunol. Methods 102, 259-274, 1987). Culture supernatant containing mAb 33G2 (approx. 10 µg/ml), was diluted 1:100. Peptide containing rods were washed in phosphate-buffered saline with 0.05% Tween 20 between all steps in ELISA. Bound antibodies were detected with a rabbit anti-human IgM-alkaline phosphatase conjugate (Sigma, St. Louis, MO) using p-nitrophenyl phosphate, disodium salt (Sigma) as substrate.

The antibody showed reactivity with four heptapeptides corresponding to amino acids 1-7 (ESVTEEI), 2-8 (SVTEEIA), 3-9 (VTEEIAE) and 4-10 (TEEIAEE) (Fig. 1a). When tested against hexapeptides the antibody recognized sequences corresponding to amino acids 2-7 (SVTEEI), 3-8 (VTEEIA) and 4-9 (TEEIAE) (Fig. 1b). Reactivity to pentapeptides was restricted to one peptide, corresponding to amino acids 3-7 (VTEEI) (Fig. 1c), while no reactivity was seen with any of the tetrapeptides (Fig. 1d). When tested against eight heptapeptides

corresponding to the sequence ESVTEEIA (amino acids 1-8), where one amino acid residue had been omitted in each peptide, the antibody could not recognize peptides where either V, T, E, E or I had been excluded (Fig. 2).

The mAb 33G2 was analyzed for reactivity against octapeptides based on the sequence ESVTEEIA, where single amino acid substitutions replaced each residue. Every residue in the parent peptide ESVTEEIA, which corresponds to residue 1-8 of the known sequence of Ag332, was replaced by the most common 20 amino acids (Fig. 3). The first (E), second (S) and last amino acid residue (A) were shown to be replaceable by any other amino acid without loosing the ability of the monoclonal 33G2 to recognize the peptides. A linear, five amino acid, sequence (VTEEI) was shown to consist of amino acids which were either essential or replaceable mainly by amino acids of resembling chemical character. Subsitution of valine (V) by C, F, I, L, P, T, W and Y, and threonine (T) by A, C, I, L, P, S, and V, gave ELISA absorbance values of 20% or more compared to the values obtained with the parent octapeptides. The pair of glutamic acids (E), contained within the epitope, were the most essential residues. The first glutamic acid (E) was totally nonreplaceable while the second glutamic acid (E) was possible to replace with aspartic acid (D), a very conserved replacement, and to some extent with cysteine (C). The last amino acid within the epitope, isoleucine (I), was possible to replace with leucine (L) and valine (V), two relatively conserved replacements. It could also be replaced to some degree by the positively charged amino acid histidine (H).

Based on the results in the replacement set analysis, pentapeptides corresponding to residue 3-7 of Ag332 were constructed in which one or severala original amino acids had been replaced simultaneously. The results from this assay showed that it was possible to replace several amino acids within the epitope, simultaneously, without loosing antibody reactivity (Fig. 4). The antibody recognized most of the pep-

tides where the modifications were consistent with the results from the replacement set analysis; ITEEI, VAEEI, IADEI, IADEV, VVEEV and LVEEV. A decrease in antibody reactivity could be seen for some peptides where several amino acids were replaced. The antibody did not react with the pentapeptide, YLDEV, indicating that not all of the reactive single amino acid substitutions can be performed simultaneously and still result in reactive peptides.

CLAIMS

1. A peptide comprising the amino acid sequence:

U-O-X-glu-Z, wherein:

U is an amino acid residue selected from val and ile;

O is an amino acid residue selected from ala and thr;

X is an amino acid residue selected from asp and glu; and

Z is an amino acid residue selected from ile and val.

2. A peptide comprising the amino acid sequence:

O-X-glu-Z-ala-glu, wherein:

O, X and Z are as defined in claim 1.

3. A peptide according to claim 1, wherein said amino acid sequence is selected from:

glu-ser-val-thr-glu-glu-ile;

ser-val-thr-glu-glu-ile-ala;

val-thr-glu-glu-ile-ala-glu;

ser-val-thr-glu-glu-ile;

val-thr-glu-glu-ile-ala;

val-thr-glu-glu-ile;

ile-thr-glu-glu-ile;

val-ala-glu-glu-ile; and

ile-ala-asp-glu-ile.

4. A peptide according to claim 3, wherein said amino acid sequence is:

val-thr-glu-glu-ile.

5. A peptide according to claim 2, wherein said amino acid sequence is selected from:

thr-glu-glu-ile-ala-glu-glu; and

thr-glu-glu-ile-ala-glu.

6. A peptide according to any preceding claim for medicinal use.

7. A peptide according to claim 6 for use as an active ingredient in a vaccine.

8. A peptide according to claim 7 for use as an active ingredient in a vaccine against malaria.

9. The use of the peptide according to any of claims 1 to 5 in the preparation of a vaccine.

10. The use of the peptide according to claim 9 in the preparation of a vaccine against malaria.

11. A composition for vaccination against malaria induced by Plasmodium falciparum, comprising a peptide according to any of claims 1 to 5 in admixture with a pharmaceutically acceptable carrier.

12. A composition according to claim 11, wherein said carrier is suitable for parenteral administration.

13. A composition according to claim 11 or 12, wherein said peptide is comprised in a larger molecule, wherein it is present in repeating units.

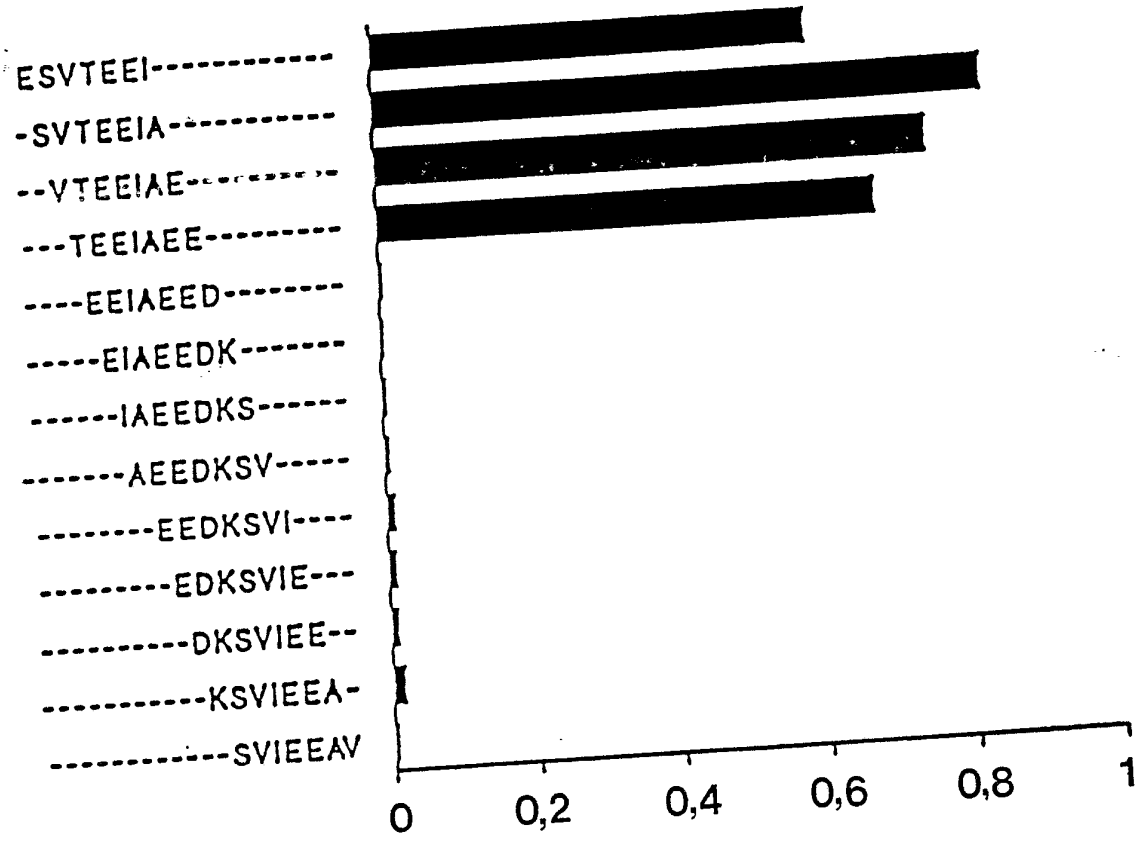
14. A composition according to claim 11 or 12, wherein said peptide is coupled to a carrier molecule.

15. A composition according to claim 11 or 12, wherein said peptide is coupled to an immunogenic carrier.

16. A method of inducing immunity against malaria induced by Plasmodium falciparum, which comprises administering to a person in need of such immunity an effective amount of the composition of any of claims 11 to 15.

17. A method according to claim 16, wherein the administration is constituted by parenteral injection.

FIG.1a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--VTEEIAE----------
---TEEIAEE---------
----EEIAEED--------
-----EIAEEDK-------
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-------AEEDKSV-----
--------EEDKSVI----
---------EDKSVIE---
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-----------KSVIEEA-
------------SVIEEAV
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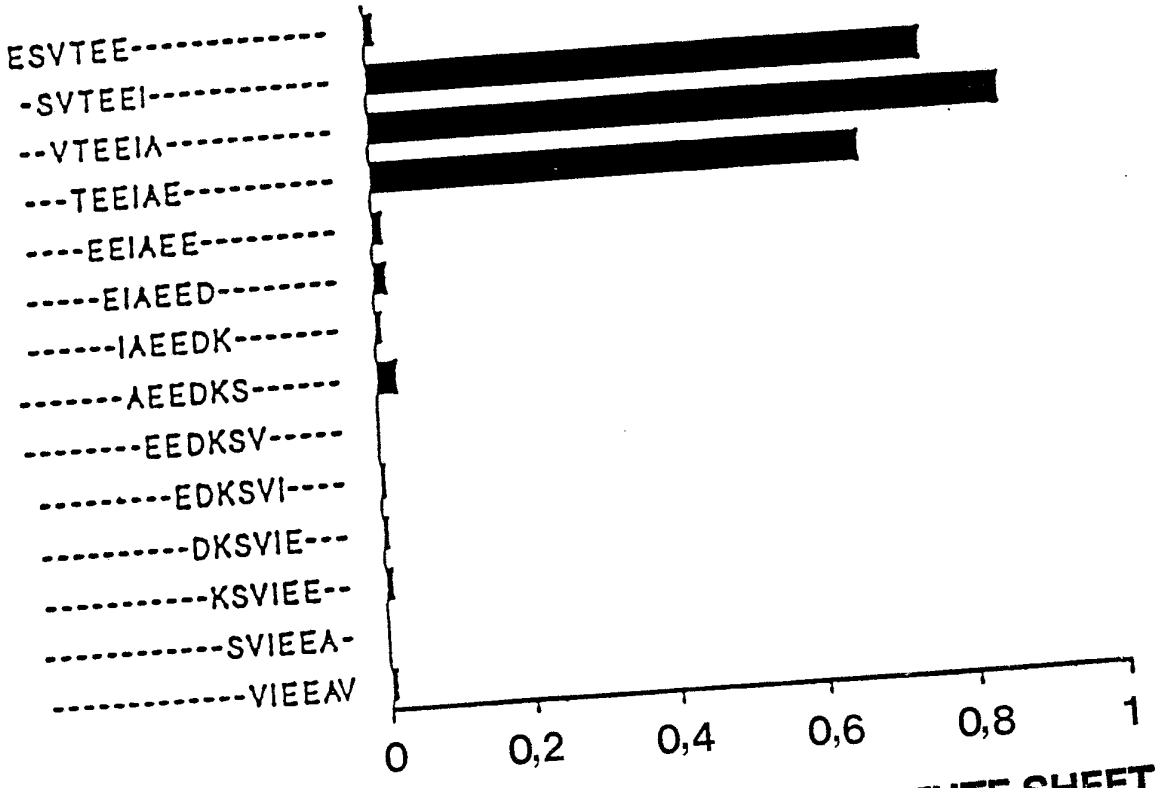
FIG.1b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-SVTEEI----------
--VTEEIA----------
---TEEIAE---------
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-----EIAEED-------
------IAEEDK------
-------AEEDKS-----
--------EEDKSV----
---------EDKSVI---
----------DKSVIE--
-----------KSVIEE-
------------SVIEEA
-------------VIEEAV
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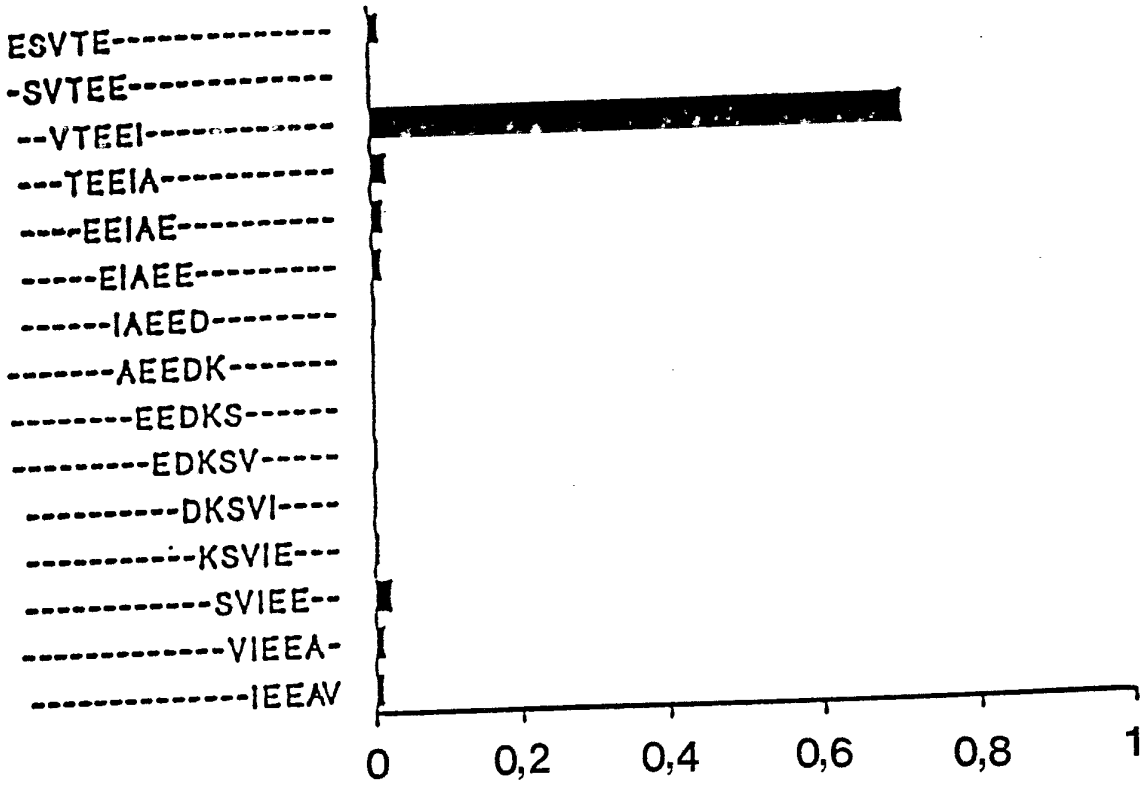
FIG.1c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-SVTEE----------
--VTEEI----------
---TEEIA----------
----EEIAE----------
-----EIAEE----------
------IAEED--------
-------AEEDK-------
--------EEDKS------
---------EDKSV-----
----------DKSVI----
----------KSVIE---
----------SVIEE--
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----------IEEAV
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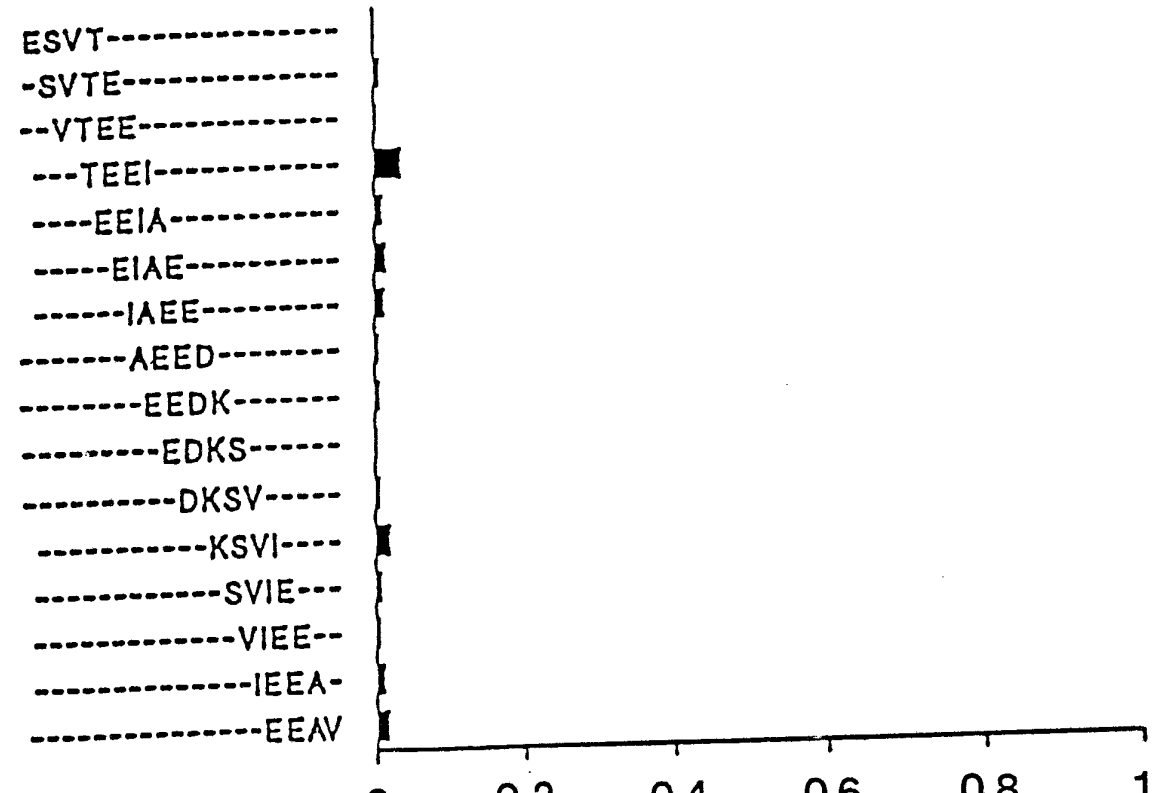
FIG.1d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--VTEE----------
---TEEI----------
----EEIA----------
-----EIAE----------
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-------AEED--------
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----------KSVI----
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----------VIEE--
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----------EEAV
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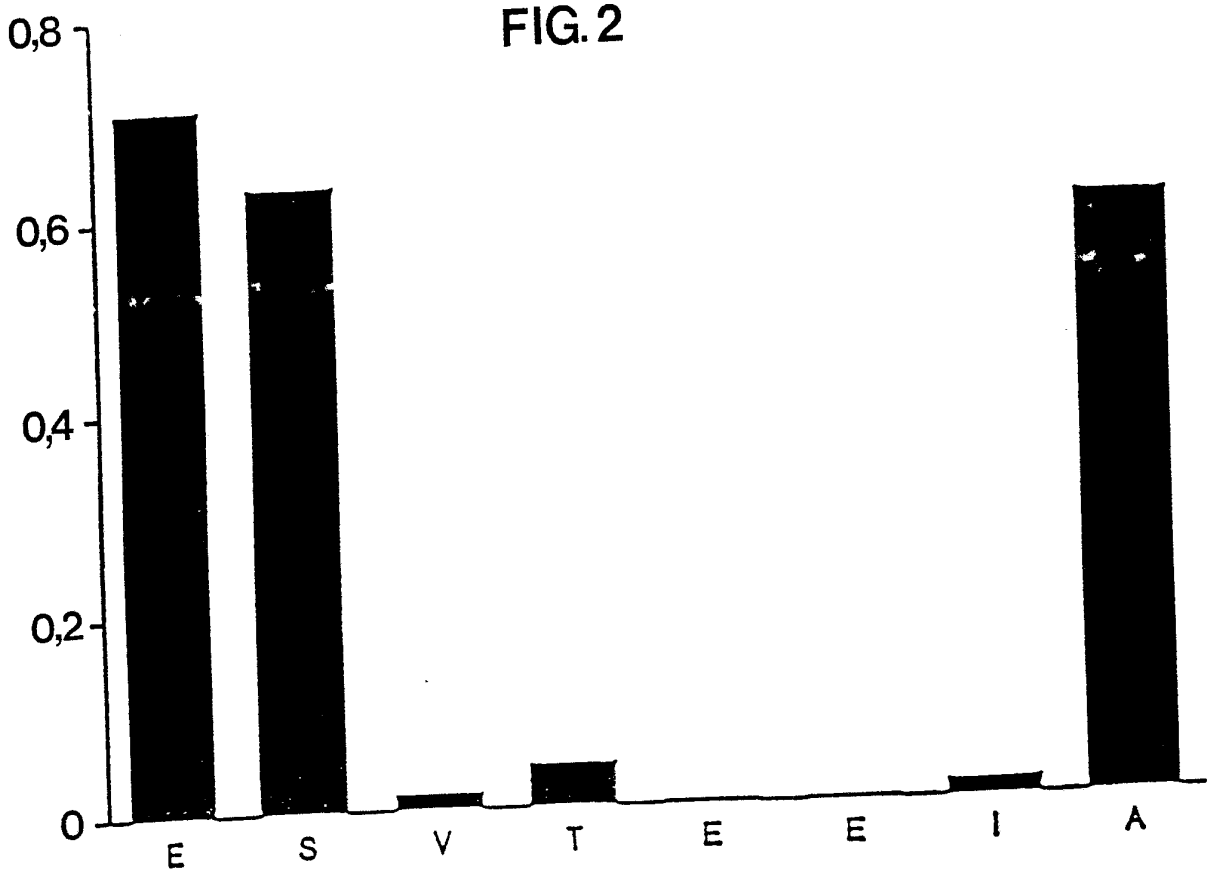
FIG. 2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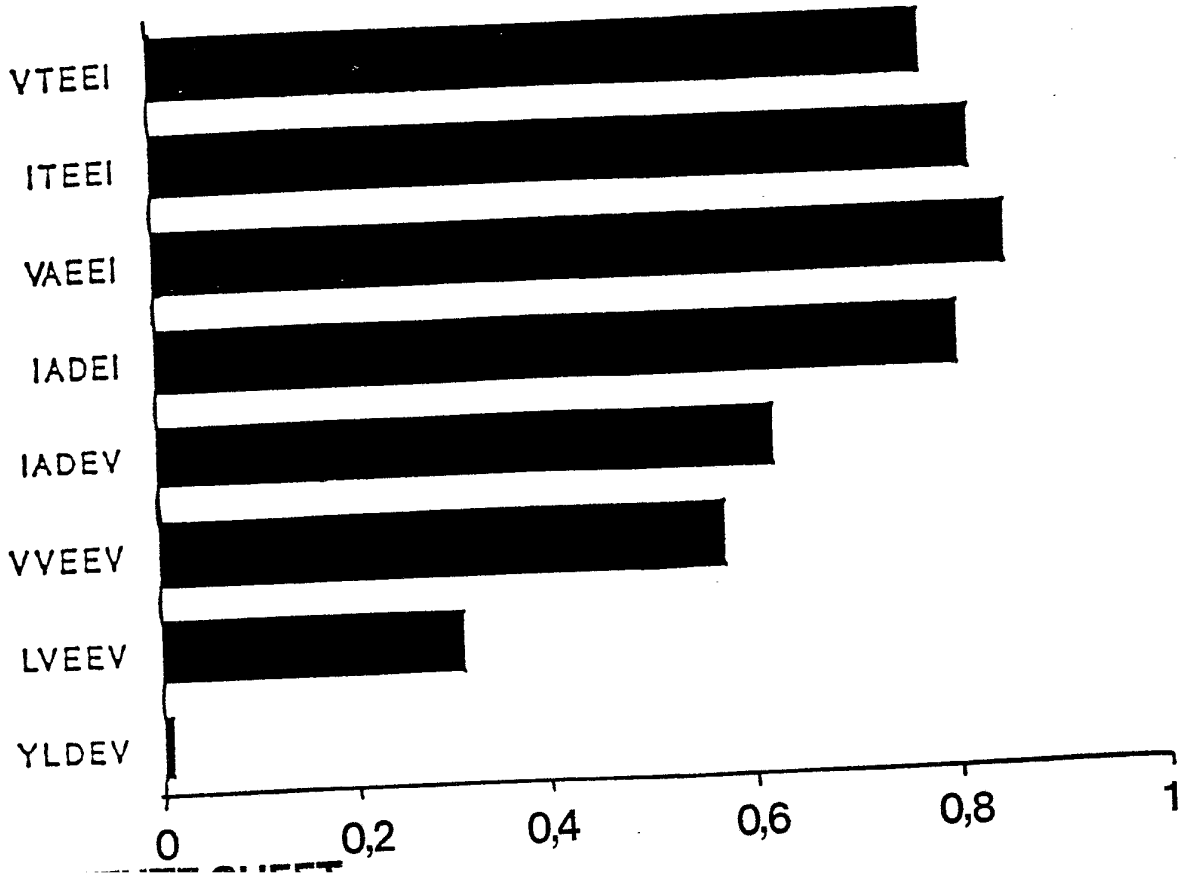
E
I
A

FIG.4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VVEEV
LVEEV
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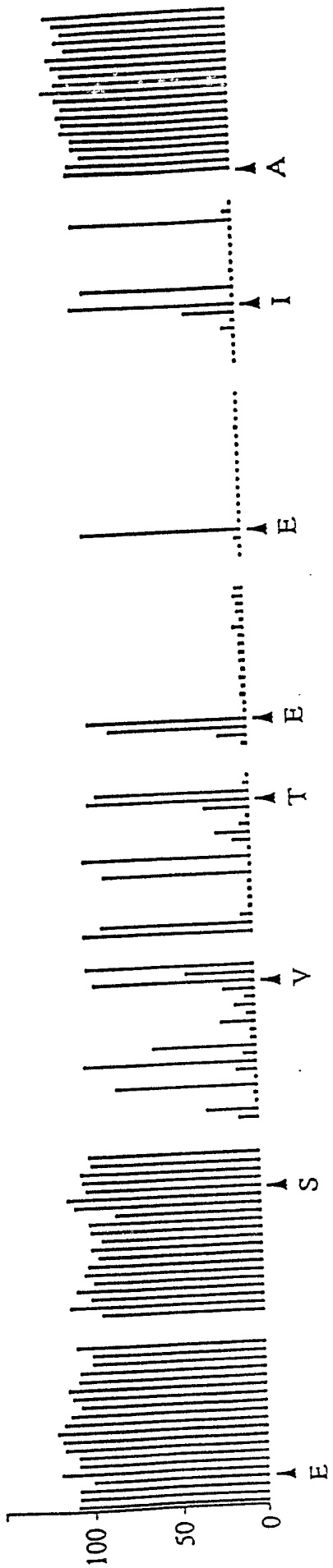

FIG.3

INTERNATIONAL SEARCH REPORT

International Application No PCT/SE 91/00541

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC5: A 61 K 39/015, C 07 K 15/0, C 07 K 7/06

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
IPC5	A 61 K; C 07 K

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in Fields Searched[8]

SE,DK,FI,NO classes as above

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category *	Citation of Document,[11] with indication, where appropriate, of the relevant passages[12]	Relevant to Claim No.[13]
X	Ann. Inst. Pasteur/Immunol., Vol. 139, 1988 P. Dubois et al.: "Structure and function of a thymic peptide is mimicked by plasmodium falciparum peptides", see page 557 - page 567 see especially p. 558, lines 25-28, p. 560, fig. 1. --	1-15
A	EP, A2, 0275196 (PATARROYO, MANUEL E.) 20 July 1988, see the whole document --	1-15
A	WO, A1, 8800597 (SARAMANE PTY. LTD. ET AL.) 28 January 1988, see the whole document --	1-15

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* Special categories of cited documents: [10]

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

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance, the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance, the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
20th November 1991	1991 -11- 26
International Searching Authority	Signature of Authorized Officer
SWEDISH PATENT OFFICE	Mikael Bergstrand

Form PCT/ISA/210 (second sheet) (January 1985)

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

V. ☒ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [1]

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☒ Claim numbers 16 - 17, because they relate to subject matter not required to be searched by this Authority, namely:

Methods for treatment of the human or animal body by surgery or therapy, as well as diagnostic methods, cf. PCT Rule 39.4

2. ☐ Claim numbers....., because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claim numbers....., because they are dependent claims and are not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. ☐ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [2]

This International Searching Authority found multiple inventions in this international application as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the the claims. It is covered by claim numbers:

4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additonal fee.

Remark on Protest

☐ The additional search fees were accompanied by applicant's protest.

☐ No protest accompanied the payment of additional seach fees.

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO.PCT/SE 91/00541

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.
The members are as contained in the Swedish Patent Office EDP file on 91-09-27
The Swedish Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
EP-A2- 0275196	88-07-20	AU-B-	603856	90-11-29
		AU-D-	1024388	88-07-21
		JP-A-	63239296	88-10-05
		US-A-	4735799	88-04-05
		US-A-	4957738	90-09-18
WO-A1- 8800597	88-01-28	AU-B-	594804	90-03-15
		AU-D-	7698287	88-02-10
		EP-A-	0273971	88-07-13
		GB-A-	2200642	88-08-10
		JP-T-	1500827	89-03-23